these nine proteins in UMP were put in the middle of BENIGN and MALIGNANT. Accordingly, immunoexpression profiles of UMP were distinct from those of BENIGN or MALIGNANT ($p < 0.05$). Besides, BENIGN, UMP and MALIGNANT were different in the number of aberrant proteins ($p < 0.05$); mean 2.06 vs. 3.78 vs. 5.27 out of nine proteins, respectively.

Conclusions: Appendiceal mucinous neoplasm can be classified into three groups, and mucinous neoplasm of uncertain malignant potential is a distinct entity in terms of molecular markers. Immunoexpression profiling may be useful to predict the clinicopathogical behavior of UMP.

691 The Potential Role of p27, Skp2, and PTEN Expression in Gastric Carcinoma

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Background: $p27^{Kip1}$ is a cell-cycle inhibitory protein and its downregulation is mediated by its specific ubiqitin subunit Skp2. PTEN is a tumor suppressor gene which upregulates p27. This study investigates p27, Skp2 and PTEN expression in gastric carcinoma (GC).

Design: The study included 127 patients with GC who underwent gastrectomy. None of the patients received any chemotherapy or radiotherapy prior to, or after surgery. Eight tumors were TNM-stage I, 39 II, 61 III, and 19 IV, whereas 95 tumors were low-grade (grade I and II intestinal type adenocarcinomas) and 32 high-grade (grade III intestinal type, and diffuse type adenocarcinomas). Formally fixed, paraffin- embedded 4μm sections were subjected to immunohistochemistry using monoclonal antibodies for p27, Skp2 and PTEN. Nuclear staining was considered as positive. Results were correlated with pathologic data and patients' survival. Mean follow-up time was 45.3 months (range 3.5-140 months).

Results: Expression of p27, Skp2 and PTEN was recorded in 78/127 (61.4%), 57/127 (44.8%) and 99/127 (77.9%), respectively. PTEN and p27 expression was higher and Skp2 expression was lower in tumors of early stage and low grade compared to those of advanced stage and high grade. Skp2 expression levels were inversely correlated to p27 and PTEN in gastric carcinomas (p=0.0039 and p=0.0068 respectively). Statistical analysis revealed a positive correlation between PTEN expression and survival (p=0.007); Skp2 expression was negatively associated with survival (p=0.015). Cox regression analysis revealed that tumor grade and stage, and PTEN expression were independent prognostic factors (CI: 0.032-0.502, p=0.03, CI:1.167-5.408, p=0.019, CI: 1.065-41.082, p=0.032, respectively).

Conclusions: The study demonstrates that in GC loss of PTEN and p27 expression and enhancement of Skp2 expression are associated with adverse pathological parameters and increased risk for tumor recurrence. Loss of p27 in gastric carcinomas may be mediated by Skp2 overexpression. PTEN is possibly involved in the regulation of p27 levels via negative regulation of Skp2.

PTEN, p27 and Skp2 expression

	PTEN	p27	Skp2
Low grade GC	87.3±11.9[a]	79.2±13.2[c]	22.41±6.3[e]
High grade GC	7.4±2.5[a]	5.3±3.1[c]	38.85±5.63[e]
TNM stages I+II	86.1±8.34[b]	73.1±7.32[d]	22.78±6.92[f]
TNM stages III+IV	12.4±1.2[b]	5.2±2.7[d]	53.01±7.34[f]

[a, d, f]:p<0.001, [b]: p=0.0029, [c]:p=0.0012, [e]: p=0.031

692 Histologic Mimics of Menetrier's Disease

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Background: Menetrier Disease (MD), a hypertrophic gastropathy characterized by giant rugal folds due to foveolar hyperplasia, with loss of parietal cells, hypochlorhydria, and protein loss, is rarely seen by most pathologists. The goal of this study was to characterize histologic mimics of MD submitted for evaluation for enrollment into a clinical trial for treatment of MD, to determine pitfalls in the diagnosis of this rare disorder.

Design: Material from 27 subjects referred for entry into a clinical trial for treatment of MD was reviewed. Pre-treatment stomach biopsies were evaluated for foveolar hyperplasia, hypermucinous change, dilatation of glands, lymphocytic gastritis; lamina propria eosinophils, plasma cells, edema, smooth muscle, granulation tissue, and presence of microorganisms.

Results: Nine of 27 subjects (33%) referred were considered to have MD. An additional adult patient was diagnosed with CMV-related MD and did not receive treatment. Foveolar hyperplasia, hypermucinous change, dilatation of glands and lamina propria smooth muscle hyperplasia were present in all patients with MD. More than half had prominent lamina propria eosinophils (5/9), and/or plasma cells (6/9), and lamina propria edema (8/9). Lymphocytic gastritis was not a feature of our MD cases. Antral biopsies were available on 2 MD patients which revealed similar changes to the fundic biopsies. Mimickers of MD included gastric involvement by juvenile polyposis (3), Cronkhite-Canada syndrome (1), proton pump inhibitor effect (1), gastric antral vascular ectasia (1), and chronic atrophic gastritis with hyperplastic polyps (7). While hyperplastic polyps and juvenile polyps were characterized by foveolar hyperplasia and hypermucinous change, lamina propria smooth muscle fibers were less prominent than in MD. For 4 patients, hypertrophic changes potentially representing MD were present, but diagnosis was inconclusive due to lack of diffuse gastric body involvement, lack of hypochlorhydria, and/or atypical clinical symptoms.

Conclusions: Foveolar hyperplasia, hypermucinous change, dilatation of glands and lamina propria eosinophilia, plasma cells, edema, and smooth muscle hyperplasia are a constellation of features characteristic of MD, but correlation with endoscopic and clinical findings remains essential for accurate diagnosis. Hyperplastic polyps and previously undiagnosed juvenile polyposis were the most common lesions confused with MD.

Genitourinary

693 IFI16: A Novel Growth Suppressor in Human Prostate Cancer

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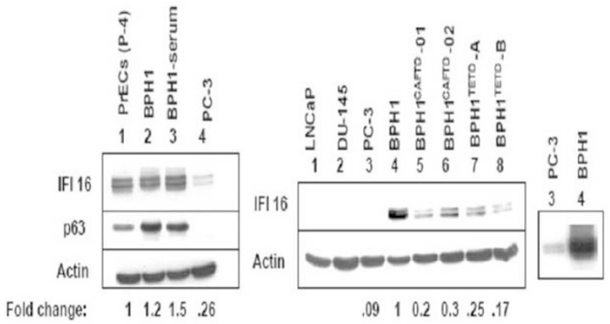
Background: Tumor suppressors (TS) provided a molecular basis for multistep carcinogenesis; human cancers have multiple genetic alterations including loss of TS. Interferon inducible IFI16 (encoded by *IFI16* gene) is a human member of the p200-protein family and its increased expression in human normal prostate epithelial cells (PrECs) leads to cellular senescence-associated cell growth arrest. Our aim is to study the role of IFI16 protein in PC, as studies of TMPRSS2 overexpression predict aggressive PC.

Design: By immunoblotting, expression of IFI16 protein was compared in total cell lysates from cultured normal PrEC, a benign prostate hyperplasia cell line (BPH-1), and derivatives from the BPH1 cell line that have acquired the ability to form tumors in nude mice (aggressive lines), and PC cell lines (RWPE-1, DU-145, PC-3 and LNCaP). Human prostate tissue array slides (Imgenex) containing 98 prostate samples and 30 of our own archival cases (total 45 BPH, 29 Gleason Score(GS)>6, 26 GS7, 24 GS<8, and 4 metastatic PC to bone marrow and lymph nodes) were stained with H&E and IHC for IFI16 (goat polyclonal sc-6050 Santa Cruz Biotech) and p63 (Ventana pre-dilute). Two pathologists independently reviewed and concurred on GS and IHC intensity and percentage of glands staining for IFI16 on a scale of 0 (no stain) to 2 (highest). Expression of p63 was used as a control in cell cultures and tissue IHC.

Results: Normal PrEC and BPH cell lines expressed IFI16 protein; however, IFI16 is reduced or lost in more aggressive BPH1 and most human PC cell lines tested. P63 was lost in all PC cells and tissue, as a control.



Loss of IFI16 protein expression is associated with the development of an aggressive form of prostate cancer

IHC expressed IFI16 weakly in BPH, and strongly in low grade PC; most high grade PC and metastasis lost the IFI16 staining. P=0.0003.

STAIN	0	1	2
BPH	22(49%)	23(51%)	0
GS<6	0	11(38%)	18(62%)
GS7	2(7%)	15(57%)	9(34%)
GS<8	13(54%)	9(37%)	2(8%)
METASTASES	4(100%)		

IHC for IFI16 – number of cases (% of cases)

Conclusions: This study shows molecular and phenotypic evidence that IFI16 loss may serve as a late hit in the multistep carcinogenesis of PC. Our model shows loss of IFI16 expression in higher grade/stage PC; this may predict aggressive PC, similar to TMPRSS2 overexpression. Further studies are warranted to confirm our findings.

694 PCA3: A Urine-Based Genetic Assay for Detection of Prostate Cancer in Men with Elevated PSA

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Background: Serum PSA has been labeled the most important tumor marker in oncology and is a valuable screening tool for prostate cancer. However, since it is not prostate-cancer specific, there is no threshold that separates men with and without cancer with a high level of accuracy. We investigated the value of PCA3 in predicting the likelihood of prostate cancer.

Design: We undertook a prospective, multi-practice, community-urologist-based, and IRB approved clinical trial to evaluate PCA3. Urine samples were obtained from 974 men with elevated serum PSA (≥2.5ng/ml) and/or abnormal digital rectal examination prior to routine 10-core prostate biopsy following standard study protocol in 30 medical practices. Urine samples were processed within 48 hours of collection. PCA3 and PSA mRNA were isolated, amplified and quantified by magnetic target capture, transcription-mediated amplification, and chemiluminescent hybridization protection assay technologies. The PCA3 value was determined using the ratio of PCA3 mRNA copy number to the PSA mRNA copy number multiplied by 1,000.

Results: In total, 380 of 974 patients (39%) were diagnosed with prostate cancer, with a mean Gleason score of 7 (range, 6-9) and 26% (range 1-100%) of specimen involvement by cancer. An additional 106 cases (11%) had only high-grade PIN and/or atypical small

acinar proliferation suspicious for cancer (ASAP), and 488 cases (50%) were benign. The mean PCA3 value in men with prostate cancer was significantly higher than in those without cancer (48 vs. 26, $p < 0.0001$). PCA3 score was associated with the presence of cancer ($p < 0.0001$) and Gleason score ($p=0.0001$), but was not associated cancer volume ($p=0.56$). Using a cutoff value of 35, PCA3 had an odds ratio of 2.6 for predicting prostate cancer. PCA3 had a specificity of 77% and a sensitivity of 44% for the diagnosis of prostate cancer, while the specificity and sensitivity for serum PSA were 22% and 87%, respectively.
Conclusions: We found that the PCA3 urine test significantly improved the specificity for the detection of prostate cancer compared to serum PSA.

695 Expression Patterns of Lewis Y Antigen in Prostate Tissue: Non-Neoplastic, Primary Adenocarcinoma and Bone Metastasis

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Background: Despite recent progress in reducing prostate cancer mortality due to improvement of surgical treatment and screening strategies for organ-confined tumors, this is still the most common cancer in males, with few therapeutical options in advanced metastatic disease. Monoclonal antibody (mAb) 3S193 was raised as a reagent against the Lewis Y (LeY) antigen, a potential target for immunotherapy. To assess a possible relevance of LeY in metastases, we searched for its immuno-expression pattern in non-neoplastic prostatic tissue (NNPT), primary prostatic adenocarcinoma (PPA) and prostatic adenocarcinoma bone metastasis (PABM) with mAb3S193.
Design: Formalin-fixed, paraffin-embedded tissue was retrieved from the files of the Dept of Pathology, Hospital das Clinicas, Sao Paulo University of Sao Paulo School of Medicine and tissue micro-arrays generated as follows: 40 cases of NNPT; 97 cases of PPA, and 22 cases of PABM. An average of 3 cores/case were arrayed. Immunohistochemistry (IHC): primary antibody: 3S193; amplification system: Novolink, polymer-base peroxidase method; semi-quantitation: estimation of percentage of positive cells , classifying according to 10% increments. Cytoplasmic and membranous immunoreactivity was individually graded . Comparison of reactivities in each compartment was assessed by non-parametric Kruskal-Wallis test.
Results: Twenty eight out of 40 NNPT (70%), 24/97 PPA (24.7%) and 13/22 (59.1%) PABM cases showed variable degrees of cytoplasmic LeY expression. For NNPT, PPA, and PABM scores for cytoplasmic LeY immunoreactivity were 12.5%±9.5, 3.4%±6.5 and 25.0%±31.4, respectively (mean + S.D., p<0,001). Regarding membranous expression, 34/40 NNPT (85%), 19/97 PPA (19.6%) and 5/22 PABM cases (22.7%) showed some degree of LeY immunostaining, with mean percentages of 12.7%±12.8, 2.1%±4.6 and 5.0%±13.3, respectively (mean + S.D., p<0,001).
Conclusions: Immunodetection of LeY by mAb3S193 occurs in larger number of cases and in higher number of cells from bone metastases than primary prostatic adenocarcinomas, both in cytoplasm and in membrane. This study further point for a possible role of mAb3S193 for treatment of subsets of metastatic prostatic adenocarcinoma.

696 Involvement of Renal Sinus Structures in Renal Cell Carcinoma, with an Emphasis on Renal Sinus Vein Invasion

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Background: The 2002 AJCC TNM staging classification of renal tumors for the first time added sinus fat invasion (SFI) to pT stage 3a, and gross invasion of muscle-containing branches of renal vein (SVBI) in the sinus to pT3b. Multiple studies on biological significance of SFI have been published since then, with conflicting results. The clinical justification of SVBI as pT3b has not been studied. We retrospectively investigated the utility of such a stage designation in a large cohort of cases.
Design: Pathology review of 214 consecutive nephrectomies performed at our institution between January, 2003 and June, 2004, with special emphasis on SVBI, was performed. Clinical follow-up was obtained from a prospectively maintained nephrectomy registry.
Results: 193 of the tumors (90%) were renal cell carcinoma (RCC), including 144 (67%) clear cell, 28 (13%) papillary, 20 (9%) chromophobe, and 1 (0.5%) unclassified subtypes. On review, 23/193 (12%) tumors were upstaged to pT3a or T3b, all because of the presence of SFI (6) or SVBI (17). Over a median follow-up of 2.9 years (range 0.1 to 5.5), 22 (11%) cases either developed or had metastases (M) at presentation. M were seen in 6/18 (33%) with SVBI without main renal vein involvement versus 7/15 (47%) with renal vein involvement (p= .493). Also, M occurred in 5/17 (29%) with SFI alone, 6/28 (21%) with perinephric fat invasion (PFI) alone, and 5/15 (33%) with both SFI and PFI. Overall survival was significantly related to SVBI, PFI and main renal vein invasion (p= .003, .002 and <.001, respectively). However on multivariate analysis, only PFI and main renal vein invasion (p= .002) remain significantly associated with death from any cause.
Conclusions: There is no significant difference in the incidence of metastases between cases with SVBI alone or with main renal vein invasion. SVBI is significantly associated with overall survival on univariate analysis, but not after adjusting for main renal vein invasion. Therefore, we are not able to support the justification of assigning pT3b stage (similar to main renal vein invasion) to SVBI alone. Additional follow-up and larger number of cases may be needed for a more definitive evaluation.

697 Immunohistochemical Screening for Lynch Syndrome in Patients with Urothelial Carcinoma of the Renal Pelvis and Ureter Does Not Correlate with Clinical Screening Parameters

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Background: Current screening practices for microsatellite instability (MSI) in colorectal carcinoma are based on the pattern of expression of four mismatch repair proteins (MMRP): MSH6, MSH2, PMS2, and MLH1. Urothelial carcinomas of the renal pelvis and ureter (UCRPU) have also been associated with Lynch syndrome (LS). We previously demonstrated a 13% incidence of abnormal expression of MMRP by immunohistochemistry (IHC) in a large cohort of UCRPU. We screened the same cohort based on clinical history parameters and compare results to the IHC.
Design: 106 UCRPU diagnosed over a period of 37 years were used to build a tissue microarray of cores measuring 2.0 mm. Resulting sections were immunostained for MSH6, MSH2, PMS2 and MLH1. Cases were scored as negative (loss of expression) or positive (protein expressed) according to convincing absence or presence of nuclear stainingin greater than or equal to 1% of tumor cells. Controls stained appropriately and slides were read by 2 pathologists. For 77 cases with available clinical charts, medical histories were reviewed for age of diagnosis, history of cancer in a first degree relative (FDR), and smoking history. Reviewers of the clinical charts were blinded with respect to IHC results. A patient was considered suspicious for LS if the UCRPU was diagnosed at age <50, or at any age if a FDR had a LS-associated cancer (i.e. colon, endometrial, ovarian).
Results: Of 77 cases, 8 (10.3%) had abnormal immunohistochemical stains (7 with absent MSH6 and MSH2 expression, 1 with absent MSH6 expression). 17 cases were considered suspicious for LS based on clinical criteria. Of these, 3 (17%) had an abnormal IHC pattern, compared to 5 of 60 cases (8.3%) that were clinically not suspicious for LS ($p=0.36$).
Conclusions: There is poor correlation between results obtained by IHC screening and clinical screening of UCRPU. These results highlight important limitations in the screening of these patients based on family history alone. Regardless of the screening method, genetic counseling and testing are necessary for adequate diagnosis of LS in patients with UCRPU.

698 *TMPRSS2-ERG* Gene Fusions in "Minimal" Prostatic Adenocarcinoma

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Background: Minimal or "insignificant" prostatic adenocarcinoma (MinPCa) is defined as tumors with insufficient virulence to threaten survival. Given recent suggestion of *TMPRSS2-ERG* gene fusion association with aggressive PCa phenotype, we aimed to evaluate incidence of *TMPRSS2-ERG* fusion in MinPCa in comparison with grade matched "non-minimal" size PCa.
Design: All 33 prostatectomies classified as containing MinPCa (2002-2003) were retrieved. Diagnosis of MinPCa (Gleason Score 6 PCa with total tumor volume < 0.5 CC, single section) was confirmed by a urologic pathologist. Tissue microarray (TMA) was constructed from the 33 cases where each tumor and paired benign tissue was represented by up to triplicate,1mm, spots. TMA sections of 59 additional archival PCa were used as controls (26 pT2 non-minimal in size, 31 pT3a and 2 pT3b). FISH analysis was performed using break-apart probes for 5' and 3' regions of *ERG*. Each spot was scored for presence of *TMPRSS2-ERG* fusion through deletion or translocation as well as for polyploidy (≥3 copies) at the *ERG* locus. At least 50 cells were scored per tumor.
Results: MinPCa: *TMPRSS2-ERG* fusion was identified in 46% (16/35) of MinPCa. In 87% (14/16) of positive tumors, fusion was due to deletion. The remaining 13% (2/16) of fusions were based on the demonstration of a split in the two juxtaposed probe signals. Ch21 polyploidy ± fusion and duplication of *ERG* deletion were not observed in any MinPCa case. Control group: *TMPRSS2-ERG* fusion was identified in 59% (35/59) of tumors. In 77% (27/35) of positive tumors, fusion was due to deletion. Ch21 polyploidy with ± fusion was present in 13/59 (22%) while polyploidy with duplicate *ERG* deletion was found in 6/59 (10%) of control tumors. On statistical analysis, there was no significant difference in *TMPRSS2-ERG* fusion incidence between the MinPCa and control groups ($p=0.2$). Statistically significant higher rates of ch 21 polyploidy ± fusion was present in control group ($p=0.0002$). A trend approaching statistical significance for higher incidence of ch21 polyploidy with duplicate deletion was also present in the control group ($p=0.052$).
Conclusions: *TMPRSS-ERG* fusion rate of 46% is present in MinPCa.The latter is not significantly different from rate of fusion in control group of non-minimal pT2 and pT3 PCa. A higher rate of Ch21 polyploidy is detected in the control group compared to MinPCa. Our finding of a comparable rate of *TMPRSS2-ERG* fusion in MinPCa and non-minmal PCa argues against its value as a marker of aggressive PCa phenotype.

699 Charcteristics of Positive Surgical Margins in Robotic Assisted Laparoscopic Radical Prostatectomy (RobRP), Open Retropubic Radical Prostatectomy (RRP) and Laparoscopic Radical Prostatectomy (LapRP): A Comparative Study from a Single Academic Center

R Albadine, JY Jeong, F Tavora, JI Epstein, M Gonzalgo, C Pavlovich, GJ Netto. Johns Hopkins, Baltimore.
Background: A positive surgical margin (SM+) is an independent predictor of PCa recurrence following prostatectomy. Reported rate of SM+ varies from 15-35% in RobRP, RRP and LapRP. Studies detailing differences in extent and location of SM+ among the 3 techniques are lacking.
Design: A retrospective "blinded" review of all 99 SM+ prostatectomies performed at our center during an 2007 calendar year, was conducted by two urologic pathologists. Our cohort included: 38 RobRP, 40 RRP, and 21 LapRP. Pathological data collected included: prostate weight and volume, tumor volume (microscopically calculated

LxWxD), Gleason Score (GS), pTNM, number and anatomic location of SM+ sites, SM+ type (at capsular incision vs EP) and total linear length of SM+. Available clinical data included: preop PSA, cTNM, BMI, nerve-sparing status, postoperative PSA/ biochemical recurrence.
Results: Initial SM+ status assigned by original pathologist was reassigned in 1/38 RobRP, 3/40 RRP, 2/21 LapRP to SM- status (5% false positive). SM+ status was confirmed in remaining 94 RP. The 3 groups were comparable in regard to preop PSA, cTNM, pTNM and tumor volume (p:NS). However, patient weight and prostate gland volume were significantly different among groups (p<0.02). Overall, solitary positive margin was present in 65% of RRP, 47% of LapRP and 49% of RobRP. The apex was the most commonly SM+ site (35% in RRP, 26% in LapRP, and 29% in RobRP). Both total linear length and number of SM+ sites significantly correlated with GS and pTNM (p<0.01). Total linear length also correlated with preop PSA (p=0.0009). No significant overall difference among the 3 groups was found with respect to: total linear length, number, laterality or location of SM+ (p:NS).
Conclusions: Urologic pathologist review detected 5% false positive margin status assignment. As expected, patient weight and prostate gland volume affected selection of surgical technique. We found no significant difference in SM+ characteristics among the three techniques.Additional studies to assess any differences in future BCR are warranted.

700 Topoisomerase IIA Status (TOP2A) in Renal Medullary Carcinoma: Immunoexpression and Gene Copy Alterations of a Potential Target of Therapy

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Background: Renal medullary carcinoma (RMC) are aggressive neoplasms with no currently available effective therapy. TOP2A is a gyrase involved in cell proliferation, DNA maintenance and repair. TOP2A is a target for agents such as Anthracyclines. Triggered by a recent RMC patient response to TOP2A inhibitors, we decided to evaluate TOP2A expression, in relation to proliferation index, and TOP2A gene copy number status in a larger series of RMC.
Design: Archival tissues from 14 RMC were retrieved from 3 academic institutions. IHC analysis was performed using monoclonal antibodies for TOP2A (Novocastra) and Ki67 (Ventana) . The percentage of positive nuclear staining was assessed in highest area of expression. A previously suggested > 5% cutoff was used for TOP2A overexpression. TOP2A gene copy number was evaluated using FISH. Locus specific TOP2A gene and chromosome 17 centromeres (CEP17) probes (Vysis) were utilized. The total numbers of TOP2A and CEP17 signals were counted in 150 cells per tumor and a TOP2A/CEP17 signals ratio was calculated. A TOP2A/CEP17 ratio 2.0 and <0.8 were used as cutoff for amplification and deletion respectively. Percentages of tumor cells with polysomic, eusomic or monosomic Chromosome 17 status were also determined.
Results: IHC Analysis: TOP2A IHC was technically inconclusive in one RMC. TOP2A was overexpressed in 11/13 (85%) RMC (median 50%, range 1-80%). As expected, a high Ki67 proliferation index was demonstrated in 13/14 tumors (median 87.5%, range 2-100%). Ki-67 was higher than TOP2A expression rate in all 13 informative tumors. Strong statistically significant correlation was found between TOP2A and ki67 expression (pairwise CC=0.9, p 0.0000). **FISH Analysis:** No TOP2A amplification was detected in any of the 14 RMC including the 11 with TOP2A overexpression. TOP2A gene deletions were encountered in 4 tumors. 2/4 deletions were in association with ch17 monosomy while the remaining 2 deletions were in Ch17 eusomic tumors.
Conclusions: TOP2A is overexpressed in 85% of RMC tumors potentially lending support for TOP2A inhibitors in this aggressive renal tumor. Our findings suggest that TOP2A overexpression in RMC is not due to gene amplification but likely to be due to transcriptional or post transcriptional modifications. The biologic significance of our findings of TOP2A deletions in 28% of RMC requires further evaluation.

701 Novel Biomarkers Expression in Patients with Superficial and Minimally Invasive Urothelial Bladder Cancer and Their Assoaciation with Tumor Outcomes

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Background: Numerous novel genes have been identified in urothelial bladder cancer (UBC) and genes including ninjurin, synuclein and neuropilin seemed to be associated with invasive tumors and aggressive behavior. This study is the first to evaluate the protein expression of these biomarkers and to reveal their prognostic value in large series of superficial (pTa) and minimally invasive (pT1) UBC cases with a long and adequate follow-up. The aims of the our study was: 1- to evaluate the expressions of each of synuclein, ninjurin and neuropilin protein in superficial and minimally invasive tumors, 2- to define their value in predicting tumor outcomes such as recurrence and progression.
Design: Tissue microarray was done on 183 paraffin-embedded tumor tissues (pTa 81, pT1 102). Immunohistochemistry for synuclein, ninjurin and neuropilin was performed on paraffin blocks. Statistical analysis was performed to define the association between each of these biomarkers, clinical data and tumor outcomes.
Results: A statistically significant association was observed between synuclein expression and tumor stage (p=0.029). In univariate Cox model among these 3 biomarkers only ninjurin was associated with tumor progression (ninjurin weak vs negative: HR 0.21, 95% CI [0.07-0.62] to ninjurin strong vs negative: HR 0.17 95% CI [0.06-0.50]; p= 0.001). Multivariate analysis revealed that only tumor grade was independent predictor of pTa/pT1 tumor recurrence and progression. Likewise, high-grade (G3) tumors are more often to progress than those well differentiated tumors with HR 23.57 (95% CI 5.64-98.53 p<0.001). In addition, high-grade tumors are more often to recur than those of low-grade tumors with HR 2.61 (95% CI 1.23-5.52, p 0.012).
Conclusions: We found that tumors with strong synuclein expressions are likely to be more advanced tumors (pT1). In addition, tumors expressing ninjurin tend to progress slower than those with no ninjurin expressions. Finally, synuclein and neuropilin failed to show any value in predicting tumor behavior. Still the best independent factor in predicting pTa/pT1 tumor recurrence and progression is tumor grade.

702 Recording the Number of Sites Involved with Gleason 8-10 in Systematic Sextant Needle Core Biopsies Allows for Stratification in Outcome of Poorly Differentiated Prostate Carcinoma

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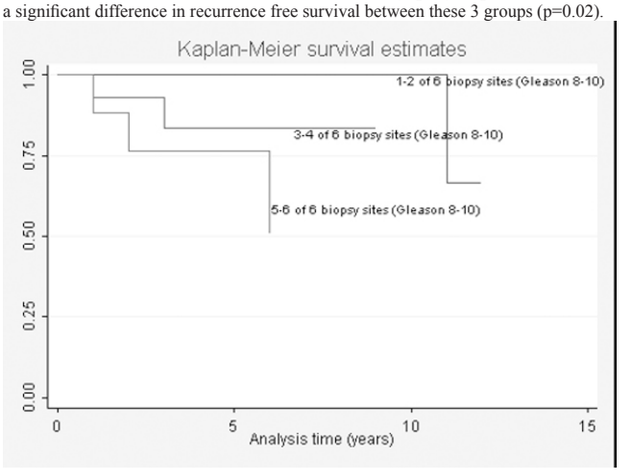
Background: Poorly differentiated (Gleason 8-10) prostate cancer (PDPCa) represents a heterogeneous patient population at risk for disease recurrence and progression despite aggressive local therapy. We sought to determine whether the number of biopsy sites involved with Gleason 8-10 provides any prognostic stratification for this high-risk group.
Design: We reviewed retrospective sextant needle biopsies from patients with PDPCa. Only patients with complete systematic sextant biopsies (right and left apex, mid and base) of the prostate were included in the study (n=85). All biopsies were reviewed by two of the authors (AA and GPP). Gleason grading was in accordance with the 2005 ISUP consensus criteria.
Results: Incidence of PDPCa stratified by location were as follows: right apex (43/85, 50%), right mid (44/85, 52%), right base (33/85, 39%), left apex (34/85, 40%), left mid (41/85, 48%), left base (33/85, 39%). Adequate follow-up was available in 64 patients (range 12-144 months). On multivariate analysis, only the number of biopsy sites with Gleason 8-10 was significantly associated with worse recurrence free survival (HR: 1.02 [1.00-1.04]). Clinical outcomes were further stratified by number of biopsy sites involved: 1-2/6, 3-4/6 and 5-6/6 cores with Gleason 8-10 (Figure). Log rank test indicated a significant difference in recurrence free survival between these 3 groups (p=0.02).



Conclusions: Prognostic stratification of patients with PDPCa on biopsy can be achieved based on the number of biopsy sites involved by Gleason 8-10 carcinoma. This study in PDPCa further emphasizes the importance of systematic biopsy sampling of the prostate and recording of Gleason score separately per site in the surgical pathology report.

703 Utility of Kidney Injury Molecule (KIM-1) Staining in a Wide Spectrum of Traditional and Newly Recognized Renal Epithelial Neoplasms: Diagnostic and Histogenetic Implications

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Background: We have recently documented KIM-1 to be a specific marker for proximal convoluted tubule associated neoplasia - papillary and clear cell renal cell carcinoma (RCC); it is not expressed in chromophobe RCC and oncocytoma. This study was performed to confirm the reproducibility of earlier reported findings in tissue microarray specimens and examine the expression in the rarer and some recently described subtypes of RCC: tubulocystic carcinoma, collecting duct carcinoma and medullary carcinoma.
Design: Antibody to KIM-1 molecule, clone AKG7 antibody (dilution 1:20) was used to immunohistochemically stain a tissue microarray section (including 25 chromophobe RCC, 23 oncocytoma, 20 papillary RCC and 22 clear cell RCC). Routine sections of 3 cases of collecting duct carcinoma, 1 case of tubulocystic carcinoma and 3 cases of renal medullary carcinoma were included and staining was evaluated for intensity (0 - 3+) and extent (focal, moderate, diffuse).
Results: KIM-1 expression was positive in 18/20 (90%) papillary RCC and 18/22 (82%) clear cell RCC; and was negative in both chromophobe RCC (0/25) and oncocytoma (0/23) . The expression was typically diffuse in papillary and clear cell RCCs with greater intensity in former. KIM-1 expression was diffuse and moderately strong in the case of tubulocystic carcinoma. Rare expression (<5%) of KIM-1 was seen in one case each of collecting duct carcinoma and renal medullary carcinoma with other cases being negative.
Conclusions: This study validates the diagnostic utility of KIM-1 for papillary and clear cell RCCs. The diffuse nature of staining in tissue microarray specimens suggests that the practical utility could be extended to needle biopsy specimens which are being

increasingly performed and in which ancillary diagnostic markers are required due to limited available tissue material. KIM-1 expression in tubulocystic carcinoma suggests association with proximal convoluted tubules and doesn't support its designation as low grade collecting duct carcinoma. The negative KIM-1 expression is largely in keeping with the putative collecting duct origin of these tumors.

704 Methylation Analysis of Four Genes in Negative Prostate Needle Biopsies Prior to Diagnosis of Cancer

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Background: CpG island hypermethylation gene silencing is a characteristic feature of prostate adenocarcinoma (Pca). It is an early genetic event, being found in most Pcas and high grade PINs. Other groups have recommended gene sets sensitive to hypermethylation that can be used in Pca. A benign biopsy does not reliably exclude the presence of Pca in men with elevated PSA. This can produce a problematic clinical scenario, especially when played out over multiple benign biopsies. The goal of this study was to assess the effectiveness of a hypermethylation assay in benign prostate biopsies that preceded adenocarcinoma as a possible tool to identify the subset of high risk patients in whom saturation biopsies would be useful.

Design: DNA from 11 benign biopsies and 4 biopsies with high grade prostatic intraepithelial neoplasia (HGPIN) / atypical small acinar proliferation (ASAP) from patients with elevated PSAs that preceded Pca was extracted. The mean time interval between the initial benign/suspicious and malignant biopsies was 15.5 months (range 2.5-36 mos). Negative control cases were benign biopsies in patients who did not have subsequent Pca over a similar/longer time period. Positive controls were the subsequent Pcas. Genes assessed for hypermethylation were *GSTP1*, *PTGS2, RASSF and APC*. The MethylLight kit was used. Methylated and unmethylated primers were concurrently and separately assessed using Real time PCR.

Results: Hypermethylation was present in *GSTP1, PTGS2, RASSF, and APC* in 1/15, 12/15, 14/15, and 6/15 biopsies that preceded Pca, respectively. In the 11 benign biopsies preceding Pca, all four genes were hypermethylated in 1 case, three genes were hypermethylated in 2 cases, two genes were hypermethylated in 5 cases, and one gene was hypermethylated in 3 cases. In the 4 biopsies preceding Pca with ASAP/HGPIN, two cases had three genes and two had 2 genes hypermethylated. Subsequent Pcas had hypermethylation of all four genes in 100% cases.

Conclusions: Hypermethylation assay using genes previously shown to be sensitive to CpG island hypermethylation appears to be useful and sensitive adjunctive assay for patients with elevated PSA and negative biopsies to identify the subset at high risk for subsequent Pca. Given the global effect of hypermethylation, the presence of hypermethylated genes in benign tissue may occur by closely adjacent Pca or high grade PIN that escaped needle sampling.

705 Carcinoma of Collecting Ducts of Bellini: Analysis of 27 Distinctive Cases of Renal Cell Carcinoma with Aggressive Clinical Behavior

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Background: Although first described in 1949 and formally recognized by the W.H.O., large series describing the detailed morphologic spectrum of these rare neoplasms is lacking.

Design: We performed a detailed clinicopathologic analysis of 27 cases; cases of renal medullary carcinoma were excluded.

Results: The age ranged from 6-87 yrs (mean 46) with marked male preponderance (4:1). The tumors primarily occurred in the medullary region with or without cortical involvement and measured 4-12 cm (mean 8). 96% of tumors extended into perinephric fat or beyond Gerota's facia. Predominantly tubular, tubulopapillary or glandular architecture characterized the tumors with occasional solid sheets (13 cases), rhabdoid morphology (4 cases) and sarcomatoid change (3 cases) . Characteristic features included a myxoid (57%), fibrotic (23%) or fibrosclerotic (20%) desmoplastic stroma and predominantly lymphocytic inflammation. The tumor cells were invariably high grade (Fuhrman nuclear grade 3) with moderate to abundant eosinophilic cytoplasm; hobnailing was present in 50% of cases. Intraluminal mucin was seen in 46% of cases; it was predominantly patchy though prominent in 11%. Geographic necrosis was seen in 60%. The immunohistochemical profile was CK7 positive (30%), CK20 negative, high molecular keratin weight positive (18%) and PAX-2 positive (6%). 50% of patients presented with metastasis; 91% of the patients died of disease (12 months median follow up). The median survival was 11 months. Metastatic sites included hilar lymph nodes, lung, bone and liver.

Conclusions: Collecting duct carcinoma is an extremely aggressive clinicopathologic subtype of renal cell carcinoma with proclivity to occur in younger males and presentation with metastatic disease. Accurate characterization is necessary for appropriate triaging of patients into ongoing clinical trials with specific therapeutic protocols.

706 EPHB2 Alterations in Prostate Carcinoma: Evaluation of Mutational Status in a Large Series of Prostate Carcinoma Tissues and Cell Lines

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Background: Ephrin receptor B2 (EPHB2) is part of the ephrin receptor family which is the largest member of the tyrosine kinase group. EPHB2 gene is located on 1p36.12 and is composed of 17 exons. Recent work has uncovered that, in prostate carcinoma (PCa), EPHB2 gene is the site of frequent mutations and that the gene locus is often deleted, suggesting a possible tumor suppressor role for this gene. In the current study we proposed to evaluate the presence of DNA alterations in EPHB2 gene in a large series of PCa cases, PCa cell lines and PCa xenografts.

Design: The study included a total of 69 cases of human PCa specimens, 6 PCa cell lines (CWR22, DU145, LnCap, LnCap104S, PC3 and Vcap) and 9 PCa xenografts (LAPC3, 4, 9, 14, 17, 18, 23, 25 and LuCap35). Fresh tissue was collected from radical prostatectomy specimens and metastatic samples and snap frozen in liquid nitrogen. DNA was extracted following microscopically controlled dissection of the frozen tissue blocks to enrich the tumor content. Sequence analysis of the coding and exon-flanking intronic regions of EPHB2 was performed using standard techniques (Agencourt Biosciences).

Results: A total number of 2856 sequence electropherograms were generated and analyzed. We identified 61 different point mutations in the EPHB2 gene. A majority of them involved introns (41) or untranslated regions (3). Five intronic mutations involved the intron/exon boundary region defined as occurring within 20 bp from the end of the exon. A total of 18 coding region mutations were found (11 silent, 6 missense and 1 nonsense). The missense mutations involved the ephrin receptor ligand binding domain (c.63A>T/ p.20Glu>Asp -1 case), the fibronectin type III domain (c.1081A>G/ p361Ile>Val -1 case and c.1273G>A/ p.425Ala>Thr -1 case) and the tyrosine kinase domain (c.1952T>C/ p.651Val>Ala -1 case, c.2035G>A/ p.679Asp>Asn -2 cases and c.2623G>A/ p.875Gln>Ser -1 case). The nonsense mutation involved the tyrosine kinase domain of DU145 cell line (c.2623G>A/ p.723Gln>X). Out of the 7 non-silent mutations, 3 were reported previously and 4 represent novel mutations of the EPHB2 gene (c.63A>T, c.1081A>G, c.1273G>A and c.2623G>A).

Conclusions: Our study successfully identified 7 non-silent mutations of the EPHB2 tyrosine kinase gene occurring in prostate cancer, 4 of which were not described previously. Functional studies to determine the biological significance of these mutations are needed in order to assess the impact of EPHB2 in prostate carcinogenesis.

707 Pathologic Features of Salvage Radical Prostatectomy Specimens Following Radiotherapy: Implications for Focal Therapeutic Options

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Background: Salvage radical prostatectomy is a therapeutic option offered to patients with clinically localized, recurrent prostate cancer following radiation therapy. Detailed knowledge of the pathologic features of recurrent prostate cancer in radical prostatectomy specimens will help to optimize the design of focal (subtotal) therapies currently under investigation.

Design: We analyzed the radical prostatectomy specimens (RPS) from 50 patients who underwent salvage radical prostatectomy following radiation therapy between 1994 and 2008.

Results: Patients' median age at surgery was 68 yrs (range 45-75). The median interval from radiation therapy to surgery was five yrs (range 1-13 yrs). The median pre-salvage serum PSA was 3.40 ng/ml (range 0.20-39.20 ng/ml). Unifocal tumors were present in 66% of the RPS. In 64% of the RPS the tumor(s) were exclusively of peripheral zone origin. Multifocal tumors of peripheral and transition zone origin were present in 22% of the RPS. The tumor was bilateral in 74% (37/50) of the cases. Bilateral involvement was due to extension of tumor across the midline in 29 RPS (58%), with or without a separate contralateral tumor focus in 8 and 21 RPS, respectively. Bilateral independent tumor foci were present in 8 (16%) of the RPS. Tumors extended from the apex to the base in 51% of the cases. Overall, involvement of the apical and base regions by prostate cancer was present in 71% and 65% of the RPS, respectively. The Gleason score was ≤7(4+3) in 21 and ≥8 in 23 cases. Therapy effect precluded grading in 6 cases. The pathologic stage was pT2: 24; pT3a:8 and pT3b:18. Six patients had nodal metastases. A positive margin was present in 8 (16%) of the RPS with the majority of the positive margins occurring in patients with seminal vesicle invasion (7/8, 87%). The median tumor volume was 0.960 cc (range 0.051-10.320 cc).

Conclusions: Recurrent prostate cancer following radiotherapy is frequently bilateral with involvement of the apex and base of the prostate. These findings represent challenges that must be taken into account in the design of appropriate templates for focal salvage therapies, as well as in the identification of optimal candidates for these therapies.

708 Prostatic Ductal Adenocarcinoma Diagnosed on Transurethral Biopsy or Transurethral Resection of the Prostate (TURP) Is Not Always Clinically Aggressive Disease

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Background: Prostatic ductal adenocarcinoma (DCa-P) is considered to be an aggressive variant of prostate carcinoma (PCa) equivalent to Gleason score 8 PCa. We studied the pathological outcome of 23 DCa-P diagnosed on transurethral biopsy or TURP.

Design: DCa-P diagnosed on transurethral biopsy or TURP was identified by searching the surgical pathology files at authors' institutions. Those cases with concomitant acinar PCa component were not included. Patients' demographic information, serum PSA levels, follow-up surgical procedures (radical prostatectomy [RP], TURP, or transrectal biopsy) and follow-up pathological outcomes were obtained.

Results: 23 patients were studies. Their man age was 67.5 (range 44-82) years. The prebiopsy PSA was 13.1 (range 0.15-65) ng/ml. DCa-P was diagnosed on transurethral biopsy in 14 patients and on TURP in 9 patients. Only ductal cancer component was present and no acinar PCa component was present in the biopsies. Three patients had a prior history of PCa. The remaining 20 patients had follow-up procedures within 4 (range 1-23) months after the initial transurethral biopsy or TURP, including 2 with transcrectal biopsy, 5 with TURP and 13 with RP. All 5 patients with prior or follow-up transrectal biopsies had biopsy-proven PCa. Of 5 patients with follow-up TURP, 4 had Gleason score≥8 PCa. However, one patient did not have residual cancer. Of 13 patients with RP, 4 had GS 7 PCa, 8 had GS≥8 PCa. Extraprostatic extension was present in 7/12, seminal vesicle invasion was present in 6/12, and lymph node metastasis was present in 2/12 patients. Three patients had GS 7, organ-confined disease. One patient did not have residual disease. Therefore of 18 patients with follow-up TURP and RP, 13/18 (72%) had high grade PCa, 3/18 (17%) had GS 7 and organ confined PCa , and 2/18 (11%) had no residual disease.
Conclusions: While the majority of DCa-P diagnosed on transurethral biopsy or TURP is associated with high grade PCa with adverse pathological features, a subset has GS7 and organ-confined disease. In addition, a subset of patients with DCa-P diagnosed on transurethral biopsy had no residual cancer. We recomment that patients with a diagnosis of DCa-P diagnosed on transurethral biopsy or TURP undergo follow-up TURP and/or transrectal prostate biopsy to confirm the presence of extensive disease before definitive therapy is offered.

709 Expanding the Morphological Spectrum of Clear Cell Papillary Renal Cell Carcinoma, a Distinct Renal Cell Carcinoma Subtype

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Background: Clear cell papillary renal cell carcinoma (CCPRCC) is a recently described histological subtype of renal cell carcinoma (RCC) with distinct morphological and immunohistochemical profile (Cheng et al, AJSP, 2008). The tumor typically consists of a mixture of cysts and papillae lined with clear cells. The tumor cells express cytokeratin 7 (CK7) and CA9, but not AMACR, and lack cytogenetic changes characteristic of clear cell and papillary RCC. We report 12 cases of CCPRCC with typical morphological features. However, they also have other unusual patterns, the presence of which may lead to misclassification of these cases as clear cell RCC.
Design: RCC cases were classified as CCPRCC based on their characteristic morphological and immunohistochemical features, namely, cysts of variable sizes mixed with tubules and papillae lined with cells with clear cytoplasm. All the tumor cells were positive for CK7 and CA9 and negative for AMACR. All the cases were stained for CK7, CA9, AMACR and TFE3. Fluorescence in situ hybridization was performed with centromeric probes for chromosomes 7 and 17.
Results: 12 CCPRCC affected 7 men and 5 women with a mean age of 60 years (range 32-78). One patient had bilateral multiple CCPRCC. One patient had diabetic nephropathy. The mean tumor size was 2.0 cm (range 1.3-4.5). Fuhrman grade was 1 in 5, 2 in 6 cases, and 3 in 1 case. The stage was T1a in 10, and T1b in 2 cases. Compressed tubules interspersed with branching tubules were present in 6, cysts of variable sizes in 10, papillae in 8. CCRCC-like area with clear cell nests separated by fine vascular septa were detected in 10 cases. CCRCC-like tumor cells had clear cytoplasm and low grade nuclei. Immunohistochemically, tumor cells lining the cysts and papillae were positive for CK7 and CA9, but negative for AMACR. The clear cells in CCRCC-like areas were positive for CK7 (10/10) and CA9 (6/9), but negative for AMACR. TFE3 was negative in all the cases. None of the cases had chromosomal gain involving 7 and 17.
Conclusions: In addition to the typical morphological pattern (cysts and tubulopapillae), clear cells nests of variable sizes resembling CCRCC can also be present in CCPRCC. These CCRCC-like areas are immunohistochemically identical to other tumor cells in CCPRCC. The presence of CCRCC-like areas in an otherwise typical CCPRCC should not lead to erroneous diagnosis of clear cell RCC.

710 Renal Carcinomas Arising in Patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD): A Clinicopathological Review

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Background: Previous studies have suggested that renal neoplasms arising in patients with ADPKD are more often bilateral and multicentric than those arising in the general population. This study was performed to determine the prevalence of the different types of renal cell carcinoma (RCC) and potential precursor lesions developing in this patient population.
Design: Kidneys excised for ADPKD over a 14 year period were reviewed to identify those with associated carcinoma. Clinical and follow up information was obtained from clinical charts and the hospital cancer registry. All tumors were histologically reviewed and characterized pathologically, aided by ancillary methods (e.g. immunohistochemistry) when necessary.
Results: Ninety five kidneys were excised from 50 patients including 32 (64%) males and 18 (36%) females (~5:3 ratio). There were 39 (78%) Caucasian (C) and 11 (22%) African-American (AA) patients (~4:1 ratio). Thirteen patients (26%) developed RCCs including 6 (46%) C and 7 (54%) AA patients (<1:1 ratio). The proportion of C and AA patients with RCC were 6/39 (15%) and (7/11) (64%), respectively (P = 0.003). The age of the patients ranged from 32 to 62 years (mean, 46 yrs). A total of 24 RCCs were identified in 15 kidneys (2 patients had bilateral tumors) with multifocal disease seen in 5 kidneys (33%). The size of the tumors ranged from 0.5-10 cm (mean, 2.3 cm). Sixteen (67%) tumors were classified as papillary RCC (type I, 9; type II, 6; mixed, 1), 4 (17%) as clear cell papillary RCC, 2 (8%) as clear cell RCC, 1 (4%) as collecting duct carcinoma with rhabdoid features, and 1 (4%) as unclassified RCC. Fifteen (63%) of the tumors had a Fuhrman grade of II with the remaining 9 (37%) being grade III. Except for one case, all tumors were confined to the kidney and without venous invasion. After a median follow up of 48 mo (range, 2-169 mo), 8 (62%) patients were alive, 4 expired (including two who died with disease after 2 and 4 months, respectively) and 1 patient was lost to follow up. One of the deceased and 7 of the living patients were cancer-free at last follow up; the status of disease was unknown in the remaining 2 patients.
Conclusions: Patients with ADPKD, especially AA patients, are at an increased risk for development of RCC which is not infrequently bilateral and/or multifocal. Most tumors are represented by papillary and clear cell-papillary RCC, are of low stage, and associated with a good outcome. Evaluation of potential precursor lesions in these cases and in ADPKD patients without carcinoma is ongoing.

711 Pseudoliposarcomatous Changes in the Perinephric Adipose Tissue of Nephrectomy Specimens Mimicking Well Differentiated Retroperitoneal Liposarcoma: Evaluation in 200 Nephrectomies

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Background: We have anecdotally observed atypical stromal cells in the fibrous septae, lymphoid aggregates and vascular changes in the perinephric adipose tissue in nephrectomy specimens. On occasions these features raised serious consideration for well differentiated retroperitoneal liposarcoma. En-bloc resections for well differentiated retroperitoneal liposarcoma may contain the kidney as perinephric fat may be involved by R-LPS. This study aims to formally evaluate the pseudoliposarcomatous changes noted in the perinephric fat.
Design: Five to eight slides each from 200 nephrectomy specimens performed for renal epithelial tumors were screened to evaluate changes in the perinephric fat. Focus was on features useful in the distinction from R-LPS. Characterization by fluorescent *in situ* hybridization for MDM2 mutation is currently underway.
Results: A spectrum of mesenchymal changes that may be observed in liposarcoma was variably seen in 22 cases. Cases showed an admixture of variably sized fibrous bands with atypical hyperchromatic stromal cells (n=22), lymphocytic aggregates (n= 12), variation in adipocyte size (n=10), and myxoid areas (n=3). All the cases demonstrated lochkern nuclei at least focally. All of these morphologic features were simultaneously present in 4 cases resulting in marked overlap with R-LPS. None of the cases showed typical lipoblasts, areas of necrosis or mitoses. The renal tumors were most commonly clear cell renal cell carcinoma and 55% of the tumors were pT1; only 2 cases were pT3. None of the cases had a preoperative biopsy.
Conclusions: The diagnosis of a well differentiated liposarcoma is fraught with challenges; various conditions such as such as fat necrosis, atrophic fat, hibernomatous changes, fat predominant angiomyolipoma or fixation artifacts may have cells that simulate lipoblasts. Pseudoliposarcomatous changes in the perinephric adipose tissue have not been previously documented and represent yet another pitfall. Awareness of these changes in nephrectomy specimens is important so as to prevent over diagnosis of well differentiated retroperitoneal liposarcoma.

712 Dedifferentiated Liposarcoma (De-Diff LPS) Presenting as a Primary Renal Mass Resulting in a Nephrectomy: A Report of 8 Cases with Discussion of Differential Diagnostic Implications

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Background: When occurring alone**,** well differentiated retroperitoneal liposarcomas (R-LPS) are relatively easy to diagnose radiologically; when dedifferentiation occurs the typical fat densities may be absent rendering the neoplasm difficult for radiologic diagnosis of an R-LPS. We describe 8 cases of nephrectomies performed for clinically and radiologically suspected primary renal neoplasms, which eventually led to the detection of R-LPS.
Design: Seven cases histologically presented as a sarcoma involving the kidney or hilar region in which histopathologic evaluation of the spindle cell malignancy and the perinephric fat resulted in the diagnosis of de-diff LPS; one was a clear cell RCC with perinephric liposarcoma.
Results: Patients' age ranged from 41-81 years occurring equally in males and females. The tumor size ranged from 5- 25cm. Three tumors showed meningothelial-like whorls mimicking a metanephric stromal tumor or follicular dendritic cell tumor resulting in a work up for these diagnoses. Four cases showed high grade spindle cell morphology including chondroid differentiation in one case, resulting in work up for sarcomatoid carcinoma and primary renal sarcoma. A panel of immunohistochemical stains, CK, S100, desmin, actin, HMB45, CD21, CD35 (all negative) was not supportive of a particular diagnosis. All cases with meningothelial whorls were CD 34 positive. Observation of atypical hyperchromatic cells and/or lipoblasts in the perinephric fat resulted in the diagnosis of liposarcoma.
Conclusions: The differential diagnoses of high grade spindle cell tumors involving the kidney include sarcomatoid change in renal cell carcinomas (RCC) and mesenchymal neoplasms primarily involving the kidney or metastatic to it. Our experience indicates that in cases not conforming to any of the more specific diagnoses, consideration should be given to the possibility of de-diff LPS with attention to the histology of the perinephric fat. The appropriate characterization in such cases has significant clinical implications as liposarcoma is typically not suspected by the urologist and the treatment approach is different than that for a primary renal spindle cell malignancy.

713 Adrenal Lymphangiomas: Clinicopathologic Review of a Rare Lesion

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Background: Adrenal lymphangiomas (AL), also known as a cystic adrenal lymphangioma, are rare benign vascular lesions that remain asymptomatic even upon reaching a large size. Although previously AL were mostly found during autopsy, currently they are more frequently detected during imaging work up and therefore

likely to immitate other adrenocortical or adrenal medullary neoplasms. We aimed to retrospectively review all AL cases at our hospital and further document their lymphatic origin by immunohistochemical staining with D2-40 antibody.
Design: A search of surgical pathology records (1984-2008) was conducted. All H&E sections were retreived from archives and reviewed by both pathologists on the study. Clinical information was gathered from electronic medical records. Representative paraffin embedded section from each case was selected for immunohistochemical analysis using monoclonal antibody D2-40 (DakoCytomation, Denmark).
Results: A total of 9 AL cases were identified (6 female and 3 male patients). All 9 patients were adults at time of diagnosis with a mean age of 42 years (range: 28-56 years). There were 7 Caucasian patients, 1 African American and 1 Asian patient. The average size of AL lesions was 4.9 cm (range: 2.0 to 13.5 cm). AL were twice more frequently located on the right side (6 right-sided and 3 left-sided). Clinically, 7/9 (77%) of lesions were asymptomatic and incidentally found during imaging studies for unrelated causes. One lymphangioma was associated with an atypical B cell proliferation that lead to its clinical discovery. The remaining AL was found during a gynecological exploration for endometriosis. Surgical removal was achieved by total adrenalectomy in 8 of the 9 lesions and partial adrenalectomy in the remaining case. No evidence of recurrence or development of contralateral lesion was encountered in any of the patients. Histologically, our adrenal lymphangiomas showed a typical multicystic architecture with dilated spaces lined by flattened, bland simple lining. The cystic channels/spaces occasionally contained protinaceous material and lacked red blood cell content. On immunostains, D2-40 cytoplasmic staining was positive in all 9 examined lesions confirming their lymphatic nature. D2-40 staining was diffuse in 7 and patchy in the 2 remaining lesions.
Conclusions: Adrenal lymphangioma are very rare benign lymphatic neoplasms with a female, right sided predominance. AL is usually found incidentally during adulthood as a large size mass (mean 4.9 cm) necessitating surgical removal to rule out other adrenal neoplasms.

714 Characterization of Gleason Grade 3 Tumor in Localized and Advanced Prostatic Adenocarcinoma Using a Panel of Functional Gene Expression Markers

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Background: The natural history of prostate cancer is variable; with prediction for individual patients difficult. For example, many patients who present with low-stage, low-grade disease have tumors that behave in an indolent fashion. However, up to 30% of these patients will develop metastatic disease within 10 years. With this in mind, we examined the gene expression profiles of areas of Gleason grade 3 tumour in localized (Gleason score 6) [LC] and advanced (Gleason score 7) [AC] prostatic adenocarcinoma [PCa] to see if there are identifiable genetic differences that might contribute to a tumors propensity to clinically progress.
Design: Using formalin fixed paraffin embedded archival material total RNA was extracted from Gleason grade 3 areas of 20 cases of LC and 20 cases of AC (Gleason scores 6 & 7 respectively) and 9 control cases of benign prostatic hyperplasia (BPH). Extractions were carried out with Ambion RecoverAll™ Total Nucleic Acid Isolation Kit. TaqMan RT-PCR was used to interrogate the cohort of samples for gene expression of designated gene targets with the following molecular functions: [i] lipid metabolism (e.g. AMACR, ACSL3, CRAT), [ii] stress response (e.g. BAX,BCL2, CDKN1A, GADD45B, HIF1A), [iii] detoxification (e.g. CAT, GPX6, GSTM2), [iv] cell cycle control (e.g. CCND1, CDK4, CDKN2A, ERB2, MDM2), and [v] PPAR regulated gene control (e.g. CITED2, MYC, CYP4A11, NFKB1, PIK3CA). Analysis of relative RNA expression data was performed using the $2^{-\Delta\Delta}$ Ct method. CDKN1B was used as endogenous control. Statistical comparison between sample cohorts was performed using the Mann-Whitney two-tailed t-test.
Results: There was significant ($p<0.05$) upregulation of ACSL3, AMACR, CDKN2A, CRAT, ERB2, GADD45B, HIF1A, MYC in cases of LC compared with BPH. Upregulation of ACSL3, AMACR, CCND1, CDK4, CDKN2A, ERB2, CRAT, HIF1A and MYC and downregulation of GSTM2 was seen in cases of AC versus BPH. There was upregulation of BAX, CCND1, CDK4 and downregulation of CDKN1A, CITED 2 and GADD45B in cases of AC relative to LC.
Conclusions: LC and AC have distinctive lipid metabolism, stress response, cell cycle control, PPAR and detoxification gene pathway regulatory expression profiles relative to each other and BPH. These markers can be used to distinguish AC from LC or BPH and may have a role in the clinical progression of PCa from localized to advanced disease.

715 HPV Infection and Correlation with the Expression of Cell Cycle Markers in Verrucous Carcinoma of the Penis

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Background: Penile verrucous carcinoma and verruciform tumours are rare and little is known of their aetiology or pathogenesis. We wished to correlate the expression of cell cycle markers with human papilloma virus (HPV) typing in verruciform carcinomas to understand possible mechanisms of their pathogenesis.
Design: In this study we examined cell cycle protein expression in a uniquely large series of penile verrucous carcinomas and their correlation with HPV infection, in comparison with usual type squamous cell carcinomas of the penis. The expression of the cell cycle associated proteins p53, p21, Rb, p16 and Ki-67 were examined by immunohistochemistry in 15 pure verrucous carcinomas, 4 verruciform carcinomas with warty features and four mixed squamous/verrucous tumours. HPV detection was performed by PCR to allow subtyping of the virus detected. Results were compared with a set of usual type squamous cell carcinomas (n=103). Samples were tested on tissue micro-array sections, and scored in a semi-quantitative manner.
Results: Of 23 verruciform tumours, p53 expression was present in 68% of cases often with a basaloid distribution pattern, p21 in 62% and p16 in 9% of patients. Rb was expressed in 83% of the tumours. The expression of p16 was significantly lower in verruciform carcinomas than in keratinizing SCC ($p=0.0008$). Ki67 also showed significantly lower expression in VC cases ($p=0.0007$) and also was predominantly basaloid in distribution. Of thirteen out of fifteen pure verrucous tumours analysed by PCR, only 3 showed HPV infection, and none for the high risk HPV types 16 or 18. Of 3 warty verruciform tumours assessed, all showed HPV infection, two showing HPV16 infection.
Conclusions: Pure penile verrucous carcinoma pathogenesis is in all probability unrelated to any HPV subtype, and this is reflected in the low p16 immunochemistry. The low Ki-67 index reflects their slow growing nature. P53 and Ki-67 expression show a different pattern with basal and supra-basal staining. However p21 and Rb immunochemistry are less useful in differentiation from keratinizing SCCs. It is uncertain whether the high expression of p53 seen is due to gene mutation or overexpression of the wild type protein, however the distribution pattern suggests the latter.

716 Extent of Prostatic Atrophy in Needle Biopsies and Serum PSA Elevation: Is There an Association with the Type of Atrophy?

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Background: In a previous published study we found a positive and significant association between extent of atrophy and free or total serum PSA elevation in prostate needle biopsies without the presence of cancer, high-grade PIN, or areas suspicious for cancer. The aim of this study is to find any possible association with the type of atrophy.
Design: The study was based on 75 needle biopsies. The only diagnosis in all biopsies was focal atrophy (partial or complete) without the presence of cancer, high-grade PIN, or areas suspicious for cancer. The subtypes of complete atrophy were classified according to a previous published study as simple, postatrophic hyperplasia, and sclerotic. The extent of atrophy was measured in two ways: the linear extent in millimeters and the percentage of linear extent showing atrophy for each biopsy. Spearman's coefficient of rank correlation and the qui-square test were used for the statistical analyses.
Results: The number of cores examined for each biopsy ranged from 7 to 18 (mean 9.4, median 8). The linear extent of atrophy ranged from 0.5 to 38 mm (mean 11, median 9.5). The percentage of linear extent ranged from 0.82% to 49% (mean 17.6, median 16.8). A positive and statistically significant correlation was found between atrophy extent and free or total serum PSA level ($p<0.01$). Based on the median value of PSA, the biopsies were divided in 2 groups: A) $\leq$ 8.2ng/mL, and B) > 8.2ng/mL. The total extent of atrophy was 321mm and 507.5mm in groups A and B, respectively. Partial atrophy, simple atrophy, postatrophic hyperplasia, and sclerotic atrophy corresponded to 12.8%, 53.9%, 19.9%, and 13.7% of the total extent in group A; and, 10.0%, 59.4%, 25.9%, and 4.7% in group B. There was no statistically significant difference in the distribution between the groups ($p=0.13$).
Conclusions: A positive and significant association was found between extent of atrophy and free or total serum PSA elevation confirming our previous study. This elevation is not related to the type of atrophy. We speculate that injurious stimuli causing atrophy, independent of the type of atrophy, may interfere in the physiologic barrier that prevent the escape of any significant amounts of PSA to the general circulation.

717 Tumor Volume in Radical Prostatectomy Specimens: Is It an Independent Prognostic Factor for Biochemical (PSA) Progression Following Surgery?

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Background: One of the most controversial aspects of the pathologic assessment of radical prostatectomy (RP) specimens is the measurement of tumor volume. There is consensus that tumor volume by itself correlates with adverse findings at radical prostatectomy, such as Gleason score, margins of resection, pathological stage and progression following RP. The critical and controversial question concerns whether tumor volume is an independent prognostic parameter once other routinely assessed variables are accounted for.
Design: The study was based on 305 whole-mount consecutive surgical specimens. Tumor extent was evaluated with a point-count semiquantitative method previously published and correlated to age, preoperative PSA, clinical stage, Gleason score in RP, positive ressection margins, seminal vesicle invasion, and biochemical progression defined as PSA $\geq$ 0.2ng/mL. The data were analyzed using the Mann-Whitney test and Fisher's exact test. Time to progression-free outcome was studied using the Kaplan-Meier product-limit analysis; the comparison between the groups was done using the log-rank test. Univariate and multivariate analysis using Cox logistic regression was performed.
Results: More extensive tumors showed higher preoperative PSA ($p<0.01$), higher clinical stage ($p=0.03$), higher positive surgical margins ($p<0.01$), higher pathological stage ($p<0.01$), higher Gleason score ($P<0.01$), and shorter time for PSA progression ($p<0.01$). Using univariate analysis, tumor progression correlated with tumor extension ($p=0.01$), preoperative PSA ($p<0.01$), Gleason score ($p<0.01$), positive surgical margins ($p<0.01$), and seminal vesicle invasion ($p=0.01$). In a multivariate analysis the p values were 0.30, 0.02, 0.01, 0.08, and 0.10 for tumor extent, preoperative PSA, positive margins, seminal vesicle invasion, and Gleason score.
Conclusions: Tumor extent did not provide additional information beyond other clinicopathologic findings in multivariate analysis. It seems that measurement of tumor

extent (a time consuming procedure) is not necessary to be performed as part of the routine pathological analysis of RP specimens.

718 CDX2 Expression in Yolk Sac Tumor of Testicular Origin

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Background: The degree and direction of the differentiation determine the histologic subtype of germ cell tumors. Yolk sac tumor (YST) shows extraembryonic differentiation and is usually a component of mixed germ cell tumors albeit rare pure YST can occur especially in metastasis. YST can show a variety of growth patterns and can mimic adenocarcinomas. CDX2 is a caudal-related transcription factor that is expressed in normal and neoplastic epithelium of intestine. Although CDX2 reactivity has been found in rare tumors of urinary bladder, prostate, lung, pancreas and ovary, it has been used as diagnostic marker of tumors of intestinal origin. However, CDX2 reactivity in testicular germ cell tumors of various subtypes has not been evaluated.

Design: Only testicular germ cell tumors were evaluated to exclude any possible CDX2+ non-germ cell tumors. There were 29 cases available, 13 with components of seminoma, 9 embryonal carcinoma, 8 YST, 1 case of immature teratoma , and 2 mature teratoma with one having mature colonic mucosa. 7 of the 29 cases also contained intratubular germ cell neoplasia unclassified (ITGCNU). Immunohistochemistry for CDX2 (clone CDX2-88, 1:10, Biogenex Laboratories, San Ramon, CA) was performed on paraffin sections using Leica Microsystems' Bond IHC staining platform. Nuclear staining was scored as negative (<1% of cell staining), 1+ (1% to 25%), and 2+ (>25%).

Results: 5 out of 8 (62.5%) YST were positive for CDX2 immunostaining, in which 3 showed 1+ positivity and two 2+ positivity. The staining pattern in some cases was notable for nuclear positivity in discrete clusters of malignant glands with intervening negative malignant glands. Others showed positive cells scattered in the tumor. One of the two cases with strongest stain resembled a well differentiated endometrioid adenocarcinoma without overt colorectal morphology. Mature colonic muscosa in one of the mature teratomas was positive for CDX2. No CDX2 immunostains were detected in ITGCNU, seminoma, embryonal carcinoma, and immature teratoma.

Conclusions: Testicular YST can show CDX2 nuclear positivity in a patchy or scattered fashion while other primitive components of germ cell tumor are negative. Although our study is confined to tumors of testicular origin, it is likely that YST of other sites would express CDX2 as well. YST should be included in the differential diagnosis of gland-forming tumors of unknown origin with CDX2 positivity, especially when the patient is relatively young, outside the gonads as metastasis from gonade or a primary.

719 Ovarian-Type Stroma in Renal Dysplasia, a Possible Pathogenic Link between Renal Dysplasia and Mixed Epithelial and Stromal Tumors of the Kidney

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Background: Ovarian-type stroma is one of the pathognomonic features of mixed epithelial and stromal tumor of the kidney. ER/PR positivity has also been reported in the epithelial component of the tumor. Recently ovarian type stroma has been described in cases of non-tumorous or tumor-associated hydronephrosis among both females and males. We investigated the presence of ovarian-type stroma in cases of renal dysplasia and compared them to hydronephrosis.

Design: Archives from two institutions were searched within a five-year period. Cases with renal dysplasia and non-tumorous hydronephrosis were selected. In the renal dysplasia group there were three female and one male patients (4 cases; age range from 3 – 53 y.o). In the hydronephrosis group five patients were female and seven patients were male (12 cases; age range from 25 weeks gestational age to 60 y.o). One case of mixed epithelial and stroma tumor (MESTK) and two normal fetal kidneys were used as positive and negative controls. Representative sections were stained for ER and PR with subsequent evaluation of the nuclear labeling.

Results: Ovarian-type stroma surrounding dilated pelvic calyces was seen in 10/12 (83%) cases of hydronephrosis and in 4/4 (100%) cases of dysplastic kidneys around tubules. ER positivity was seen in both groups in all but one case of hydronephrosis (94%). PR labeling was present in only 6/12 (50%) cases of hydronephrosis and in 4/4 (100%) of cases with dysplasia. Interestingly, in all 4/4 (100%) cases of dysplasia the epithelium of the dysplastic tubules focally showed strong PR and in one case both ER and PR labeling. It is important to note that even in cases of hydronephrosis with secondary dysplastic changes, such as metaplastic cartilage and thick-walled vessels, the epithelial component was negative for ER/PR receptors. Two normal fetal kidneys did not stain for ER or PR. MESTK showed ER/PR positive stroma as expected.

Conclusions: We report the presence of ovarian-type stroma in renal dysplasia. In addition to the ovarian-type stroma, also seen in hydronephrosis, cases of dysplasia showd focal strong epithelial staining for ER and PR receptors. Our finding suggests that unlike the ovarian-type stroma in hydronephrosis which most likely represents a metaplastic response to mechanical forces, the presence of ovarian type stroma and positive epithelial staining for ER/PR in dysplastic kidney and MESTK may indicate a possible pathogenic link between the two entities.

720 Smoothelin Expression Is a Useful Adjunct for Assessing Muscularis Propria Invasion in Bladder Cancer

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Background: The invasion of muscularis propria (MP) in bladder carcinoma is a critical factor in determining appropriate therapy and prognosis. Although MP invasion can usually be determined by H&E morphology, it is often difficult to distinguish MP from the muscularis mucosa (MM), especially if the MM is hypertrophic in small biopsy and TURBT samples. Recently a novel immunohistochemical (IHC) marker, smoothelin, has been demonstrated to be preferentially expressed in MP versus MM in normal bladder tissue. However, no studies to date have explored the utility of smoothelin expression in diagnostic specimens to assess the extent of tumor invasion or its presence within the MP.

Design: We analyzed TURBT and cystectomy specimens from patients with urothelial carcinoma in which smoothelin IHC was performed from 4/08 to 9/08. These represented cases with morphologic features strongly suggestive of MP invasion, a question as to whether MP was present in the biopsy, or a question as to whether tumor was invading MP or hypertrophic MM. We semiquantitatively graded strength of reactivity as negative (0), weak (1+), moderate (2+), or strong (3+). For each specimen, vessels, MM, and MP were each scored individually.

Results: Smoothelin IHC was performed on 26 tumor cases (1 low grade and 25 high grade urothelial carcinomas) from 25 patients, and included 19 TURBTs and 7 cystectomies. 13 cases invaded the MP, 10 invaded to the LP, and 3 were non-invasive. Reactivity patterns are listed below.

Reactivity	Vessels (%)	MM (%)	MP (%)
0	2/26 (8)	3/26 (12)	0/26 (0)
1+	23/26 (88)	22/26 (85)	2/26 (8)
2+	1/26 (4)	1/26 (4)	7/26 (27)
3+	0/26 (0)	0/26 (0)	17/26 (65)

In every case, MP demonstrated distinctly more intense smoothelin immunoreactivity than vessels or MM. MM intensity was similar to that of vessels and never showed strong reactivity. In one consult case, smoothelin results supported an alternative diagnosis. In four cases in which there was equivocal MP involvement by H&E, smoothelin helped establish MP invasion. In all nine cases in which MP invasion was strongly suspected by H&E, smoothelin immunoreactivity was confirmatory.

Conclusions: In cases of urothelial carcinoma, smoothelin IHC is very useful in confirming the presence of MP invasion, arriving at the correct diagnosis in equivocal cases, and distinguishing between MM and MP. Furthermore, vessels within the bladder wall have similar reactivity with MM, and can be utilized as an internal intensity control for distinguishing MM and MP.

721 Primary Vascular Tumors of the Kidney: A Clinicopathologic Analysis of 26 Cases

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Background: Vascular tumors of the kidney are distinctly rare, and to date no large series has been reported. We analyzed a large series of primary vascular tumors of the kidney in order to further delineate their clinicopathological features and identify organ-specific morphologic features, if present.

Design: 26 renal cases previously coded as "hemangioma", "hemangioendothelioma" and "angiosarcoma" were retrieved from our archives and re-evaluated histologically. Tumors were classified according to the 2000 WHO classification of tumors of soft tissue and bone. Clinical information was obtained.

Results: The tumors occurred in 17 males and 7 females (M:F = 2.4:1) ranging from 21 to 95 years (mean 57.4 years). Lesions ranged from 0.4 to 30 cm (mean 6.0 cm) and were tan-brown, cystic and hemorrhagic. On re-review, cases were classified as arteriovenous malformation (AVM, N=3), capillary hemangioma (CH, N=15) and angiosarcoma (AS, N=8). AVM were identical to their somatic soft tissue counterparts. Renal CH typically lacked a well-formed lobular pattern and showed a "sieve-like" arrangement reminiscent of the splenic sinusoids; all were non-infiltrative and lacked cytological atypia and mitotic activity. AS were diffusely infiltrative with extensive parenchymal destruction; all showed at least small areas of conventional vasoformative growth, but were frequently dominated by spindled and epithelioid histologies. All cases were positive for some combination of CD31, CD34, Fli-1, GLUT-1 and D2-40. Cytokeratin expression was absent in all AS. Follow-up was available for 14 cases: all patients with AVM and CH were disease free; 4 patients with AS were dead of disease at 1 mo, 1 mo, 6 mos and 11 mos, respectively.

Conclusions: Unlike in somatic soft tissue, where benign vascular tumors greatly outnumber malignant ones, a very significant number (31%) of renal vascular tumors are AS, with a very poor prognosis. Capillary hemangiomas of the kidney are morphologically distinctive tumors that often show "spleen-like" features, differing from their somatic soft tissue counterparts. In well-differentiated vascular lesions, renal cell carcinoma with angiomatoid growth, and in poorly-differentiated lesions, sarcomatoid renal cell carcinoma enter into the differential diagnosis.

722 Renal Cell Carcinoma Associated with Prominent Leiomyomatous Proliferation Appears Not To Be a Variant of Clear Cell Renal Cell Carcinoma

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Background: A few renal cell carcinomas composed of epithelial elements resembling clear cell and stromal elements with angioleiomyoma-like and leiomyomatous features have recently been described. The aim of this study is to investigate the immunohistochemical and molecular profile of three additional cases of these tumors and to evaluate their relationship with clear cell renal cell carcinoma.

Design: Immunohistochemistry was performed on the tumors with antibodies to detect CD10, cytokeratin 7 (CK7), high molecular weight cytokeratin 34bE12, aacemase (AMACR), S100A1, parvalbumin, HMB45, desmin, α-smooth muscle actin (α-SMA) and caldesmon. Interphase fluorescence in situ hybridization analysis was performed with a centromeric probe for chromosome 3 and a subtelomeric probe for 3p25 in order to evaluate 3p deletion.

Results: The epithelial elements in all three tumors showed positive reactions for CD10, CK7 and S100A1. Two tumors were positive for 34bE12. All of the other markers gave negative reactions in the epithelial components. The stromal elements displayed strong immunoreactivity for α-SMA and caldesmon. The clear cell component did not show 3p deletion.
Conclusions: We concluded that: 1) renal cell carcinomas associated with prominent leiomyomatous proliferation show immunoreactivity for CK7 and 34bE12 which usually are not present in clear cell renal cell carcinoma; 2) the epithelial elements do not show the 3p deletions typical of clear cell renal cell carcinoma; 3) the results suggest the epithelial elements in renal cell carcinoma associated with prominent leiomyomaotus proliferation are not a variant of clear cell renal cell carcinoma.

723 Core Biopsies of Renal Tumors: Accuracy for Histopathological Evaluation

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Background: The expanding treatment options and integration of clinico-pathological factors into prognostic and therapeutic algorithms have stimulated renewed interest in renal tumors biopsy. In this study we evaluated the accuracy of core biopsy for histopathological evaluation of renal tumors.
Design: After radical or partial nephrectomy renal tumor biopsies were performed using 18 gauge needles in 50 consecutive cases from Anatomia Patologica of the University of Verona. At least two cores per tumor were obtained. All biopsies were performed after preparation of the kidney ex situ on back table visually guided. The biopsies were evaluated on hematoxylin-eosin stained slides by 4 pathologists with variable expertise in uropathology. Clinical data and tumor diagnosis on standard whole sections were blinded.
Results: Biopsy material was considered insufficient for evaluation in 5-9 (mean 6; 12%) cases due to the absence of tumor or the presence of only a few scattered neoplastic cells. Four pathologists assessed a confident morphological diagnosis from biopsies in respectively 80% (35/44), 84% (38/45), 82% (37/45), 76% (31/41) of cases with available neoplastic material. According to four pathologists, clear cell renal cell carcinoma was correctly assessed in respectively 86%, 90%, 95%, 95% (mean, 90%), papillary renal cell carcinoma in respectively 44%, 67%, 77%, 77% (mean, 66%), chromophobe renal cell carcinoma in respectively 77%, 77%, 83%, 100% (mean, 84%). Renal oncocytoma was correctly assessed in respectively 80%, 80%, 80%, 100% (mean, 85%). Overall, from 66% to 95% of available tumourous material a correct diagnosis may be referred from biopsies. In 8 cases (16%), although the presence of tumor was recognized, the correct subtyping was not reached (1 clear cell, 1 papillary, 1 chromophobe, 2 unclassified RCCs, 1 simple cyst, 2 Wilms' tumors).
Conclusions: Although the expertise in uropathology is related to the accuracy of diagnosing and subtyping renal tumors on core biopsies, they provide adequate diagnostic material in, on average, 80% of the cases.

724 P16 Expression in Small Cell Carcinoma of the Urinary Bladder

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Background: Small cell carcinoma (SmCC) of the urinary bladder is a rare malignancy accounting for less than 1% of all urinary bladder carcinomas. The diagnosis is usually made on morphologic grounds with the help of immunohistochemistry for neuroendocrine differentiation. However, staining for neuroendocrine markers is inconsistent and ranges between 30-70%. Alterations in the p16-Rb pathway and overexpression of p16 tumor suppressor protein is present in a high percentage of SmCCs of the lung and is absent in the majority of poorly differentiated squamous cell carcinomas of the lung. These data suggest that p16 immunohistochemistry - as part of an immunohistochemical panel - could be useful in the diagnosis of urinary bladder SmCCs. To our knowledge, this is the first study investigating p16 expression in SmCCs of the urinary bladder.
Design: We identified 14 cases of SmCCs of the bladder (either pure or admixed with other high grade components) and 16 cases of high grade urothelial (transitional cell) carcinoma (HG-TCC) of the bladder diagnosed at our institution. After reviewing the H&E slides, the following immunohistochemical panel was performed on a representative block from each case: p16, p63, CK20, CK7, chromogranin (Chr), synaptophysin (Syn), and CD56 (N-CAM).
Results: P16 expression was significantly higher in SmCCs (92.8%) compared to HG-TCCs (43.7%). P63 and CK20 on the other hand, were positive in majority of HG-TCCs (80% and 53.3%, respectively). None of the SmCCs stained with p63 and only 15.4% of cases were focally immunoreactive with CK20. CK7 immunostain was positive in 61.5% of the SmCCs and in 80% of the HG-TCCs. The sensitivity of the traditional neuroendocrine markers was low, ranging between 28.6% (Chr) and 69.2% (CD56) in SmCCs.

Percent of positive cases

	P16	P63	CK20	CK7	Chr	Syn	CD56
SmCC	92.8%	0%	15.4% (focal)	61.5%	28.6%	64.3%	69.2%
HG-TCC	43.7%	80%	53.3%	80%	0%	6.7% (rare cells)	0%

Conclusions: A p16 +, p63 - and CK20 - immunohistochemical profile is typical of SmCC of the urinary bladder, whereas HG-TCC is p16 +/-, p63 + and CK20 +. Overexpression of p16 in SmCC of the urinary bladder also provides further evidence for a link between alterations in the p16-Rb pathway and neuroendocrine differentiation.

725 SALL4 Is a Novel Sensitive and Specific Diagnostic Marker for Testicular Germ Cell Tumors

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Background: Most of testicular germ cell tumors (TGCTs) are malignant but they are curable with modern therapy. The diagnosis of TGCTs is usually straightforward but sometimes it can be challenging necessitating ancillary markers. Earlier markers such as AFP, PLAP, C-KIT and CD30 show only moderate sensitivity and specificity. Recently novel stem cell markers OCT4, NANOG and SOX2 have emerged as more sensitive and specific markers for certain TGCTs. More recently SALL4 has been identified as a new member in the family of OCT4, NANOG and SOX2. However, no study has investigated the utility of SALL4 as a potential diagnostic marker in TGCTs.
Design: Ninety-eight primary TGCTs were retrieved including 54 pure tumors (44 seminomas, 5 embryonal carcinomas (EC), 5 pure yolk sac tumors (YST)) and 44 mixed germ cell tumors (with seminoma in 18 cases, YST in 26 , EC in 34, teratoma in 24 including 5 with immature elements, and choriocarcinoma in 6). Fifty cases also contained intratubular germ cell neoplasia (ITGCN) for SALL4 staining. To test SALL4 specificity in testis, 10 Leydig cell tumors (LCT), 4 Sertoli cell tumors (SCT), 3 adenomatoid tumors, and 2 primary diffuse large B cell lymphomas (DLBCLs) were also stained. One to 2 formalin fixed paraffin embedded tissue blocks per case were used to prepare unstained slides for immunostain with a SALL4 monoclonal antibody. Only nuclear staining was counted as positive. The staining intensity was scored as weak or strong. The percentage of tumor cells stained was scored semiquantitatively as: 0 (no tumor cell staining), 1+ (<=30% tumor cells), 2+ (31-60%), 3+ (61-90%), and 4+ (>90%).
Results: All 50 ITGCNS, 62 seminomas, 39 ECs, and 31 YSTs showed strong 4+ (>90% tumor cells) SALL4 staining. SALL4 staining was seen in the mononucleated trophoblastic cells in 6 of 6 choriocarcinomas (1+ weak in 1, 3+ strong in 3, 4+ strong in 2). Syncytiotrophoblastic cells in choriocarcinomas and in other TGCTs were negative for SALL4 staining. The teratomatous glands in 22 of 24 teratomas showed 1+ weak SALL4 staining. The immature elements showed 1+ weak SALL4 staining in 4 of 5 cases. In contrast, no SALL4 staining was seen in all LCTs, SCTs, adenomatoid tumors and DLBCLs. The only non-neoplastic cells within the testis stained with SALL4 were spermatogonia (weak staining).
Conclusions: SALL4 is a novel sensitive and specific marker for TGCTs and should be included in the diagnostic panel in difficult cases.

726 SALL4 Is a Novel Sensitive and Specific Marker for Metastatic Yolk Sac Tumor and Other Germ Cell Tumors

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Background: Metastatic germ cell tumors (MGCTs) from testis, ovary and extragonadal sites are rare. Correct diagnosis is critical because MGCTs can be effectively treated and even cured with modern therapy. The correct diagnosis can sometimes be challenging without ancillary markers. Previous makers such as PLAP, AFP, C-Kit and CD30 only show moderate sensitivity and specificity. Recently stem cell markers OCT4, NANOG and SOX2 have emerged as more sensitive and specific markers for MGCTs. More recently SALL4 has been identified as a novel stem cell marker in the family of OCT4, NANOG and SOX2. The goal of this study is to investigate the utility of SALL4 as a potential diagnostic marker for MGCTs.
Design: Ninety-one MGCTs from testis (75), ovary (13) and extragonadal sites (3) were retrieved including 22 seminomas, 7 dysgerminomas, 22 embryonal carcinomas (EC), and 15 yolk sac tumors (YSTs), 7 choriocarcinomas and 18 teratomas. Unstained slides prepared from 1 to 2 paraffin blocks from each case were stained with a SALL4 monoclonal antibody. To test SALL4 specificity, 170 metastatic non-germ cell malignancies (6 head and neck carcinomas (CAs), 8 thyroid CAs, 12 lung CAs, 8 breast CAs, 7 hepatocellular CAs, 3 cholangiocarcinoma, 2 ampullary adenocarcinomas, 10 pancreatic adenocarcinomas, 18 gastric adenocarcinomas, 15 esophageal CA, 10 renal cell CAs, 10 urothelial CAs, 12 prostatic adenocarcinomas, 18 ovarian CAs, 6 uterine CAs, 13 colonic adenocarcinomas, and 12 melanomas) were also stained. Only nuclear staining was counted as positive. The staining intensity was scored as weak or strong. The percentage of tumor cells stained was scored semiquantitatively as: 0 (no cell staining), 1+ (<=30% cells), 2+ (31-60%), 3+ (61-90%), and 4+ (>90%).
Results: All 22 seminomas, 7 dysgerminomas, 22 ECs, and 15 YSTs showed strong 4+ (>90% tumor cells) positive for SALL4. Weak to strong SALL4 staining was seen in the mononucleated trophoblasts in 5/7 choriocarcinomas (1+ in 3, 3+ in 2); no SALL4 staining was seen in syncytiotrophoblastic cells (0/7). Nine of 17 teratomas showed 1+ positive SALL4 staining in teratomatous glands (weak) and immature elements (weak to focal strong). In contrast, only 10 (5 esophageal, 4 gastric, and 1 colonic CAs) of 170 metastatic somatic tumors showed focal 1+ weak SALL4 staining.
Conclusions: SALL4 is a novel sensitive and specific diagnostic marker for metastatic YST and other germ cell tumors.

727 PITX2 Promoter Methylation Status Is an Independent Prognostic Marker for Biochemical Recurrence in Patients with Prostate Cancer after Radical Prostatectomy

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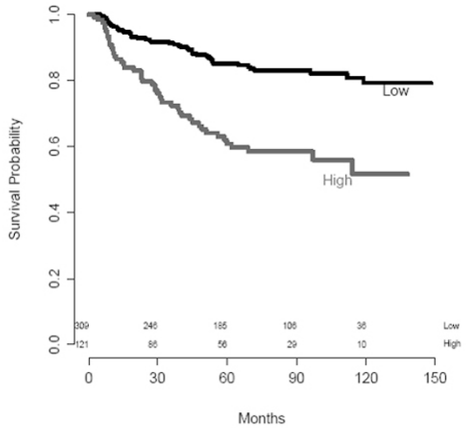
Background: We have developed a test that measures the methylation status of the PITX2 gene promoter qualitatively, that correlates with the probability of biochemical recurrence of prostate cancer after radical prostatectomy. The assay uses FFPE tumor tissue samples and the Affymetrix Genechip system, with a customized microarray

(Epichip PITX2).

Design: Here we present the results of a clinical investigation including almost 500 patients from four different cohorts in the USA and Europe.

Results: We demonstrated a significantly higher risk for biochemical recurrence in patients with a high methylation status result, when compared to patients with low methylation status (HR = 3.0, 95% CI: 2.0 – 4.5, p < 10-5). The probability of being free of biochemical recurrence at 5 years after surgery was 85% for patients in the low methylation group and only 61% for patients in the high methylation group. In the group of patients with Gleason score 7 the relative risk of suffering from a biochemical recurrence is twice as large for a patient from the high methylation group when compared to the low methylation patient group (HR = 2.0, 95% CI: 1.2 – 3.3, p = 0.005). The probability of being free of biochemical recurrence at 5 years after surgery was 79% for patients in the low methylation group and only 62% for patients in the high methylation group.



Kaplan-Meier curves depicting the differences in the risk for biochemical recurrence in patients with prostate cancer treated with radical prostatectomy according to the methylation values for the PITX2 gene promoter. The black line represents the survival analysis of patients with low methylation value, showing a low risk of biochemical recurrence. Meanwhile, patients with a high methylation value show a significant higher risk of biochemical recurrence ($p < 10^{-5}$).

Conclusions: The PCMCT assay defines a group of patients most likely to experience biochemical recurrence. This test adds to the information currently provided by clinical and histological analysis to stratify the patient population, and especially patients diagnosed with Gleason score 7 could benefit from this new tool for biochemical relapse risk stratification.

728 Antigen Processing Machinery Alterations in Bladder Carcinoma: A Tissue Microarray Study of 167 Cases

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Background: T-lymphocytes may be important in controlling the growth of many carcinomas including bladder carcinoma. Tumors may avoid detection and destruction by down-regulating antigen processing machinery (APM) proteins. The aim of this study is to investigate defects in APM using a comprehensive panel of monoclonal antibodies.

Design: Paraffin blocks from 167 cystectomies for primary bladder carcinoma were used to create a tissue microarray. Immunohistochemistry was performed for 15 APM components, (ß2-microglobulin, calnexin, calreticulin, Delta, Z, MB1, LMP2, LMP7, MICA, LMP10, HLA Class I, tapasin, TAP1, TAP2 and ERp57). Full sections of normal urothelium from 6 subjects were also examined. Most tumors were urothelial carcinoma (n=152); the remainder were mixed urothelial or other types of bladder carcinoma. 58 tumors were Stage I/II and 105 were Stage III/IV. Survival data were available for 128 patients with pure urothelial carcinoma; of these 28 were Grade 1/2, 83 were Grade 3 and 17 were Grade 4.

Results: Delta, MB1, Z, LMP2 and LMP7 had high staining scores in superficial and deeper layers of normal urothelium, with calreticulin high in superficial urothelium alone. All APM components except MB1, LMP2 and TAP2, had significantly lower staining in urothelial carcinoma than in either superficial or deeper urothelium, or both. No significant differences were found in APM staining between different grades of urothelial carcinoma. Squamous cell carcinoma had the highest APM scores, with urothelial carcinoma intermediate and other types of bladder carcinoma lowest. High-stage urothelial carcinoma demonstrated significantly lower staining for calnexin, LMP2, LMP7 and LMP10. On average 3.6 year follow-up, significant survival differences were associated with the Delta score in urothelial carcinoma (p=0.0369) and the calreticulin score in all tumor types (p=0.0267).

Conclusions: With a few exceptions, both superficial and deep normal urothelial layers have relatively low APM component staining. High-stage urothelial carcinomas have lower scores for APM immunoproteasome components compared with low-stage urothelial carcinomas. Delta and calreticulin staining scores are associated with survival differences in urothelial carcinoma and in all types of bladder carcinoma respectively. These findings delineate specific defects in APM proteins and may provide insight to guide future immunotherapeutic approaches for controlling bladder malignancies.

729 Evidence for Polyclonal Origin of Multifocal Clear Cell Renal Cell Carcinoma

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Background: With the increased use of nephron sparing surgical excision of renal neoplasms, concern has been expressed that limited surgical resections may not encompass multifocal renal cell tumors, specifically those that are too small to be visualized radiologically, with the result that such unresected tumors would ultimately require additional treatment. Consequently, a clear understanding of the genetic relationships between multifocal renal tumors in the same patient and a reasonably accurate knowledge of the malignant potential of each lesion could have important diagnostic, therapeutic, and prognostic implications.

Design: A total of 62 tumors from 26 patients who underwent radical nephrectomy were examined. All patients had multiple separate clear cell renal carcinomas. Loss of heterozygosity analyses were performed using five microsatellite polymorphic markers that represent putative tumor suppressor genes on chromosome 3p14 (D3S1300), 7q31 (D7S522), 8p22 (D8S261), 9p21 (D9S171) and 17p13 (TP53). X-chromosome inactivation analyses were also performed on the renal tumors from the 10 female patients. Chromosome 3p deletion status was determined by dual color interphase fluorescence in situ hybridization analysis in all tumors.

Results: Nineteen of the 26 (73%) patients with multifocal clear cell renal cell carcinoma showed allelic loss in at least one of five microsatellite loci in separate tumors analyzed. A disconcordant pattern of allelic loss between coexisting kidney tumors was observed in 7 cases. Six cases showed discordant 3p deletion patterns by dual color interphase fluorescence in situ hybridization analysis. Of the eight informative female cases studied by X-chromosome inactivation, one showed a discordant non-random pattern of X-chromosome inactivation. Overall, evidence of independent origin of the multifocal renal tumors was observed in 12 of 26 cases (46%).

Conclusions: Our data suggest that in a significant number of cases of multifocal clear cell renal cell carcinoma, the spatially separate tumors are of different clonal origin and arise independently.

730 Genomic Hypomethylation and CpG Island Hypermethylation in Prostatic Intraepithelial Neoplasm

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Background: Altered DNA methylation in cancer cells is characterized by focal CpG island hypermethylation and diffuse genomic hypomethylation. Both types of aberrant methylation are frequently found in human prostate adenocarcinoma (PCa). Prostatic intraepithelial neoplasm (PIN), a precursor lesion of PCa, has been demonstrated to contain CpG island hypermethylation, but little is known about the role of DNA hypomethylation.

Design: We analyzed the methylation status at 12 CpG island loci and at two repetitive DNA elements (*LINE-1* and *SAT2*) from normal prostate (n=20), PIN (n=25), and PCa (n=35) tissues using MethyLight assay or combined bisulfite restriction analysis.

Results: The methylation levels in *LINE-1* and *SAT2* decreased with progression of lesion types from normal prostate to PIN to PCa ($P<0.05$), whereas promoter CpG island loci displayed increased methylation. Ten genes were found to be hypermethylated in a cancer-specific manner and were further analyzed in another set of PCa tissues (n=64). The number of methylated genes was closely associated with TNM stage, Gleason sum, and preoperative serum PSA levels ($P=0.020$, 0.073, 0.033, respectively).

Conclusions: These results suggest that genomic hypomethylation and CpG island hypermethylation, common among PCas, are early events in prostate carcinogenesis and may be implicated in the development of PIN.

731 P16 Expression Can Not Be Used To Differentiate between Squamous Cell Carcinomas of Uterine Cervix and Urinary Bladder

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Background: p16 is a widely used immunohistochemical marker in gynecologic pathology. Strong and diffuse cytoplasmic and nuclear expression of p16 in squamous cell carcinomas (SCC) of the female genital tract is strongly associated with high-risk human papilloma virus infection and neoplasms of cervical origin. However, p16 can be expressed in other neoplasms as well as in several normal human tissues. Occasionally, SCCs may involve both uterine cervix and urinary bladder. Accurate identification of site of origin in such cases has therapeutic and prognostic implications. We investigate the potential value of p16 expression in this distinction.

Design: We reviewed 74 SCCs, 38 (51%) from urinary bladder and 36 (49%) from uterine cervix obtained between 2003 and 2008. Of the 38 cases of bladder carcinoma, 21 occurred in women and 17 in men. Immunohistochemical analysis for p16 (DAKO M7247, clone 484, dilution of 1:50) expression was performed in all cases using the LSAB method.

Results: Strong and diffuse nuclear and cytoplasmic p16 positivity was observed in 45 cases (61%), 14 (31%) from urinary bladder and 31 (69%) from uterine cervix. Of the 38 SCCs in urinary bladder, 14 (37%) expressed p16 (8 men, 6 women). Of the 36 SCCs of uterine cervix, 31 (86%) were positive for p16.

Conclusions: 1) The majority of SCCs of uterine cervix express p16. 2) More than a third of urinary bladder SCCs express p16. 3) SCCs of urinary bladder express p16 independent of gender. 4) p16 immunohistochemical expression alone cannot be used to discriminate between SCCs arising from uterine cervix versus urinary bladder.

732 Carbonic Anhydrase IX as a Novel Target for Cytological Detection and Therapy of Low Grade Urothelial Cell Carcinoma

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Background: Carbonic Anhydrase IX (CA9) is a hypoxia-inducible member of the carbonic anhydrase family of proteins that regulates intracellular pH level. Overexpression of CA9 has been observed in a number of solid tumors, and several therapeutic agents including chimeric monoclonal antibody cG250 and antibodies conjugated with cytotoxic agents have been developed. Recently, our study of urothelial cell carcinoma (UCC) tissue microarray (TMA) encompassing 543 primary UCCs and 135 normal urothelial tissue samples showed that over 71% of UCCs demonstrate overexpression of CA9 relative to normal urothelium. Strikingly, the expression is significantly higher in low grade versus high grade UCC (P<0.001). Cytological diagnosis of low grade UCC is notoriously difficult. While past efforts for identifying specific low grade UCC markers have been unsuccessful, this finding prompts a hypothesis that CA9 may be a marker for cytological detection of low grade UCC.

Design: A total of 185 archived voided urine cytological slides were retrospectively selected from the period of 1994-2007, with subsequent histological diagnoses of negative (66), reactive (29), low grade UCC (38), or high grade UCC (48). Immunocytochemical staining of CA9 was performed after removal of the cover slip. The results of the immunostains were considered positive if five or more urothelial cells showed cytoplasmic granular staining.

Results: CA9 was positive in 89% of the low grade UCCs, which was significantly higher than high grade UCC (48%, P<0.05 by Chi-square test), reactive (34%, P<0.01), and negative (P<0.01) patient samples.

Staining Results for Archived Voided Urine Cytological Slides

Histological Diagnosis	Positive Stain	Negative Stain	Total
Negative	13 (20%)	53 (80%)	66
Reactive	10 (34%)	19 (66%)	29
Low Grade UCC	34 (89%)	4 (11%)	38
High Grade UCC	23 (48%)	25 (52%)	48

* 4 cases were excluded due to staining artifact

Conclusions: CA9 is a potentially useful adjunctive urine cytological marker of low grade UCC. The findings are consistent with TMA analysis. Additional prospective studies are warranted to further validate this finding. An algorithm of CA9-based detection and therapy of low grade UCC may be on the horizon.

733 Rhabdomyomatous Tumors (RT) after Chemotherapy for Metastatic Testicular Germ Cell Tumors: A Study of 7 Cases

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Background: In advanced testicular germ cell tumors (TGCT), retroperitoneal lymph node dissection (RPLND) is often performed for the resection of residual post-chemotherapy masses. Teratoma is a good prognostic finding but sarcoma, including rhabdomyosarcoma, carries a poor prognosis. Rarely, sizable nodules of differentiated skeletal muscle are found, and it is unclear if these should be considered as teratoma or sarcoma.

Design: A computer search for RPLND specimens having substantial foci of pure rhabdomyomatous elements and lacking non-teratomatous GCT elements identified 4 cases that were confirmed on retrospective slide review. Cases of rhabdomyosarcoma, based on mitotic activity and a primitive cell component, were excluded. In addition, 3 cases that were previously reviewed by the senior author were included, although the slides were unavailable for review.

Results: Clinical and pathological features are summarized in the Table.

Clinical & Pathologic Features

Age at RPLND	TGCT	Interval to RT	Size of RT	Rx after RPLND	F/U after RPLND
27 yrs	T, YST	4.7 yrs	5 cm	ChemoRx	Mediastinal T, 1.7 yrs
28 yrs	S, T	0.3 yrs	2 cm	None	NED, 2.6 yrs
23 yrs	YST, CC, EC	0.2 yrs	1.5 cm	None	NED, 3.4 yrs
19 yrs	T, scar	0.5 yrs	2-3 cm	None	NED, 2.2 yrs
18 yrs	YST, T, EC, CC	0.7 yrs	?	None	RP T, 1.3 yrs; lung nodules*, 3.7 yrs
18 yrs	T, PNET, RMS, YST	0.3 yrs	?	?	Lost
?	?	?	?	?	Lost

CC - choriocarcinoma; EC - embryonal carcinoma; F/U - follow-up; NED - no evidence of disease; PNET - primitive neuroectodermal tumor; RMS - rhabdomyosarcoma; RT - rhabdomyomatous tumor; RP - retroperitoneal; S - seminoma; T - teratoma; * clinically teratoma but not confirmed

The RTs consisted of nodular to diffuse aggregates of fetal-type rhabdomyocytes with central to peripheral nuclei and abundant, eosinophilic, fibrillar cytoplasm with occasional cross striations. Elongated myotubes with multiple nuclei in a common sarcoplasm occurred at least focally in all cases. Mild to moderate nuclear atypia, including nuclear enlargement and nucleolar prominence, was present, but mitotic activity, necrosis and a primitive cellular component were absent. All but one case were associated with other teratomatous elements.

Conclusions: RTs in RPLND specimens after chemotherapy for metastatic TGCTs exhibit clinical behavior similar to teratoma rather than rhabdomyosarcoma.

734 Teratoma with Malignant Transformation in Adult Testis: 25-Years Experience at Istituto Nazionale Tumori of Milan

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Background: The presence of teratoma with malignant somatic transformation (TMT) is a rare phenomenon: though lacking large-scala data, TMT seems to associate with shorter survival and in metastatic cases best treatment modality is not still established. We reviewed our 25-years experience in primary TMT of the testis and in cases with development of malignant somatic transformation in metastatic sites following chemotherapy for advanced testicular germ cell tumors.

Design: A total number of 28 patients with TMT was recorded from consultation or surgical pathology files at Istituto Nazionale Tumori of Milan from 1983 to 2007. The case histories and slides were reevaluated.

Results: 10 out of 28 patients had TMT in the primary only, 2 both in primary and metastatic sites and 16 had post-chemotherapy metastatic malignant transformation. The histology of TMT in the testis was: chondrosarcoma (2 cases), rhabdomyosarcoma (2), leiomyosarcoma (1), sarcoma Nas (5),while two cases (1 nephroblastoma and 1 PNET) occurred with simultaneous metastases . Among TMT in metastatic sites (retroperitoneum, lung, lymphnodes, mediastinum and bone) we observed rhabdomyosarcoma (4 cases), PNET (2), adenocarcinoma (4), leiomyosarcoma (2), sarcoma with neural differentiation (3) and 1 sarcoma Nas. All the 10 patients but one (91.6%) with TMT only in primary tumor were free of disease (mean follow-up 223.5 months). One patient died perioperatively. 14 out of 16 patients who had TMT in metastases were evaluable: these who were submitted to a radical excision of metastases had better outcome (7/8). Seven patients died after chemiotherapy and surgery (1 rhabdomyosarcoma, 2 PNET, 2 adenocarcinomas, 1 sarcoma with neural differentiation and 1 nephroblastoma). Rescue chemotherapy was able to cure 4/11 of not radically resectable patients.

Conclusions: Stage of disease at transformation and the feasibility to perform a radical surgical removal of disease seems to be the strongest disease-related prognostic factors in TMT of the testis. Histology of TMT at the primary site seems not to be a significant predictor of outcome, while the development of PNET in metastatic sites following chemotherapy is associated with a poor prognosis.

735 Estrogen and Progesterone Receptor Positive Stroma in Pediatric Cystic Renal Dysplasia: An Immunohistochemical Study of 18 Cases

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Background: Estrogen receptor (ER) and progesterone receptor (PR) positive stroma is found in a variety of renal neoplasms including cystic nephroma, mixed epithelial-stromal tumor (MEST), and angiomyolipoma with epithelial cysts. A recent study has reported ER and PR positive stroma in non-neoplastic renal parenchyma and suggested that it represents a metaplastic response related to obstruction (Tickoo S. et al. Mod Pathol 2008;21:60-65). We studied a series of pediatric multicystic renal dysplasia, a non-neoplastic lesion with characteristic spindled stroma surrounding abnormal tubules, to assess expression of ER, PR, CD10, calretinin, and inhibin.

Design: Cases of cystic renal dysplasia were identified from surgical and autopsy archives. Patient age, sex, kidney laterality, and pre-operative diagnoses were recorded. H&E sections were reviewed to confirm the diagnoses. Immunohistochemical stains for ER, PR, CD10, calretinin and inhibin were performed and scored as percent nuclear reactivity (ER and PR) or qualitatively (0-3+) in the spindled stroma.

Results: 15 surgical and 3 autopsy cases were identified (n=18). Ages ranged from 3 days to 13 years (mean: 3 years) and the male:female ratio was 14:4. Only two cases (both autopsy) had bilateral disease. Evidence of urinary obstruction (pelviectasis or megaloureter) was present in 5 (28%) cases. At least focal ER expression was seen in 15/18 cases (83%) including all four females, and 11 males. ER staining ranged from <10% to 70% of spindled cells. PR staining was seen in only three (17%) cases (2 female, 1 male) and all were focal (<10%). Only the three autopsy cases of very young or premature neonates had no ER expression. CD10 expression was seen in 12/18 cases (67%), but no correlation with ER expression was found. Not all cases with obstruction expressed ER, and strong ER reactivity was detected in cases without hydronephrosis. Immunostains for calretinin and inhibin were negative in all cases, consistent with the absence of luteinized stroma.

Conclusions: These findings support that a subset of non-neoplastic renal stromal proliferations express ER and PR. As recently proposed, incidental non-neoplastic stromal proliferations in adults may represent a metaplastic response to obstruction or injury, similar to obstructive or segmental dysplasia in childhood.

736 Invasive Urothelial Carcinoma with Chordoid Features: A Clinicopathologic Study of 12 Cases

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Background: Variant forms of urothelial carcinoma are well-described. We have recently encountered urothelial carcinomas with a unique chordoid morphology characterized by extensive myxoid stromal change and cellular cording, which to our knowledge, has not been described previously.

Design: 12 urothelial carcinomas with myxoid stroma and cellular cording were identified. The patient age, patient sex, tumor stage, association with typical urothelial carcinoma, and clinical outcome were recorded. Immunostains for CK20, CK34BE12, p63, calponin, GFAP, S-100 protein, oncofetal protein glycipan-3, and brachyury were performed in 7 cases with available blocks.

Results: 11 of 166 (7%) consecutive invasive urothelial carcinomas had foci with chordoid morphology, and 1 consult case was added (n=12). The patients' ages ranged from 50 to 85 (mean: 68) years; 8 were male and 4 were female. 7 had cystectomies, 4 transurethral resections, and 1 nephroureterectomy. Morphologically, each case had at least focal areas (range 5-95%) with chordoid morphology. When well-developed, the neoplastic cells had scant eosinophilic cytoplasm and were arranged into cords closely mimicking chordoma, extraskeletal myxoid chondrosarcoma, myoepithelioma, or yolk sac tumor. No sarcomatoid component, no intracytoplasmic mucin, and no glandular formation were present in any case. All 12 cases had foci of typical urothelial carcinoma present at least focally. Immunophenotypically, 7 of 7 cases showed immunoreactivity for p63 (nuclear) and cytokeratin 34BE12 (cytoplasmic). S-100 protein showed only focal cytoplasmic staining in 1 of 7 cases. Immunostains for CK20, calponin, GFAP,

oncofetal protein glypican-3, and brachyury were negative in the 7 cases studied. 11 of 12 cases were high stage (pT4: 5, pT3: 4, pT2: 2, and pT1: 1). 6 of the 8 cases (75%) with nodal sampling had metastatic disease. In 1 case, the lymph node metastasis had areas with chordoid morphology. 9 of 12 patients had available follow-up: 2 died of disease (1 and 10 months), 4 alive with disease (2-8 months), 3 NED (2-108 months).
Conclusions: Urothelial carcinomas can show an unusual chordoid morphology mimicking other myxoid neoplasms, but they maintain an immunophenotype typical of high grade urothelial carcinoma. Foci of typical urothelial carcinoma were present, facilitating the diagnosis, but the chordoid component may comprise the majority of the tumor and may be present in metastatic foci.

737 Defective DNA Repair in Renal Cell Carcinoma

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Background: One of the mechanisms involved in carcinogenesis is somatic mutation of tumor suppressor genes. Cellular metabolites, such as free radicals and N-nitroso compounds, can generate mutagenic DNA base lesions 8-oxoguanine and 0^6-methyl-guanine respectively. A mismatch between guanine and thymine (G:T mismatch) will occur in DNA if 8-oxoguanine and 0^6-methyl-guanine are not promptly removed by their respective repair enzymes, 8-oxoquanine DNA glycosylase 1 (hOGG1) and 0^6-methyl-guanine DNA methyltransferase (MGMT). These mismatches can be corrected by a mismatch repair enzyme, hMLH1. Persistence of DNA mismatches due to insufficient hMLH1 mismatch repair can lead to permanent somatic mutations. The kidney is very prone to DNA damages of various forms because it disposes, and is exposed to, most toxic metabolites produced in the body. In this study, we aim at characterizing the gene expression levels of 3 important DNA repair genes, hOGG1, MGMT and hMLH1 in clear cell renal cell carcinoma as compared to matched normal renal parenchyma.
Design: Total RNA was extracted from 14 fresh RCC tissue samples and their matched benign renal parenchyma. Gene expression levels for hOGG1, MGMT and hMLH1 genes were analyzed by real-time RT-PCR quantitation with ABI Fast 7500 Real-time PCR System, using Taqman β-actin as an internal controls for normalization.
Results: The mRNA expression levels were significantly lower in RCC than those seen in adjacent normal parenchyma in 12 of 14 cases (85.7%) for hOGG1 gene, 11 of 14 cases (78.5%) for the MGMT gene and 11 of 14 cases (78.5%) for the hMLH1 gene. Overall, the mRNA expression levels in RCC is about 28% for hOGG1 gene, 53% for MGMT gene and 42% for hMLH1 gene of those seen in normal renal tissues. The mean relative mRNA levels for hOGG1, MGMT and hMLH1 in all 14 cases were 0.78, 1.0 and 1.4 respectively as compared to a mean of 2.72, 1.9 and 3.4 for normal renal parenchyma. The differences in mRNA expression between RCC and adjacent normal tissues for hOGG1, MGMT and hMLH1 genes are statistically very significant ($p < 0.01$).
Conclusions: Significant defects in DNA repair occurs commonly in CC-RCC as seen in 85.7% cases for hOGG1 gene and in 78.5% cases for MGMT and hMLH1 genes. These results indicate that insufficient DNA repair may play crucial roles in the development and/or progression of renal cell carcinoma.

738 The Value of P16NK4a as a Histomarker for HPV in Subtypes of Penile Squamous Cell Carcinoma (SCC). A Study of 158 Tumors

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Background: The histomarker p16NK4a has been postulated as a valid surrogate for HPV in vulvar carcinomas. Penile and vulvar carcinomas are morphologically similar. At both sites, about a third of the tumors showing a warty (condylomatous) or basaloid pathological features are considered to be HPV related.
Design: To explore the value of P16NK4a as a simpler and less expensive marker for HPV in major subtypes of penile carcinomas we compared results of HPV testing and P16 staining of 158 penile tumors from Paraguay. HPV-DNA was amplified at the ICO (Barcelona) by SPF-10 broad spectrum primers PCR followed by DEIA and genotyping by LiPa 25 (version 1). P16ink4A was carried out using standard procedures (CIN TECH provided by MTM, Heidelberg), and tested blinded to HPV status. P16 positivity was a diffuse and continuous strong staining throughout the tumor or at basal layer. Patchy staining or discontinuous staining were considered negative. Tumors were grouped as follow: basaloid, warty and warty basaloid SCCs (B/W/WB), 57 cases, and usual, verrucous and papillary SCCs (non B/W/WB), 101 cases.
Results:

SCC type	No of Cases	HPV+ (%)	p16+ (%)	Sensitivity	Specificity	PPV	NPV
B/W/WB	57	34 (59.6)	37 (64.9)	88.2	69.6	81.1	80.0
Non B/W/WB	101	20 (19.8)	10 (9.9)	30.0	95.1	60.0	84.6
Total	158	54	47	66.7	90.4	76.6	83.8

There was no difference between the number of HPV and p16 positive B/W/WB SCCs ($p=0.7$). The difference was also no significant in the non B/W/WB group ($p=0.07$). Using HPV as a reference, sensitivity of p16 was 88.2% in B/W/WB and 30% in non B/W/B carcinomas. Conversely, the specificity of p16 was low in the B/W/WB group, but over 95% in the non B/W/WB cases.
Conclusions: These data indicate that in B/W/WB group the positivity of p16 correlates well with the presence of HPV but the sensitivity and specificity are not sufficient to substitute HPV testing for p16 staining. P16 may be used as a complement in the HPV related histological diagnosis.

739 Distinctive Association of HPV with Special Types of Penile Squamous Cell Carcinoma (SCC) and Giant Condyloma. A Study of 211 Cases

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Background: There is variation in the reported contribution of HPV in penile SCC (from 22 to 86%) probably related to technical differences utilized in the detection of the virus.
Design: We are presenting a reliable method for HPV identification using paraffin blocks from patients diagnosed in Paraguay (203 carcinomas and 8 giant condylomas). Detection was done by SPF-10 PCR followed by DEIA and genotyping by LIPA 25 (version 1). Samples were tested at HPV laboratories at ICO (Barcelona). The WHO-AFIP histological classification was used specially recording mixed tumors to ascertain the influence of focal tumor patterns in HPV detection.
Results: HPV was detected in 71 of 211 cases (34%). Thirteen tumors had multiple HPV types. HPV 16 was observed in 41 cases (7 with multiple HPV subtypes), HPV 6 in 8 and HPV 18 in 3 cases. HPV was frequently found in giant condylomas (88%) low risk types (6,11,45,74), warty-basaloid carcinomas (86%) and basaloid carcinomas (75%). A group of tumors of intermediate to low prevalence of HPV (from 39 to 16 %) were mixed usual-warty-basaloid, warty, usual and papillary SCCs. HPV was not detected in verrucous, mixed verrucous- papillary, pseudohyperplastic and pseudoglandular SCCs. In 85 cases there were at least focal features of basaloid and/or warty carcinoma and HPV was detected in 50 cases (59%). Tumors without features of warty or basaloid cancers were less likely to harbor HPV DNA (22 of 128 cases (18%) compared to those with such features ($p\ 0.000001$).
Conclusions: The presence of warty and/or basaloid features, even focally, is a strong predictor of presence of HPV and should be reported. The consistent absence of HPV in verrucous, mixed verrucous and pseudohyperplastic carcinomas in this and other studies suggest an alternative HPV unrelated pathogenesis.

740 Primary Urethral Adenocarcinomas: A Clinicopathological Review

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Background: Compared to metastases and direct extension from adjacent sites, primary urethral adenocarcinomas are rare. The aim of this study was to comprehensively examine the clinical and pathological features of these neoplasms.
Design: A computerized search of the surgical pathology files at 2 medical centers was performed to identify cases of primary urethral adenocarcinoma. Review of the clinical, radiological, and pathological findings served to confirm their urethral origin and to exclude other entities in the differential diagnosis. The tumors were classified as clear cell (CCA), enteric (EA) or signet-ring adenocarcinoma based on the presence or absence of characteristic morphological findings. Tumors that did not fit in any of these categories were classified as adenocarcinoma, not otherwise specified (NOS). The expression of CK7, CK20, CDX-2, villin and β-catenin, among other markers, was also used for evaluation. Follow up information was obtained from clinical charts and tumor registry data.
Results: Eleven patients have been identified to date including 4 males and 7 females. The mean age at presentation was 66 yrs (range, 46 to 82 yrs). The most common symptoms were urinary obstruction and hematuria seen in 9 (82 %) and 7 (64 %) patients, respectively. Three patients had more than one transurethral resection and 4 had a radical surgical excision following diagnosis. Based on morphology, 3 cases were classified as CCA, 7 as EA, and one as adenocarcinoma, NOS. Presumed precursor lesions including urethritis glandularis with intestinal metaplasia, villous adenoma, or adenocarcinoma in-situ were identified in 6 of the EAs, but in none of the CCAs. Mucin production was limited to non-CCA cases with intracellular mucin seen only in EAs. Expression of CK20, CK7, CDX-2, villin, and β-catenin was seen in 6/6, 2/6, 5/6, 4/5, and 4/4 cases of EA, respectively. Cases of CCA displayed a CK7+/CK20-/villin- profile and 1 of 2 cases expressed CDX2. After a median follow up of 7.3 mo (range, 2.5 to 135 mo), 6 patients were alive with disease, one (with EA) expired with disease after 26 mo; the cancer status was unknown in the remaining 4 patients (3 alive and 1 dead).
Conclusions: These preliminary findings suggest that the 2 major types of primary urethral adenocarcinoma share similar clinical presentations; however, putative precursor lesions are only seen in EA which also has an immunohistochemical profile distinct from CCA. The expression of additional markers and inclusion of other potential cases is ongoing.

741 TMPRSS2-ERG Gene Fusion in Small Cell Carcinoma of the Prostate

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Background: The TMPRSS2-ERG gene fusion has been frequently found in prostatic adenocarcinoma. Small cell carcinoma of the prostate is a highly aggressive disease with distinct pathologic and clinical features that are significantly different from those of conventional prostatic adenocarcinoma. In this study, we examined the TMPRSS2-ERG gene fusion in small cell carcinoma of the prostate.
Design: We searched our pathology files from 1986 to 2008 and identified 14 patients with small cell carcinoma of the prostate. The specimens from the patients included prostate biopsy specimens (n=4), radical prostatectomies (n=5), and biopsy specimens of metastatic sites (n=2). The TMPRSS2-ERG gene fusion was evaluated by fluorescence in situ hybridization (FISH) using the ERG gene break-apart probe. Four cases of small cell carcinoma of the urinary bladder were also included for comparison.

Results: The average age of the 14 patients was 68.9 years (range, 44.0-85.0 years). Eleven patients had a previous diagnosis of prostate adenocarcinoma. In 13 cases, focal high-grade prostatic adenocarcinoma was also present in the specimens. In 1 case, no adenocarcinoma was present. In 12 cases, neuroendocrine differentiation of small cell carcinoma was confirmed by positive immunohistochemical staining for synaptophysin, chromogranin, and CD 56. Rearrangements in the ERG gene were present in eight cases (57%) of small cell carcinoma of the prostate. In all 8 cases, the rearrangement was associated with a deletion of the 5' end of the ERG gene. The rearrangement in the ERG gene was not present in any of the 4 cases of small cell carcinoma of the bladder. Clinical follow-up was available for 12 patients. The patients had received hormonal therapy (n=12), chemotherapy (n=12), radiation therapy (n=11), and radical prostatectomy (n=5). Eleven patients developed distant metastasis. Ten patients died at a mean of 64.8 months after diagnosis (range, 5.0-168.0 months). Two patients were alive with the disease at 55 and 58 months after diagnosis, respectively.
Conclusions: The rearrangement in the ERG gene was frequently present in small cell carcinomas of the prostate. The high prevalence of the TMPRSS2-ERG gene fusion in small cell carcinoma as well as conventional adenocarcinoma implies that small cell carcinoma may evolve from conventional adenocarcinoma in the prostate. Our finding also suggests that the TMPRSS2-ERG gene fusion may be helpful in distinguishing small cell carcinoma of prostatic origin from those of other origins.

742 Fascin Regulates Prostate Cancer Cell Invasion and Is Associated with Metastasis and Biochemical Failure in Prostate Cancer

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Background: Prostate cancer (PCA) metastasis to secondary organs is considered an initial event in the development of hormone refractory disease and remains the major cause of death among PCA patients. In this study, we investigated the role of fascin, a cytoskeleton actin-bundling protein involved in the formation of filopodia and cell migration, in prostate cancer progression.
Design: Fascin protein expression was examined by immunohistochemistry in a cohort of 196 patients with localized PCA and across several stages of disease progression, including hormone refractory disease. Cellular changes were also assessed in vitro and in vivo in DU145 PCA cell line using fascin gene silencing.
Results: Fascin epithelial expression was significantly upregulated in localized and hormone refractory prostate cancer compared to benign prostate tissue ($p<0.05$). Furthermore, high fascin expression was associated with an increased rate of PSA recurrence following radical prostatectomy ($p=0.075$) signifying more aggressive clinical course, thus, supporting a function for fascin in PCA progression. In cellular models, fascin gene silencing using siRNA in the androgen-independent PCA cell line DU145 decreased cell motility and invasiveness while increasing cell adhesive properties. In addition, fascin siRNA expressing DU145 cells implanted orthotopically in mouse prostate showed significantly decreased growth ($p<0.005$) and drastically prevented the formation of lymph node metastases ($p<0.001$) compared to their matched controls.
Conclusions: Our data demonstrate a function of fascin in the regulation of PCA progression and emphasizes the importance of fascin as a prognostic marker for aggressive disease and as a potential therapeutic target for advanced androgen independent disease.

743 TMPRSS2-ERG Fusion Is Frequently Observed in Gleason Pattern 3 Prostate Cancer in a Canadian Cohort

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Background: *TMPRSS2-ERG* gene fusion was recently reported as the most common gene rearrangement in prostate cancer (PCA). However, its significance as a prognostic marker for clinical practice beyond current pathological staging is still needs to be validated in larger cohorts.
Design: Using break-apart FISH assay to indirectly assess the fusion of *TMPRSS2-ERG*. We sought to characterize the incidence, pathological features and clinical parameters of *TMPRSS2-ERG* gene fusion in a cohort of 196 Canadian men treated by radical prostatectomy for localized PCA, and to investigate its potential as a biomarker in PCA.
Results: In this cohort 163/196 prostate samples were available for evaluation by FISH. 41% of the patients showed positive gene fusion status in their PCA. The *TMPRSS2-ERG* gene fusion status was homogenous within the same cancer focus and 82% of fusion positive PCA were present in GS 6 or 7 vs. 14% in GS 8 ($p= 0.004$). Moreover, *TMPRSS2-ERG* fusion was present in 42% of Gleason pattern 3 vs. 27% of Gleason pattern 4 ($p = 0.014$). However, in this study, no significant association was noticed between *TMPRSS2-ERG* fusion status in relation to pathological stage, surgical margin or biochemical failure.
Conclusions: The significance of *TMPRSS2-ERG* gene fusion as a prognostic marker for men with localized prostate cancer beyond current pathological staging needs further investigation. The higher association of *TMPRSS2-ERG* with Gleason score 6 and 7 should be further validated. This finding could have significant clinical impact in further stratifying patients with PCA should the *TMPRSS2-ERG* be confirmed as prognostic marker linked to worse clinical outcome.

744 EAAC1 Aspartate Transporter Expression in Normal, Hyperplastic and Neoplastic Human Prostate Gland

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Background: It is essential for prostate glandular cells to possess a plasma membrane-associated aspartate uptake transport system to keep the high citrate concentration gradient of ~40-1 between intra and extracellular compartments. We previously identified that excitatory amino acid carrier (EAAC1) plays an important role in L-aspartate transport process in prostate metabolism. The goal of the present work is to compare the expression of EAAC1 in normal, BPH and prostate adenocarcinomas (PCa).
Design: EAAC1 protein expression in prostate tissue sections were determined by IHC using anti-EAAC1 antibody. Sections prepared from archival paraffin blocks of 11 normal core biopsies, and 22 cases of PCa in which normal prostatic glands were identified in 5 cases and BPH in all cases (n=22) were investigated. The appearance of membrane-associated immuno-positivity of the glandular epithelial cells was used for scoring as: negative, no; +, < 10%; ++, 10-50% and +++, > 50% positive cells. The mean scores were analyzed by the Student's t-Test.
Results: Normal, BPH and PCa glandular epithelium exhibit immuno-positive EAAC1 staining localized mainly at cell membranes. Analysis of the cases for the presence of glands that exhibit membrane EAAC1 positivity showed that the protein is expressed in normal (16/16), BPH (21/22) and PCa (22/22). The density of expression of EAAC1 showed significant difference between normal and BPH as one group and PCa as second group ($p<0.001$). Acini composed of >10% positive cells reveals that normal and BPH glands exhibited this criterion in 58% (22/38) of cases compared to 91% (20/22) for the PCa.
Conclusions: The present work revealed that EAAC1 is highly expressed in malignant prostate glandular epithelium. In normal prostate, which accumulates citrate for secretion, aspartate is the source of oxaloacetic acid (OAA) for citrate synthesis. In PCa, the citrate level decreases because of increased oxidation due to loss of zinc and its inhibition of mitochondrial aconitase. Citrate is also a major source of acetyl CoA for lipid synthesis which is required for membraneogenesis and proliferation of cancer cells. The citrate used for lipid biosyntheses is not re-cycled to OAA, thus a readily available source of OAA is needed for citrate synthesis to support cell proliferation. We speculate therefore, that the EAAC1 aspartate uptake transporter is up regulated in prostate cancer cells to provide aspartate which is converted to OAA for citrate synthesis.

745 Are Nephrogenic Adenomas Renal Stem/Progenitor Cell-Derived Lesions? An Immunohistochemical Study

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Background: Nephrogenic adenoma (NA) is a benign tumor-like lesion of the urinary tract that histologically resembles the developing distal nephron. Recent evidence suggests that NA is truly a "nephrogenic" lesion, arising from downstream seeding of shed renal tubular cells with implantation and proliferation in areas of damaged urothelium. This proposed pathogenesis and the rarity of the lesion suggest the possibility that NAs arise from kidney stem/progenitor cells that retain the ability to proliferate and develop into renal tubule-like structures when implanted at a distant site. Renal stem/progenitor cells have recently been identified in adult kidney tubules with several markers, including CD133 and PAX2. In our study, we investigate the expression of stem cell surface markers CD133 and CD44 as well as renal-specific transcription factors PAX2 and PAX8 by immunohistochemistry.
Design: Twenty-nine cases of NA from 2000 to 2004 were retrieved from the tissue archives, 18 of which were from urinary bladder and 19 from prostatic urethra. CD133, CD44, PAX2, and PAX8 immunohistochemical staining was performed using the avidin-biotin peroxidase method following antigen retrieval. Complete circumferential membranous staining was considered positive for CD133 and CD44. Distinct nuclear staining was required for PAX2 and PAX8 positivity.
Results: All NAs were positive for renal-specific transcription factors PAX2 and PAX8, consistent with previous studies. CD133 staining was detected focally in eight of 29 (28%) cases. The CD133 positive cells were seen in papillary surfaces, small tubules, and occasionally in the stroma. CD44 staining was detected in seven of ten cases, including five CD133 positive lesions. In the CD44 positive/CD133 positive cases, CD44 was present in the corresponding CD133 areas. CD44 expression, however, was also seen in other areas and in two CD133 negative cases. No staining for these for markers was identified in the epithelium or stroma in prostatic glands, prostatic urethra, or urinary bladder.
Conclusions: Stem cell markers CD44 (70%) and CD133 (28%) were identified in a subpopulation of cells in nephrogenic adenomas, all of which were also positive for renal-specific transcription factors PAX2 and PAX8. Therefore, we suggest that nephrogenic adenomas may arise from transplantation and proliferation of primitive renal cells into an extrarenal stem cell niche. The expression of additional stem cell markers in this regard is currently under investigation.

746 Impact of Bladder Biopsy Second Review on Pathological Stage and Subsequent Patient Management

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Background: The interpretation of bladder biopsy and transurethral resections are fundamental to determining patient management with overstaging having the possible consequence of unnecessary radical cystectomy. Identifying and classifying diagnostic errors in these specimens may help reduce future mistakes. Little data exists on the impact of re-evaluation of referred bladder material on clinical management. As such, we compared referred diagnoses to an academic center, to determine the rate of diagnostic discrepancies, identify problematic areas in interpretation, and assess the impact on patient management.

Design: A retrospective review was performed of the previous 83 consecutive bladder biopsies and transurethral resections referred to our sub-specialized academic institution for patient treatment. Diagnoses from referring institution were compared to the subsequent review and discrepancies in presence of tumor, depth of invasion, tumor grade, and type of tumor were quantified. For specimens with multiple parts, the most malignant diagnosis from each institution was compared as differences in diagnoses between parts would not affect patient care.
Results: Major diagnostic discrepancies were identified in 12% of cases (10/83). Surprisingly, 9 of the 10 differences affected tumor stage. Only one discrepancy in grade was identified and this case also had a concurrent difference in level of invasion. The remaining case was misdiagnosed as prostatic instead of urothelial carcinoma. Our review resulted in upstaging 3 patients (T0 to Tis, n=1; Ta to T1, n=2) and downstaging 6 patients (Ta to T0, n=1; T1 to Ta, n=2; T2 to T1, n=3). In total, misinterpretation of depth of invasion accounted for 70% of the discrepant diagnoses (n=7). Additional transurethral resections of the 3 tumors downstaged from T2 to T1 have shown muscularis propria invasion in 1 specimen. This patient received a cystectomy while the other 2 patients were able to avoid radical surgery.
Conclusions: A significant number of discrepant diagnoses were identified upon review of bladder biopsies and transurethral resections. Most discrepancies were due to misinterpretation of depth of invasion, with overcalls being more frequent than undercalls. Review of bladder specimens changed the clinical management in a substantial portion of our patient cohort.

747 Biallelic VHL Gene Alterations Promote Cancer Progression in Early-Stage Clear Cell Renal Carcinoma through Nuclear Localization of Hypoxia-Inducible Factor-1A

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Background: Von Hipple-Lindau gene (*VHL*) inactivation represents the most frequent abnormality in clear cell renal carcinoma (cRCC). *VHL* encods two different proteins: VHL30 and VHL19 arising as a result of an alternative translation initiation. Hypoxia-inducible factor-1 (HIF-1) is a heterodimer composed of a α and ß subunit. HIF-1ß is constitutively expressed, while HIF-1α expression is regulated by O_2 level. In normal O_2 conditions VHL binds HIF-1α, after hydroxylation in ODD (oxygen-dependent degradation domain) and it allows HIF-1α proteasomal degradation. In this domain a SNP at codon 582 is located. In hypoxia, VHL/HIF-1α interaction is abolished and HIF-1α activates target genes in the nucleus. The study analyzes the *VHL* and codon 582 of HIF-1α gene (*HIF-1α*) status in cRCC, correlating genetic alterations, protein expression and subcellular localization with prognosis.
Design: Tissues microarray fom 136 intracapsular cRCC cases (8ys FU) were constructed and two anti-pVHL Ab (Ig32 and Ig33) and a polyclonal anti-HIF-1α Ab were used for IHC analysis. It was performed mutational analysis for the whole *VHL* and for *HIF-1α* SNP (C1772T), promoter methylation of *VHL* and LOH (3p25). The results were correlated with survival (TSS).
Results: *VHL* gene alteration was found in 57% of cases. *VHL* mutations, methylation and LOH were detected in 51%, 11% and 17% cases. Patients with biallelic *VHL* alteration had a short TSS (p=0.01). We found a statistical association between biallelic *VHL* alteration and no pVHL expression (p=0.004) and nuclear localization of HIF-1α (p=0,04). The SNP was associated with a only cytoplasmic localization of HIF-1α (p=0.007). pVHL negativity and HIF-1α nuclear positivity were correlated with a short TSS.
Conclusions: *VHL* and *HIF-1α* alterations influence their protein expression and localization. Biallelic *VHL* gene alterations with loss of pVHL expression are associated to nuclear localization of HIF1-a and poor prognosis in early-stage cRCC. Alteration of pVHL/HIF-1α pathway is an early event in cRCC carcinogenesis and it's involved in cancer progression through nuclear localizzation of HIF-1α.

748 Renal Oncocytomas with Atypical Features: A Clinicopathologic Analysis of 34 Cases

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Background: Oncocytomas comprise approximately 5 % of all neoplasms of the renal tubular epithelium. Most occur sporadically with a benign clinical course. Some oncocytomas show atypical histopathologic features, including vascular invasion, perirenal fat infiltration, necrosis, marked nuclear pleomorphism, mitotic activity and focal nests of cells with cytoplasmic clearing/ perinuclear halos (CC/PNH). CC/PNH can be particularly problematic in the differential diagnosis with chromophobe renal cell carcinoma (RCC) and eosinophilic variant of conventional RCC. Classification of such cases in the "undetermined malignant potential" category has been proposed; however, the existent outcome data are sparse and controversial regarding oncocytomas with atypical features. Here, we evaluated atypical histopathologic features in regards to clinical outcome.
Design: We retrospectively identified 34 oncocytomas in our files and reviewed them to identify cases with atypical features (vascular invasion, perirenal fat infiltration, necrosis, marked nuclear pleomorphism, mitotic activity and focal nests of cells with CC/PNH. Clinical and follow-up data were collected from patient charts and were correlated with histopathological features.
Results: Of the 34 cases, 26 were males and 8 were females. Age range was 52 to 91 years (mean: 70.0 years). Twenty-one tumors were in the right, 12 were in the left kidney. One case was bilateral. Tumor size ranged from 1.0 to16.0 cm (mean: 4.3cm). Four cases (11%) were multifocal. Seventeen cases (50%) had atypical features including 10 cases with PNH resembling, but not diagnostic of, chromophobe RCC, one with focal CC, two with focal papillary areas, seven with marked nuclear pleomorphism, three with perirenal fat invasion and one with focal necrosis. No mitotic activity or vascular invasion is identified. Follow-up was available from 0.5 to 96 months in 30 patients. Only one patient (0.02 %) died of disease at 55 months of initial diagnosis with liver, bone and lung metastases. Focal PNH was the only atypical feature found in this case. Two patients died of unrelated causes. Others, including 17 classical oncocytomas, are free of disease.
Conclusions: Atypical features in oncocytomas are common (50%). Rarely, there is a risk of metastasis to liver, bone and lungs, warranting long-term follow-up. Overall, classical oncocytomas and the great majority (99.98 %) of those with atypical features behave in a benign fashion.

749 Radical Prostatectomy (RP) Findings in Patients Who Fail Active Surveillance of Prostate Cancer

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Background: Little data is available on the pathologic findings at RP in men who progress following active surveillance for minimal prostate adenocarcinoma on biopsy.
Design: 470 men were enrolled in our active surveillance program since 1995. Men undergo annual repeat needle biopsies to look for progression defined as either: 1) any Gleason pattern grade 4 or 5; 2) >50% cancer on any one core; or 3) cancer in >2 cores. 51 men subsequently have had a RP because of progression and slides were available for review in 48 of these cases. All RPs were serially sectioned and totally submitted.
Results: Mean age at time of presentation was 62 years (52-70). The average time elapsed between the 1st prostate biopsy and RP was 29.5 months (13-70), and the average number of biopsies performed during active surveillance was 3.1 (2-8). Almost half of the patients progressed after the 2nd biopsy (46%), and 75% by the 3rd biopsy. 31 (65%) cases were organ-confined at RP and 25 cases (52%) were Gleason score (GS) 6. 17/48 (35%) of the RPs had extra-prostatic extension (EPE)(7 focal, 10 non-focal), with one of these cases having seminal vesicle involvement and two others with lymph node metastases. 7/48 (15%) had positive margins, all with EPE. 8/48 (17%) men were predicted to have a >50% likelihood of biochemical progression based on grade, stage, and margin status. The mean total tumor volume in the RPs was 1.3 cm^3 (range 0.02 cm^3 - 10.8 cm^3). 33/48 (68%) had a tumor volume of <1 cm^3. 13/48 (27%) of the tumors were potentially clinically insignificant (dominant tumor <0.5 cm^3 and all tumor organ confined with no Gleason pattern 4 or 5). 24% of the RPs with a dominant tumor nodule <0.5 cm^3 demonstrated EPE, yet all had Gleason pattern 4. There was no difference in RP tumor volume whether the needle biopsies contained only 1 or 2-3 criteria for progression.
Conclusions: 1. Most men who "progress" after active surveillance do so 1-2 years after diagnosis suggesting undersampling of more aggressive tumor rather than true progression of indolent tumor. Hence, extended biopsy sampling is required prior to active surveillance. 2) Even with progression, most tumors are organ confined, GS 6, have negative margins, and relatively low tumor volumes. 27% are potentially insignificant cancers. 3) Despite minimal tumor on biopsy and yearly biopsies, 17% have tumors that are more likely to progress with a few cases of advanced cancer. 4) Men considering active surveillance should be counseled on the risk of progression and the likelihood of cure if progression occurs.

750 Utility of Double Immunohistochemical Staining for Smoothelin/ Pancytokeratin in the Staging of Urothelial Carcinomas

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Background: Pathologic staging of urothelial carcinomas reflects the depth of tumor invasion and is important for both therapy and prognosis. The distinction between muscularis mucosae (pT1) and detrusor muscle (at least pT2) involvement can be challenging in unoriented and limited specimens, such as biopsies and transurethral resections (TURBT). Smoothelin was recently described as a promising marker of terminally differentiated contractile smooth muscle cells not expressed in muscularis mucosae. The goal of our study was to evaluate the contribution of double immunohistochemistry (DIHC) for smoothelin and cytokeratin in staging of invasive urothelial carcinomas especially in fragmented, cauterized or scant TURBT material.
Design: Twenty-six pT3 cystectomies, 25 TURBT specimens and a tissue microarray of 120 TURBT specimens from 118 patients with urothelial carcinoma invasive into the detrusor muscle were stained with DHIC for cytokeratin (combination of Cam 5.2 and AE1/3, Biocare Medical, red chromogen) and smoothelin (Chemicon International, brown chromogen). Staining intensity was graded on a scale 0-3+ (0- <5%, 1+ 5-10%, 2+ 11-50%, 3+ >50%).
Results: All cystectomies (26/26) and TURBTs (25/25) showed a significant difference in smoothelin staining between the detrusor muscle (3+ cytoplasmic staining) and muscularis mucosae (0-1+ staining). Staining of variable intensity (1-2+) was also observed in the smooth muscle of vessel walls, especially in large caliber arteries of muscularis propria. Smoothelin did not stain desmoplastic areas in lamina propria invasive urothelial carcinomas. Twenty one cores (21/120) of the tissue microarray contained an area of detrusor muscle invasion; carcinoma cells infiltrating detrusor muscle fibers were highlighted by smoothelin/cytokeratin DIHC in 20/21 cases.
Conclusions: (1) Smoothelin proved to be a reliable marker to differentiate detrusor muscle from muscularis mucosae, desmoplastic areas in lamina propria or smooth muscle of vessel walls; (2) smoothelin/cytokeratin DIHC helped to highlight invasion into detrusor muscle in limited material of TURBT and tissue microarray; (3)smoothelin/ cytokeratin cocktail may be a useful diagnostic tool in the assessment of challenging bladder biopsies.

751 Clear Cell Renal Cell Carcinoma (CCRC): Prognostic and Therapeutic Value of Angiogenesis

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Background: Progress in the treatment of locally advanced or metastatic CCRC has been reported due to the emergence of anti-angiogenic therapies. Their efficacy raises a number of issues: better understanding of action mechanisms, identification of biological predictive factors for response/non response.

Design: Antiangiogenic drugs target CCRC angiogenesis via the VHL/VEGF/HIF pathway. The aim of the study is to analyze the prognostic value of angiogenesis in a prospective series of CCRC in correlation with clinicopathological factors and survival. 75 CCCR were included. Intratumoral vasculature was defined after histologic examination and CD31 immunostaining as type 1 mature and type 2 immature angiogenesis, in comparison with non tumoral kidney vascularization. Intratumoral microvascular density (MVD) was analyzed after immunostaining with CD31 as well as expression of VEGF-A, CAIX,and pVHL. VHL gene mutational analysis was performed using PCR/DNA sequencing and MLPA.

Results: Type 2 immature angiogenesis was significantly associated with high Fuhrman grade ($p<0.0001$), T stage ($p<0.001$), lymph node metastasis ($p<0.03$, M stage ($p<0.002$), low CAIX expression ($p<0.002$), high pVHL expression ($p<0.001$) and VEGF overexpression ($p<0.0001$). Type 2 vasculature was also associated with a low MVD (10+/-27.5) whereas type 1 angiogenesis was associated with high MVD (37.4 +/- 41.04) ($p<0.0001$). A significant association was found between VHL mutated CCRC, a less aggressive tumor profile and a type 1 angiogenesis ($p<0.01$). Moreover, patients with type 1 angiogenesis had improved survival, with a significant statistical difference compared with patients with type 2 angiogenesis ($p<0.003$).

Conclusions: CCRC seem to develop different pattern of angiogenesis: an immature vasculature associated with poor prognostic, and a mature angiogenesis associated with a favorable outcome. These types of blood vessels were also associated with different intratumoral expression of the VHL/HIF/VEGF pathway proteins. With the impact of antiangiogenic drugs, determination of the type of angiogenesis could define predicitve factors of response/non response, and patients who could benefit from these therapies. This work was supporting by grants of PNES REIN of Institut National du Cancer, France.

752 Characterization of New Immunohistochemical Markers of Renal Angiomyolipoma

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Background: Renal angiomyolipoma (AML) is a benign neoplasm composed of blood vessels, spindle cells and mature fat in varying proportions. Rarely, these tumors exhibit epithelioid or atypical features which may have malignant behavior and which may be confused with renal cell carcinoma. Currently used markers such as HMB54 may show a very focal expression in AML causing diagnostic difficulties. Recent gene expression microarrays showed two genes highly expressed in AML in comparison with normal kidney tissues and other renal tumors; these are GREM1 and 5HER2B. The GREM1 gene encodes gremlin 1, belonging to the cysteine knot superfamily, and 5HER2B encodes 5-hydroxytryptamine (serotonin) receptor 2B.

Design: We evaluated immunohistochemical gremlin 1 and 5HER2B expression in 69 cases of renal AML (24 routine sections and 45 cases on tissue microarrays). Immunohistochemical staining was scored semiquantitatively (0 for no staining, 1 for weak staining, 2 for moderate staining and 3 for strong staining); where present, adjacent kidney tubules served as a positive internal control while vessels and kidney stromal cells served as a negative internal control.

Results: The tumors were divided into three morphological groups: 44 spindle cell predominant, 7 classic, and 18 epithelioid types. Gremlin 1 demonstrated cytoplasmic and nuclear staining in 66 of 69 cases (96%; 23/24 = 96% large sections; 43/45 = 96% TMA), which was focal in 5 cases. 5HER2B showed cytoplasmic and nuclear staining in 68 of 69 cases (99%; 24/24 = 100% large sections; 44/45 = 98% TMA), which was diffuse in all but one. Intensity of staining differed by subtype of AML. Both antibodies showed weak staining (0, focal 1+ or 1+) in the spindle cell group. Of 24 cases negative or staining weakly for Gremlin 1, 19 were spindle cell type (79%). Of the 15 cases negative or staining weakly for 5HER2B, 13 were spindle cell predominant (87%). Additionally, tumors with focal epithelioid features showed stronger staining.

Conclusions: We have verified by immunohistochemistry that both 5HER2B and Gremlin 1 are overexpressed in angiomyolipoma. Further, staining is decreased in tumors which are predominantly composed of smooth muscle and stronger in those with epithelioid features. These findings may be useful diagnostically, and also may suggest different mechanisms of tumorigenesis among various AML subtypes.

753 Seminal Vesicle Invasion at Radical Prostatectomy: Correlation with Magnetic Reasonance Images

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Background: Seminal vesicle invasion (SVI) in newly diagnosed prostate cancer (PC) is associated with a poor prognosis and treatment decisions may rely on preoperative radiologic detection. Endorectal coil magnetic reasonance imaging (MR) is reported to be accurate in demonstrating SVI prior to prostatectomy. In this study, we characterized SVI histologically with whole mount prepared radical prostatectomy specimens and correlated the findings with MR.

Design: Prostate glands (PG) from 100 consecutive patients with biopsy proven PC who underwent MR were selected for histologic review. The PG slices were kept intact and sections prepared on 2"x3" slides and the seminal vesicles (SV) were sliced on cross section. Features of SVI evaluated were number of tumor foci, location and tumor size (measured microscopically). MR evaluation of SVI was obtained from MR reports; a reading was considered positive if SVI was considered "present, probable, suspicious, possible or cannot be ruled out.".

Results: Of the 100 patients, 8 had histologically identified SVI (3 bilateral, 5 unilateral); MR agreed in 3 cases (1 present, 1 probable, 1 possible). Of these 3 cases, 45-80% of the PG was involved by tumor and the Gleason score (GS) in 2 cases was 9(4+5) and in one case was 7(4+3) tertiary 5. SVI was only present (by direct extension) at the PG-SV junction and the foci measured at least 2cm. In 5 cases, SVI was not identified by MR. The PG involvement in 4 of these 5 cases ranged from 5-15% and one PG had 70% tumor involvement; tumor was at the PG-SV junction only in 1 case, in the free SV wall only in 3 cases, and in both the PG-SV junction and free SV wall in 1 case. In 4 cases the GS was 7 (3 cases 3+4; 1 case 4+3 (+5)) and in one case it was 9(4+5). The foci of tumor in the free SV wall and PG-SV junction ranged from ≤ 1mm-4mm. The SV wall was not distorted in any case. MR reported SVI in an additional 4 cases (3 possible, 1 cannot rule out); however, no SVI was identified at pathologic review.

Conclusions: MR detection of SVI is more likely to be accurate when described as "present or probable" and tumor volume and GS are high. MR detects tumor at the PG-SV junction when the tumor nodule measures >1cm. Small foci of carcinoma ≤4mm in the SV wall or at the PG-SV junction, and without microscopic distortion are unlikely to be detected by MR. These smaller foci may not be detected when seminal vesicles are randomly sampled.

754 Relationship between COX-2 Expression, Angiogenesis and Apoptosis in Prostate Carcinoma

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Background: COX is the enzyme that responsible for the production of prostaglandins from arachidonic acid. It plays role in tumorigenesis of a variety of human malignancies by stimulating cell proliferation, inhibiting epithelial differentiation, inhibiting apoptosis, mediating immune suppression. M30 is a monoclonal antibody which is used to detect the cleavage of cytokeratin 18 that occurs as an early event in epithelial cells that undergo apoptosis.The objective of this study is to determine if there is relationship between COX-2, apoptosis and angiogenesis in prostate carcinomas.

Design: COX-2, Bcl-2, VEGF and M30 were studied immunohistochemically at 49 prostate adenocarcinoma. The results of staining for COX-2, Bcl-2 and VEGF were analyzed semiquantitatively by using an immunohistochemical scoring system (HSCORE) that combines the percentage of immunoreactive cells (quantity score) and an estimate of staining intensity (staining intensity score). The number of M-30 positive cells per 1000 cells was expressed as apoptotic index.

Results: The age of the patients ranged from 48 to 73 years old (average, 63.8±6.4). Twenty-one (42.8%) were Gleason score ≤ 6 and 28 (57.2%) were Gleason score ≥7. COX-2 expression was detected in 81.6% of cases. It was significantly correlated with Bcl-2 expression ($r=0.49$; $p<0.0001$). There was no correlation between COX-2 and VEGF expression ($p>0.05$). Gleason score was negatively correlated with M30 ($r=-0.28$; $p=0.04$). No significant relation between Gleason score, VEGF and Bcl-2 was observed. Negative correlation between COX-2 expression and mean survival was also observed ($p=0.007$).

Conclusions: Our study showed an overexpression of COX-2 in prostate carcinoma. Unlike some reports, we found no relationship between COX-2 and angiogenesis (VEGF). However, COX-2 was significantly correlated with bcl-2. This raise the possibility that COX-2 may influence tumor progression in prostate carcinoma through inhibiting apoptosis rather than the promotion of angiogenesis. Selective agents targeting apoptosis and COX-2 may play role in the treatment. Additionally, expression of COX-2 is associated with a significantly worse survival. In this regard, overexpression of COX-2 may be useful for assessing the biologic behaviour of prostate carcinoma.

755 Immunohistochemical Profiling of the mTOR Pathway in PIN and Prostatic Adenocarcinoma (PCa): A Tissue Microarray (TMA) Study

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Background: The Mammalian Target of Rapamycin (mTOR), a constituent of the AKT pathway is activated in many cancers. AKT phosphorylates (p) mTOR (p-mTOR) leading to the activation of downstream targets p70 Ribosomal Protein S6 Kinase 1 (p-S6K1) and Ribosomal Protein S6 (p-S6RP). This study correlates the immunohistochemical (IHC) expression of p-mTOR and its downstream targets p-S6K1 and p-S6RP in PIN and PCa using TMAs, and identifies potential therapeutic targets.

Design: Three TMAs were constructed from radical prostatectomies: PIN (35 cases), Gleason (GL) 7 (3+4) (35 cases) and High Grade (HG) PCa (GL 8, 9, 10) (37 cases). Each case was represented by three 1mm cores. IHC was performed for p-mTOR, p-S6K1, and p-S6RP on serial sections. Scanned TMAs were scored for the percentage of positive cells. Positive areas in each core were correlated for the 3 markers. Greater than 5% immunoreactivity in a core was considered positive. P values less than .05 were considered significant.

Results:

IHC Expression of p-mTOR, p-S6K1, and p-S6RP in PIN, GL7, and HG PCa

Number of Cases Positive	PIN	GL7	HG
Cytoplasmic p-mTOR	29/35 (83%)	22/35 (63%)	20/37 (54%)
Nuclear p-S6K1	35/35 (100%)	26/35 (74%)	31/37 (84%)
Cytoplasmic p-S6RP	29/35 (83%)	20/35 (57%)	29/37 (78%)

The highest expression of all three markers was observed in PIN. A Cochran-Armitage Trend analysis demonstrated decreasing p-mTOR activity progressing from PIN through GL7 to HG PCa. p-S6K1 and p-S6RP showed statistically significant differences among the 3 groups, however a trend was not observed. There was considerable intratumoral IHC heterogeneity within an individual case but statistically significant correlation for

all 3 markers was observed in each core. In addition, for PIN, GL7, and HG PCa specific areas of positivity/negativity in each core correlated for all 3 markers.
Conclusions: p-mTOR, p-S6K1, and p-S6RP were highly expressed in PIN and may provide novel targets for its treatment. A decreasing trend in p-mTOR IHC was observed progressing from PIN through GL7 to HG PCa. No identifiable trends were observed for p-S6K1 and p-S6RP. Extensive heterogeneity of expression within individual cases may limit the use of mTOR and components of this pathway as predictive of expression in limited biopsy samples. These findings demonstrate potential novel therapeutic targets for PIN and PCa.

756 Methods of Submitting Cores and Gleason Score as Contributory Factors to the Fragmentation of Prostate Core Biopsies Containing Adenocarcinoma

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Background: Fragmentation of prostatic core biopsies containing prostatic carcinoma may complicate quantification of the number cores with cancer and/or the amount of tumor in a core.
Design: A search for prostate biopsy cases containing fragmented cores with cancer and cancer cases present only in unfragmented cores was performed on cases from the consult service of one of the authors. Cases were not consecutive cases but were enriched for fragmented cases to better evaluate causes of fragmentation. Cases of prostatic adenocarcinoma with fragmented cores containing cancer (n=463) and cases lacking fragmented cores (n=200) were evaluated in regards to number of parts per case, the number of cores per specimen, number of parts containing cancer, the number of parts fragmented, and the highest Gleason score in the fragmented and unfragmented cores.
Results: Mean number of fragmented parts was 1.3 (1-6). Mean number of parts was 7.7 (1-32). The mean number of parts per case were 8.1 and 7.5 in the unfragmented and fragmented cases respectively ($p=0.1$), yet there were significantly more fragmented cores in cases with >6 vs ≤6 parts ($p<0.05$). Mean number of parts with cancer was 2.4 (1-13). There was a significantly higher number of parts containing cancer in the fragmented cores compared to cases without fragmented cores (2.8 vs 1.6, $p<0.0001$). Mean number of cores per part was 2.5 (1-10). The cases containing fragmented cores with cancer had a higher number of cores per part (2.6 vs 2.1, $p=0.004$). Mean Gleason score was 6.5 (6-10). A higher mean Gleason score was associated with the cancer in the fragmented cores when compared to the unfragmented cores (6.6 vs 6.2, $p< 0.0001$). Multivariate analysis showed an association of fragmentation with the following variables: number of parts with cancer (odds ratio 1.7, $p<0.0001$); cores per part (odds ratio 1.3, $p<0.0001$); and Gleason score (odds ratio 1.3, $p<0.04$).
Conclusions: The more parts with cancer the more likely that at least one of the parts may show fragmented cancer. Higher Gleason score is also associated with fragmented cores, with for example large cribriform Gleason pattern 4 with little supporting stroma often fragmented. While these factors cannot be controlled, pathologists and urologists can limit the number of parts per specimen and the number of cores submitted per part to reduce the likelihood of fragmentation of cores containing prostatic adenocarcinoma.

757 Estrogen Receptor-α and β Expression in Prostate Cancer: Effect of Hormonal Therapy, Tumor Grade, and Ethnicity

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Background: Estrogens (E), acting through estrogen receptor (ER) subtypes α and β, are involved in the growth, differentiation, and maintenance of the prostate, and have been implicated in prostate carcinogenesis. We aimed to assess by IHC the effect of androgen deprivation therapy (ADT) on the expression of ER-α and β in prostate cancer (PCA) and to evaluate their expression in different ethnic groups and in relation to Gleason Score (GS).
Design: TMA containing 336 PCA, 121 treated with ADT (tPCA) and 212 untreated (uPCA), were stained for ER-α and β. Of the uPCA, 62 were Black, 27 Japanese, and 123 White. The uPCA GS distribution was: 39 GS6, 163 GS7, and 10 GS≥8. The % of immunolabeled nuclei was determined: ER-α was scored as 0 (<5%), 1 (5-25%), 2 (26-50%), and 3 (>50%); ER-β as present (1) or absent (0).
Results: ER-β locates in prostate epithelium; ER-α is detected in prostatic stroma. The results are summarized in the table. Although ER-α expression was significantly different between tPCA and uPCA ($p<0.001$), no difference was appreciated in the distribution of ER-β between the two groups. Within the uPCA, the immunoreactivity for both ER-α ($p<0.001$) and β ($p=0.002$) was significantly different among race categories. The expression of neither ER-α nor β was different among GS categories.

				uPCA				
		tPCA (n=121) %	uPCA (n=212) %	Black (n=62) %	Japanese (n=27) %	White (n=123) %	p value	
ER-α	0	15.7	24.1	25.8	51.9	17.2	B vs. J: p=0.06	
	1	14.8	30.7	41.9	25.9	26.0	J vs. W: p=0.001	
	2	19.1	26.9	29.0	14.8	28.5	B vs. W: p<0.0001	
	3	50.4	18.4	3.2	7.4	28.5		
	P value	p<0.001		p<0.001				
ER-β	0	82.6	85.5	82.3	65.4	91.6	B vs. J: p=0.09	
	1	17.4	14.5	17.7	34.6	8.4	J vs. W: p=0.001	
	P value	p=0.5		p=0.002				B vs. W: p=0.06

Conclusions: The different expression of ER-α and β among different ethnic groups, independent of the GS, may be responsible for different incidence and behavior of PCA in various populations. In view of the upregulated expression of ER-α after ADT, its potential role as therapeutic target and marker for response to hormonal manipulation should be further investigated.

758 Can Saturation Biopsy Predict Significant Cancer Nodule Localization in Radical Prostatectomy Specimens: A Correlative Study and Implications for Focal Therapy for Prostate Cancer

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Background: Focal therapy for prostate cancer using non-prostatectomy approach, such as cryoablation, has been increasingly gaining acceptance for patients who are not candidates for radical prostatectomy (RP). The success of focal therapy relies on the accurate mapping, using transrectal biopsy, of the significant cancer nodules in the prostate gland before the procedure.
Design: Seventy-two consecutive patients underwent saturation needle biopsy of the prostate (Bx) followed by RP for PCa performed at our institution from August 2003 to October 2007. The biopsy protocol consisted of traditional sextant, plus an additional 12 cores focused in the lateral peripheral zone and apex. All RP specimens were reviewed by a single pathologist, who mapped the tumor outline, determined the number of separate foci of PCA, their volume (TV), zone of origin and Gleason score (GS). The locations of tumor on Bx and RP were correlated.
Results: The median age and preoperative PSA of the patients was 60 years (range 38-75) and 6.9 ng/mL (range 1.2-27.7), respectively. The prostate specimen median weight was 48 g (range 25-274). In 37 (51.4%) men, the Bx findings correlated with the RP findings. In the remaining 35 patients (48.6%), 51 PCA foci, ranging from 1 to 4 per RP, were missed by Bx. Of the 51 missed PCA foci, 32 were significant PCA (>5 mm^3 and GS≥6). The significant PCA were GS6 (n=22) and GS7 (n=10); their TV ranged from 8 to 200 mm^3; the location was distributed as follows: 14 transitional zone (TZ) (44%), 7 apex/anterior zone (22%), 7 mid peripheral zone(22%) and 4 posterior peripheral zone (12%). In 39/72 (54.2%) cases, the Bx was positive only on one side (right in 26 and left in 13 cases), meanwhile the RP specimen showed significant PCA in both sides in 22 specimens (false negative [FN])(negative predictive value [NPV] = 43.6%). There was no statistical difference in patient's age, PSA, and prostate specimen weight between FN and true negative cases.
Conclusions: A negative saturation needle biopsy does not confirm the absence of PCA in the corresponding side of the RP and cannot be used as single determinant when considering a patient for focal treatment. Considering that most of the PCA foci missed by Bx were located in the transitional zone, TZ biopsies could be suggested before taking surgical decision such as focal therapy.

759 Mass-Forming Intraductal Carcinoma of the Prostate

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Background: The defining features of and clinicopathologic outcomes associated with intraductal growth of prostatic carcinoma (IDC-P) are controversial, with most considering it a late event in prostate cancer (CA) evolution. Mass-forming IDC-P, accounting for the bulk of tumor volume, has not been well studied.
Design: We identified 3 radical prostatectomy (RP) and corresponding needle biopsy (NB) specimens in which IDC-P represented ≥ 70% of the tumor mass and evaluated architectural, cytologic, and topographic features of these lesions.
Results: RP: IDC-P comprised 70 to 90% of total tumor volume, with retained basal cell layers confirmed by immunohistochemistry (IHC) in all cases (RP and NB). Intraductal tumor masses were predominantly located in the peripheral zone, with one (case 3) extending to transition zone, periurethral ducts and urethral surface. A spectrum of architectural patterns was observed, including micropapillae and loose cribriforming (case 1), dense arborizing micropapillae (case 2), and dense cribriforming/solid growth (case 3), the latter accompanied by comedonecrosis. Cases 1 and 3 showed marked nucleomegaly in >70% of the IDC-P, while case 3 also had significant pleomorphism/frequent mitoses. The same cases showed 10% flat growth of IDC-P within acini. Case 2 showed marked atypia in 15% of the IDC-P and abundant macronucleoli in the remainder of the lesion. Gleason score of the invasive component was 3+4=7 for cases 1-2 (>80% pattern 3) and 4+3=7 for case 3 (– equal patterns 3 and 4). Extraprostatic extension was seen in cases 2-3. NB: Between 6 and 11 NB cores revealed IDC-P. In cases 1 and 3, IDC-P was recognized at diagnosis, while in case 2, IDC-P was identified retrospectively. In cases 1 and 3, IDC-P involved 10-95% of cores with minimal to no invasive CA. Micropapillary and loose cribriform (case 1) or dense cribriforming/flat growth (case 3) was observed, along with an equivalent level of nucleomegaly to that seen at RP. On careful review, positive NB from case 2 showed 5-20% IDC-P and up to 75% invasive CA, with arborizing micropapillary IDC-P architecture reminiscent of that seen at RP and moderate to focally marked cytologic atypia.
Conclusions: Intraductal spread of prostate CA may form expansile lesions. The limited and predominantly low grade invasive CA seen with mass-forming IDC-P suggests that intraductal growth is a primary pathway of prostate cancer spread. IDC-P is identified on needle biopsy by recognition of complex architecture +/- marked cytologic atypia, the latter of which may manifest as flat intra-acinar growth.

760 Immunohistochemical Expression of BRCA1 in Prostate Cancer

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Background: BRCA1 is a multifunctional protein involved in DNA repair, gene transcription and the regulation of cell-cycle check-points. While germline mutations of BRCA1 are rare in prostate cancer and seem to play a limited role in tumor susceptibility, BRCA1 expression has not been investigated to date.

Design: We analyzed the immunohistochemical expression of BRCA1 in paraffin embedded samples from 524 men with prostate cancer belonging to the Physicians' Health Study and the Swedish Watchful Waiting cohorts of prostate cancer patients. High density tissue micro-arrays (TMA) including at least three tumor cores for each case were utilized for the immunohistochemical staining with the monoclonal MS110 antibody specific for the N-terminus of the 220 kDa BRCA1 protein. Cases were scored as negative or positive for BRCA1 immunostaining. The Ki67 proliferation index was also assessed on the same TMAs and evaluated by quantitative image analysis.

Results: A positive nuclear immunostaining for BRCA1 was revealed in 62 of 524 (11.9%) patients while normal prostate control cores were all negative. BRCA1 positive tumors were associated with 4 times greater proliferation rate compared to BRCA1 negative tumors ($p \sim 0.0003$). In addition, we found a linear trend such that tumors with greater number of TMA cores expressing BRCA1 had stronger extent of proliferation. Men with BRCA1 positive tumors had a slightly higher Gleason's score (mean 7.5) compared to those negative for BRCA1 (mean 7) No significant correlation was found between BRCA1 staining and cancer-specific death.

Conclusions: BRCA1 protein is expressed in a small subset of prostate cancers characterized by high proliferation index but not in normal prostate tissue. Expression of BRCA1 might be acquired in selected tumors to prevent DNA damage in actively replicating cells. A different role independent of germline mutations might be disclosed for BRCA1 as cell cycle regulator in prostate cancer.

761 Prostatic Adenocarcinoma and Status of Resection Margin of Radical Prostatectomy Associated with Core Biopsies with Atypical Small Acinar Proliferation or Minimal Cancer

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Background: The finding of atypical small acinar proliferation (ASAP) in prostate biopsy cores has been previously investigated with repeat biopsies that show a rate of prostatic adenocarcinoma (PAC) ranging from 15 to 40%. The relationship between cancers missed on biopsy and cores positive for ASAP, minimal cancer, and absence of PAC has not been examined. In this study we use a model of hemiprostates corresponding to 5 cores associated with no cancer, ASAP, and minute cancers.

Design: The PAC biopsy negative hemiprostates of radical prostatectomy (RPs) specimens with biopsy proven contralateral PAC were examined. PAC negative hemiprostates with biopsy cores corresponding to minute cancers (group 1), ASAP (group 2) and no cancer (group 3) were obtained retrospectively from 2002 to 2008 from our institution. Groups 2 and 3 were subdivided into 2a/3a and 2b/3b for hemiprostates with or without PAC respectively.

Results: The interval from biopsy to RP ranged from 1 to 8 months. Of 637 total RPs, there were 31, 14, 18, 86 and 75 hemiprostates that fulfilled the criteria for groups 1, 2a, 2b, 3a and 3b respectively. There was a higher rate of PAC, including clinically significant PAC, in the hemiprostates associated with ASAP compared to non-ASAP hemiprostates. However, this difference was not statistically significant. Positive resection margins were observed in 2 cases. The biopsies for these 2 cases had demonstrated minute cancer and negative for cancer.

Conclusions: ASAP in needle core biopsy appears to be associated with an increased rate of PAC, however this increase was not statistically significant in the current study. There was no difference in the surgical resection margin status of RPs performed soon after an ASAP positive biopsy or following a delay of up to eight months. Furthermore, biopsies with ASAP were not associated with a higher rate of positive margins when compared to biopsies with minute cancer or negative for cancer.

762 Urothelial Carcinoma Associated with Clinically Diagnosed Prostatic Adenocarcinoma

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Background: Prostatic adenocarcinoma (PAC) and urothelial bladder carcinoma (UC) are the two most common malignancies of the lower urinary tract in males. There is an elevated incidence of incidental PAC in patients with UC who undergo cystoprostatectomy. An increased incidence of UC is also observed in patients diagnosed with PAC. The aims of this study were 1) to validate the association between these two groups of tumors and 2) to further characterize the relationship of UC with PAC, with regards to their clinical impact (incidental versus clinically symptomatic PAC) in a population living in a Canadian city.

Design: All cases of UC and PAC between 2002 and 2008 were retrieved from the archives of our institution and retrospectively analyzed.

Results: There were a total of 790 patients with biopsies positive for UC (n = 670) or who underwent cystoprostatectomy for invasive UC (n = 120). The UC was of low grade (LGUC) in 408 of the cases and high grade (HGUC) in 382 of the cases. Of the 120 cystoprostatectomies for UC, 78 (65%) had incidental findings of PAC of which 50 were clinically insignificant and 28 were clinically significant. A total of 2568 patients were diagnosed with PAC over the six year period. Twenty-one (0.8%) of these patients (ages: 76+/- 8) had both high grade/advanced stage PAC and UC. Categorization of the twenty-one UC cases included 16 non-invasive LGUCs, 3 non-invasive HGUCs, and 2 superficially invasive HGUCs. In addition, three patients had concurrent UC and secondary PAC invading into the urinary bladder. All UC cases occurred within 6 years prior to or 1-2 years following the diagnosis of PAC.

Conclusions: Cystoprostatectomy for the management of invasive UC was associated with incidental PAC in 65% of cases, although the majority of these PACs were clinically insignificant. Conversely, a subset of patients underwent further investigations for symptomatic high grade or clinically detectable PAC. These patients were more likely to be diagnosed with incidental UC, typically of low grade.

763 MicroRNAs as a Powerful Diagnostic Tools for the Differential Diagnosis of Kidney Tumors

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Background: Renal cell tumors are a group of tumors that differ both in morphologic appearance and biological behavior. In some cases, despite morphologic and immunohistochemical assessment, the pathological differential diagnosis can be difficult. Accurate diagnosis is essential for proper management of the patient. Additional diagnostic tools are therefore required. MicroRNAs are non-coding RNAs that regulate gene expression and display remarkable tissue specificity. Rosetta Genomics has developed technologies for extracting and profiling of microRNAs from FFPE samples. This technological platform was applied to differentiate between common types of kidney tumors.

Design: Two independent sets of kidney tumors FFPEs were collected and reviewed by a pathologist specializing in urological pathology. Histologically, renal cell tumors included: clear cell RCC, chromophobe RCC, papillary RCC (both subtypes) and oncocytoma and were classified using H&E stains. High-quality RNA was extracted using a protocol developed to preserve the fraction of microRNAs. Expression levels of microRNAs were measured on microarray and qRT-PCR platforms developed at Rosetta Genomics.

Results: Expression levels of over 800 microRNAs in a training set of over 70 kidney tumors identified differentially expressed microRNAs between the different histological types of renal cell tumors. We defined a simple algorithm for classification that uses a set of only 6 microRNAs to classify clear cell, chromophobe, papillary and oncocytoma tumors. The classifier was then tested on an independent validation set including the same histological types, and diagnosed correctly 91% of the tumors. Technical validation was performed using qRT-PCR showing a high correlation to the results obtained using microarrays.

Conclusions: Expression levels of certain microRNAs are highly specific to subtypes of kidney tumors. This evidence adds to the accumulating evidence on the specificity of microRNA expression in tissues, tumor types and developmental stages. A combination of 6 microRNAs can successfully answer specific differential diagnosis of morphologically similar renal cell tumors. The results we present provide a basis for the development of microRNA based diagnostic assay for renal oncology.

764 Splice Variants and Functions of TMPRSS2-ERG Fusion, a Common Genomic Alteration in Prostate Cancer

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Background: *ERG* oncogene is overexpressed in the majority of prostate cancers due to genomic rearrangements placing the ERG gene under the control of the androgen regulated TMPRSS2 promoter. However, the biological functions of *ERG* in CaP are not well understood. This study was designed to evaluate the function of ERG and to reveal the forms of ERG in human prostate tumors.

Design: ERG was knocked down in TMPRSS2-ERG expressing VCaP cells. Cell proliferation and tumor growth was assayed by in vitro and in vivo models, respectively. Gene expression changes were evaluated by microarray and by quantitative PCR. ERG splice variants were cloned from a Lambda-Zap cDNA library from TMPRSS2-ERG expressing prostate tumors from six patients.

Results: We demonstrated that ERG activates C-MYC oncogene and negatively regulates the expression of prostate differentiation markers in prostate cancer. The predominant form of ERG in human prostate tumors is a novel type (type II) form that lacks DNA binding domain.

Conclusions: The primary consequence of ERG overexpression in prostate tumors is the suppression of prostate differentiation genes. ERG positively regulates C-MYC oncogene contributing to the suppression of differentiated cellular phenotype. Detection of prostate cancer by Type I and II variants in human prostate tissues is superior to the detection of tumors by the TMPRSS2-ERG fusion junction.

765 Elevated Secreted Protein, Acidic, and Rich in Cysteine (SPARC) Expression in Prostate Cancer Correlates with Tumor Metastasis after Radical Prostatectomy

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Background: Comparative gene expression signatures of well differentiated and poorly differentiated prostate cancer (CaP) along with knowledge based gene analysis highlighted alterations of SPARC, and genes linked to it, in poorly differentiated CaP. Quantitative determination of SPARC gene expression levels in prostate tumor cells has been associated with an increased risk of PSA recurrence, with poorly differentiated carcinoma with overall Gleason score 8-9. We hypothesized that

determination of SPARC protein expression levels in prostatectomy specimens by immunohistochemistry (IHC) may have the potential to predict aggressive clinical behavior in post prostatectomy patients.

Design: Fifty four prostatectomies matched by Gleason grade and pathologic stage were studied. Twenty seven patients with metastasis after the surgery were compared to 27 without metastasis. All specimens were processed as whole mounts and stained for SPARC by immunohisto-chemistry. The sections were incubated with anti-SPARC mouse monoclonal antibody (Zymed Laboratories, Inc., CA, USA) at a dilution of 1:160 for 1 hour, followed by 30 minutes in biotinylated horse antimouse (Vecto, Burlingham, CA) at a dilution of 1:400, and ABC (Vector, Burlingham, CA) Vector VIP was used as chromogen. SPARC expression was scored by % of tumor cells positive on a scale of 1-4, staining intensity on a score of 1-3, and a combination of both. These scores were correlated with clinical-pathologic features.

Results: Higher SPARC protein expression was significantly associated with metastases compared to non-metastasis group after the prostatectomy by using Fisher exact test (p=0.0076) and ROC (AUC=0.789). SPARC protein expression was able to predict the development of metastases.

Conclusions: High SPARC expression in CaP is associated with an increased risk of tumor metastasis in this patient cohort. Quantitative determination of SPARC protein expression levels in radical prostatectomy specimens may have prognostic utility and may help stratify and treat patients with locally advanced CaP.

766 Estrogen Receptor-alpha (ERalpha) and – beta (ERbeta) Expression in the Stroma Associated with Adenocarcinoma of the Prostate

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Background: ERalpha and ERbeta are expressed in the stroma of prostatic adenocarcinoma. Recently there has been an increased interest in the role of tumor-associated stroma in prostate tumorigenesis, but little is known about the respective roles of ERalpha and ERbeta in prostate cancer. This study evaluated the expression of ERalpha and ERbeta in the tumor-associated stroma and the stroma surrounding benign prostate. The correlation between their expression and clinicopathological factors is also investigated.

Design: Immunohistochemical analysis with antibodies against ERalpha and ERbeta were used. Two sets of prostatic cancer cases are included. In the first set, whole tissue sections from 47 prostate cancer cases were studied to compare stromal ER levels in prostate cancer and benign prostate on the same slide. In the second study set, ER expression were studied in 200 samples of prostate cancer on a tissue microarray (TMA). The samples on TMA were stratified by clinicopathological factors. The levels of ER nuclear expression were scored as 0 negative, 1 weak, 2 moderate and 3 strong expression.

Results: The expression of both ERalpha and ERbeta is significantly lower in tumor-associated stroma (ERalpha: $p < 0.01$; ERbeta: $p=0.01$) compared with stroma around benign glands. When stratified with clinicopathological factors, the level of ERalpha expression in tumor-associated stroma shows a positive correlation with Gleason score ($r = 0.93$, $p<0.05$). The expression of ERalpha is higher in prostate cancer with advanced tumor stage ($p = 0.05$). The level of ERbeta expression in tumor-associated stroma is decreased in older patients ($p=0.01$). No significant correlation between the expression of ERbeta in tumor-associated stroma and any other clinicopathological factors was detected.

Conclusions: This study demonstrated significant down-regulation of ERalpha and ERbeta expression in tumor-associated stroma of prostate cancer. However, ERalpha expression level in tumor-associated stroma shows a positive correlation with Gleason score and it is slightly increased in cases with advanced tumor stage. The level of ERbeta expression in tumor-associated stroma is decreased in older patients but not correlated with Gleason score or stage. These findings suggest that ERalpha and ERbeta plays different roles in the tumorigenesis and progression of prostate cancer.

767 Correlation of Gleason Score and Tumor Size with Magnetic Reasonance Image-Detected Prostate Cancer

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Background: Endorectal coil (ERC) magnetic resonance imaging (MR) has become important in the care of patients with prostate cancer (PC). In this study, we histologically characterized foci of PC with whole mount prepared radical prostatectomy specimens and correlated the findings with MR.

Design: Twenty-one patients with biopsy proven PC voluntarily enrolled in an IRB-approved study assessing 3T ERCMR prior to surgery. The prostate gland (PG) slices were kept intact and sections prepared on 2"x3" slides. On each slide, GS, size (greatest 2-dimensional), and pattern of tumor growth (A= < 10% benign glands; B= ≥ 10-50% benign glands; C=small cluster of glands "alone"; D=<10 glands infiltrating between benign glands) of each tumor focus was recorded. The genitourinary (GU) pathologist was blinded to the MR results. Two GU radiologists, blinded to pathology results, independently reviewed the ERCMR of each patient. Possible foci of carcinoma were graded on a 1-5 scale (1=no cancer; 5=positive). A score of 4 or 5 by at least one radiologist was considered a positive result. Each PG slice was spatially mapped to the corresponding MR slice for each case.

Results: Twenty-one cases were available for review and 81 separate foci of PC were identified histologically. Of these, 43 foci were seen by MR (MR+) and 38 were not (MR-).

MR positive

GS	# of foci	Tumor size		Growth pattern			
		≤7mm	>7mm	A	B	C	D
6(3+3)	15	6	9	6	8		1
7(3+4)	23	3	20	10	13		
7(4+3)	3	1	2	2	1		
7(4+3)5	1		1	1			
8(4+4)	1		1	1			

MR negative

GS	# of foci	Tumor size		Growth pattern			
		≤7mm	>7mm	A	B	C	D
6(3+3)	33	33		7	7	2	17
7(3+4)	5	4	1	1	1		3

Of the ≤7mm MR+ cases, the tumors ranged from 4-7mm and of the >7mm MR+ cases, 4 foci were 8-9mm, 22 were 1-2cm and 7 were >2cm. Of the MR- cases, 10 foci were ≤1mm, 25 were 2-5mm and 2 were 6-7mm. No histologic reason for a 1.6cm MR- focus of PC was found. MR reported 15 additional foci of PC lacking pathology concordance. Most MR+ PC foci were GS ≥7 (65%), ≥1cm (67%) and present as a dominant tumor nodule. Thirty-three of 34 (97%) foci > 7mm were MR+; all GS ≥ 4+3 were MR+. Most MR- PC foci are GS 6 (87%) and ≤7mm (97%), and more than half are present as a few glands infiltrating between benign glands.

Conclusions: The PC foci identified by MR tend to be larger and higher grade and may prove to be more clinically relevant. ERCMR may be useful for guiding patient management, including "watchful-waiting" protocols.

768 CAIX Expression in Renal Neoplasms: Correlation with Tumor Type and Grade

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Background: Carbonic anhydrase IX (CAIX), a hypoxia-induced protein, is expressed in some renal cell carcinomas (RCC) and is reported to be an independent predictor of outcome. In this study, we assessed CAIX expression in renal neoplasms (RN) and correlated it with tumor type and grade.

Design: The study group consisted of primary and metastatic RN. The diagnosis and grade of the tumor were recorded from review of slides or pathology reports. Papillary RCC (PRCC) were classified as Type 1 or 2. CAIX immunostaining was performed on one representative section of tumor from each case with the M75 antibody. CAIX expression was divided into two categories, >85% tumor cells positive (high) or ≤85% tumor cells positive (low); cytoplasmic membrane staining was considered positive. Fisher's exact tests were used to determine associations between CAIX expression and tumor type and Fuhrman grade.

Results: Three hundred fifty-one cases were available for analysis; there were 302 primary RN, 42 metastatic RCC, and 7 cases site unknown. The distribution of tumor type was as follows: 186 clear cell RCC (CCRCC), 52 PRCC, 35 chromophobe RCC, 20 unclassified RCC (URCC), 26 oncocytomas, 2 metanephric adenomas, 1 urothelial carcinoma, 1 mixed epithelial and stromal tumor, and 1 angiomyolipoma. Twenty-seven cases were either unknown tumor type or more than 1 tumor type. Variable staining for CAIX was seen in CCRCC, PRCC and URCC. Only one chromophobe RCC showed focal, weak staining. No staining for CAIX was seen with other tumor types. High CAIX staining was seen in 71% of CCRCC compared to 7% of all other tumors. There was an association between CAIX positivity (high vs. low) and tumor type (CCRCC versus other) when all cases were considered ($p<0.01$), as well as primary ($p<0.01$) and metastatic ($p=0.025$) cases analyzed separately. In CCRCC, there was a correlation between CAIX and grade ($p<0.01$); more grade 1(92%), 2(85%) and 3(76%) tumors expressed CAIX than grade 4(42%) tumors. There was no statistically significant association between CAIX and grade of PRCC ($p=0.282$) or between CAIX and type of PRCC ($p=1.00$).

Conclusions: CAIX expression is more common in CCRCC than other tumor types and is associated with grade. CAIX may be a useful marker to distinguish CCRCC from chromophobe RCC. Although PRCC may express CAIX, there was no association with grade or type 1 or type 2 PRCC.

769 Trend of Radical Prostatectomy and Clinical Pathologic Features of Prostate Cancer: Experience from a Single Tertiary Hospital in the Last Ten Years

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Background: Radical prostatectomy (RP) is standard treatment for clinically localized prostate cancer and has been proven to provide excellent long-term cancer control in patients with organ confined disease (pT2). Widespread use of PSA screening and biopsy, availability of predictive models and advances in surgical techniques, particularly robotic-assisted laparoscopic procedure has resulted in significant changes in the surgical management of prostate cancer. This study summarizes the trend of radical prostatectomies and clinicopathologic features of prostate cancer in a single hospital in the last ten years.

Design: We reviewed the clinicopathologic data of RP at one tertiary hospital from 1999 to 2008. The cases were divided into three periods: 1999-2002, 2003-2005, and 2006-2008. Type of prostatectomy, patient age, tumor stage (based on 2002 AJCC staging manual), surgical margin, and status of nodal dissection were obtained from hospital records and pathology reports.

Results: Of the 2828 patients with prostate cancer treated by RP, the patient mean age was 60.4 years. Data on tumor pathologic stage, nodal dissection and status, surgical margin, seminal vesical invasion, and type of procedure divided by three periods were summarized in Table 1.

Table 1. Trend of Radical Prostatetomy and Pathologic Feature (1999-2008)

Years (# cases)	Mean Age	Robotic RP* (%)	≤pT2 (%)	+LN (%)	Nx* (%)	PSM* (%)	SVI* (%)
99-02 (n=985)	60.1	6 (1)	709 (72.0)	12 (1.2)	44 (4.5)	114 (11.6)	62 (6.3)
03-05 (n=811)	60.8	125 (15.4)	606 (74.7)	26 (3.2)	165 (20.3)	104 (12.8)	55 (6.8)
06-08 (n=1032)	60.4	597 (57.8)	863 (83.6)	25 (2.4)	349 (33.8)	107 (10.4)	66 (6.4)

*RP-Radical Prostatectomy; LN-lymph node; Nx-no nodal dissection; PSM-positive surgical margin; SVI-seminal vesical invasion

Conclusions: *da Vinci* robotic-assisted laparoscopic RP has been rapidly adopted as the dominant surgical technique for the surgical treatment of prostate cancer. There is continued trend of more localized prostate cancer treated by RP. Less and less patients undergo nodal dissection as a part of RP. However, positive margin rate and incidence of seminal vesicle invasion remains at a constant level. These results suggest that pathologic examination remains the key component for determining the postsurgical treatment plan.

770 Glucose Regulated Protein-78 (GRP78) Expression in Urothelial Carcinoma: A Potential Marker for Chemotherapy Resistance

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Background: The majority of patients who recur after radical cystectomy will die from their disease, even with aggressive multi-agent chemotherapy. One potentially important mechanism for chemotherapy resistance involves the induction of stress response proteins referred to as glucose-regulated proteins (GRP). GRP78, the best characterized GRP, has been implicated in tumor proliferation and anti-apoptosis that may play a key role in resistance to systemic therapies. The aim of this study was to analyze the expression pattern of GRP78 in urothelial carcinoma of the bladder in radical cystectomy specimens.

Design: From February 2003 to September 2007, 110 patients underwent radical cystectomy with pelvic lymphadenectomy for urothelial bladder cancer. A total of 19/110 (17.3%) patients presented with metastasis in the lymph nodes (LN). Tissue microarrays were prepared using a representative section of the primary tumor, benign mucosa, and lymph node (involved or uninvolved) from formalin-fixed, paraffin-embedded blocks. Anti-GRP78 antibody was used for immunohistochemical analysis using the streptavidin-biotin-peroxidase complex method. Slides were interpreted by an uropathologist, blinded to the clinical outcome, recording the intensity of GRP78 staining (0 to 3+) as well as the fraction of cells stained (<15%, 15-50%, >50%).

Results: The presence of strong staining in both tumor and LN was almost always associated with mortality on follow up (7/8 patients, 88%), while weak staining of tumor and LN was associated with mortality in 3/5 patients (60%). The weak staining in either the tumor or LN tissue was associated with mortality in only 50% of these high-risk cases.

Conclusions: To our knowledge, this represents the first report of GRP78 expression in urothelial carcinoma. Underexpression of the GRP78 in the tumor and lymph node metastasis correlated with a favorable response to surgery with adjuvant chemotherapy. This intriguing correlation shows that GRP78 may have a potential role in the pathophysiology of urothelial carcinoma, and may also be of help in the approach to treatment of these patients.

771 Prostate Cancer of Transitional Zone Lacks TMPRSS2-ERG Gene Fusion

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Background: Recent studies have revealed that the majority of prostate cancers have a unique chromosomal rearrangement leading to the TMPRSS2-ERG gene fusion. Whereas most prostate cancers develop in the peripheral zone (PZ), some arise in the transitional zone (TZ). The role of TMPRSS2-ERG gene fusion in zonal origin of prostate cancer remains unclear. Therefore, we studied TMPRSS2-ERG gene fusion in prostate cancer of different zonal origin.

Design: Radical prostatectomy specimens were obtained from 30 patients who received treatment of clinically localized prostate cancer at our institution. Every specimen contained at least two foci of the tumor, one in the PZ and the other in the TZ. The PZ and TZ tumor foci were separated by at least 4.5 mm. The TMPRSS2-ERG gene fusion was evaluated in each tumor focus by fluorescence in situ hybridization (FISH) using the ERG break-apart probes, and was related to their pathological features including zonal distribution.

Results: The average age of the patients was 58.7 years (range, 46.0-71.0 years). None of the patients had received neoadjuvant hormonal or radiation therapy. The average number of tumor foci in the specimens was 3.3 (range, 2.0-5.0). Two separate tumor foci in each specimen, one in the PZ and the other in the TZ, were selected for the analysis. The PZ tumor foci selected for the analysis had a mean Gleason score of 6.8 (range, 6.0-7.0) and a mean tumor volume of 1.2 cm^3 (range, 0.1-4.6 cm^3). The selected TZ tumor foci had a mean Gleason score of 6.7 (range, 5.0-8.0) and a mean tumor volume of 4.0 cm^3 (range, 0.5-9.0 cm^3). In all cases, the TZ tumors had a normal FISH signal pattern without evidence of rearrangement in the ERG gene. In 13 cases (43.3%), the PZ tumors demonstrated rearrangements in the ERG gene. In 10 cases, the rearrangement was associated with a deletion of the 5' end of the ERG gene. This deletion was not present in the other 3 cases.

Conclusions: The TZ prostate cancer was negative for rearrangements in the ERG gene, implicating the absence of TMPRSS2-ERG gene fusion in the prostate cancer arising in the TZ. In contrast, PZ prostate cancers frequently exhibited rearrangements in the ERG gene, suggesting that the TMPRSS2-ERG fusion play a role in the pathogenesis of prostate cancer arising in the PZ.

772 Prostate Atrophy, but Not Prostate Carcinoma, Is Characterised by a Relative Infiltration of Tregs

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Background: Regulatory T cells (Treg), a subset of CD4+ lymphocytes are thought to control tolerance to self-antigens and tumor-antigens, possibly acting by inhibiting the function of CD8+ (killer) T lymphocytes. Increased Treg have been reported in various malignancies, suggesting that Treg may facilitate tumor evasion from the immune response.

Design: The transcription factor Foxp3 is a specific immunohistochemical marker of Treg. We investigated the distribution of Foxp3+, CD4+ and CD8+ T cells using 4 tissue microarrays containing 1480 matched normal, atrophic and neoplastic prostate tissue samples from radical prostatectomies.

Results: CD4+ and CD8+ cells were increased in both atrophy and cancer compared to normal, denoting an increased inflammatory cell component in both pathologies. The CD4/CD8 ratio was inverted in normal prostate tissue. The CD4/CD8 ratio in atrophy was markedly different than in normal, PIN or cancer, approaching a more normal ratio. Foxp3+ cells were also significantly increased in atrophy. The Foxp3/CD8 ratio was increased in atrophy (mean 25.30, median 19.80), as compared to normal prostate (mean 14.43, median 9.45), but was not markedly increased in prostate cancer, contrary to what was expected. No correlation between Foxp3/CD8 ratio and Gleason score or tumor grade was found.

Conclusions: Our results contradict previous data suggesting a role for Treg in prostate tumor progression. Here, we did not observe an increased ratio of Treg/CD8 in prostate cancer. We found T cell infiltrates in the prostate gland biased towards CD8 T cells, regardless of the presence or absence of tumor. A marked elevation of Treg was found in atrophy as compared to normal, both absolute and relative to CD8+ lymphocytes. These data show that proliferative inflammatory atrophy (PIA) represents a unique immunological state, with an inversion in the "expected" CD4/CD8 ratio and a marked Treg infiltrate. This implies that PIA is characterized by an ongoing and tightly regulated immune response, a finding supported by recent data showing clonal populations of CD8 T cells in the prostate gland.

773 Comprehensive Assessment of *TMPRSS2* and *ETS* Family Molecular Aberrations in Histologic Variants of Prostate Carcinoma

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Background: Histologic variants of prostate carcinoma (PCa) account for 5 to 10% of all PCa and typically seen in association with ordinary acinar PCa. These variants often differ from latter in clinical, immunophenotypic, genetic and biologic potential. We comprehensively analyzed gene rearrangement for the ETS family member and their known 5' fusion partner TMPRSS2 in histologic variants and compared with associated acinar PCa to understand the frequency, subtype, and clonality of this common molecular event in histologic variants of PCa.

Design: A tissue microarray representing 69 cases of variant morphology (foamy gland (N=17), large duct (N=18), mucinous (N=18), small cell neuroendocrine (N=7), glomerulation (N=9)) and paired acinar morphology when present, were analyzed using break apart (TMPRSS2, ERG, ETV1, ETV4, ETV5) fluorescence in situ hybridization assays.

Results: Overall 55% of variants PCa demonstrated TMPRSS2: ETS aberrations. ERG, the most common genetic rearrangement observed in acinar PCa, was identified in 82%, 71%, 56%, 33% and 29% of mucinous, small cell, large duct, glomeruloid and foamy variant morphologies respectively (Table 1). ERG rearrangement through intronic deletion of 5'was observed exclusively (100%) in small cell and 56% of large duct PCa. Out of 54 cases that harbored paired acinar PCa, concordance of gene rearrangement between variant morphology and paired PCa was observed in 91% of cases.

Conclusions: Our result suggests that variant morphology represents a clonal expansion of primary acinar PCa. The frequency and spectrum of these genetic aberrations differ between variants. Exclusive ERG rearrangement through deletion observed in small cell PCa supports that 5' ERG rearrangement through deletion represents an aggressive molecular subtype of PCa.

Table 1. Summary of ETS rearrangment in hostologic variants of prostate carcinoma

ERG				ETV1
Histologic Variants	Rearranged	through deletion	through translocation	
foamy gland	29% (4/14)	25% (1/4)	75% (3/4)	0% (0/13)
large duct	56% (9/16)	56% (5/9)	44% (4/9)	0% (0/16)
Glomerulation	37% (3/7)	0% (0/3)	100% (3/3)	14% (1/7)
Mucinous	82% (14/17)	36% (5/14)	64% (9/14)	0% (0/16)
small cell	71% (5/7)	100% (5/5)	0% (0/5)	0% (0/7)

774 Comprehensive FISH Assessment Shows Association of PTEN Deletion with ERG Rearrangement during Prostate Cancer Development

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Background: Whereas *ERG* rearrangement and *PTEN* deletion are two of the most common genomic aberrations in human prostate cancer, little is known about the link between these two genomic events. Hence, we comprehensively evaluated a wide spectrum of benign tissues, premalignant and malignant lesions to characterize *ERG* rearrangement and *PTEN* deletion during prostate cancer carcinogenesis and progression.

Design: Fluorescence in situ hybridization (FISH) was employed to assess aberrations of *ERG* and *PTEN* in a cohort of 282 localized prostate cancer and 47 androgen-independent metastatic prostate cancer patients, along with 89 high grade prostatic intraepithelial neoplasia (HGPIN) foci and 73 non-neoplastic prostate tissues.

Results: *ERG* rearrangement was present in 47% of localized prostate cancer, 35%

of metastasis and 15% of *HGPIN*. *PTEN* deletion was identified in 17% of localized cancer and 54% of metasetasis, of which homozygous deletion was observed in 6% and 24%, respectively. Only 9% of HGPINs harbored *PTEN* deletion, all of which were hemizygous. Further, immunohistochemistry revealed correlation of *PTEN* aberrations with decreased *PTEN* protein expression ($p<0.05$). Notably, *PTEN* deletion is significantly associated with *ERG* rearrangement both in localized cancer ($p<0.001$) and metastasis ($p<0.05$). Concomitance of *ERG* rearrangement and *PTEN* deletion was observed in 50% (6/12) of cases in a selective cohort of HGPINs, all of which were adjacent to cancer. Of note, *ERG* aberration, but not *PTEN* deletion, was identical both in localized cancer and adjacent HGPIN. Similarly, whereas 5% (2/41) of metastatic cancers showed discordance for *PTEN* deletion across multiple sites from the same patient, all metastasis (43/43, 100%) shared the same *ERG* status across multiple sites.
Conclusions: Our study suggests that *ERG* rearrangement and *PTEN* deletion may cooperate but contribute through different ways in prostate carcinogenesis and progression. Understanding the molecular cross-talk between these two genetic events may provide insight into understanding of prostate cancer development.

775 Telomere Variability Is Common in Neoplastic Urothelium and Associated Morphologically Normal Urothelium

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Background: Telomeres are situated at the ends of chromosomes and serve to prevent aberrant chromosomal fusions and to regulate cellular senescence. Variable telomere lengths, including both abnormally short and long telomeres, have been reported in pre-invasive and invasive carcinomas and have been proposed to contribute to the genetic instability characteristic of these lesions. Urothelial carcinomas (UCCs) generally arise from well-characterized precursor lesions and are often multifocal in nature. In order to determine the role of telomere variability in UCC, we examined not only invasive carcinoma and flat urothelial carcinoma in situ (CIS), but also in adjacent morphologically normal urothelium.
Design: Telomere lengths were quantitatively assessed by the Telomere-Immunostaining Fluorescence in Situ Hybridization (TELI-FISH) technique followed by use of a custom software plugin (http://bui2.win.ad.jhu.edu/telometer/) written for the open source image analysis software program ImageJ (http://rsbweb.nih.gov/ij/). Fifteen to twenty nuclei from stromal cells and accompanying normal or lesional tissue were quantified.
Results: We examined 10 invasive UCCs, 5 matched CIS cases, 10 matched morphologically normal urothelium adjacent to UCC, and 5 cases of normal urothelium from patients with no precedent or subsequent urothelial malignancy. True normal urothelium demonstrated an average telomere length of 0.73 relative to stroma, whereas urothelium adjacent to UCC or CIS demonstrated markedly shortened telomere lengths in 3 cases (30%) of 0.35, 0.37 and 0.41. Shortened telomeres were also present in 3/5 CIS lesions (range 0.2 to 0.41) and 5/10 invasive UCCs (range 0.18 to 0.55). In contrast, 2 cases of invasive UCC demonstrated increased telomere lengths relative to associated normal stroma. The remainder of cases (1 CIS, 3 invasive UCCs) demonstrated telomere lengths within the range of normal stroma.
Conclusions: Telomere variability, predominantly telomere shortening, commonly occurs in pre-invasive and invasive UCC, supporting an early pathogenic role of telomere dysfunction in bladder cancer. Importantly, a subset of morphologically normal urothelium adjacent to in situ and invasive UCC also demonstrated shortened telomeres, suggesting telomere dysfunction as a potential key step in the earliest neoplastic transformation of the urothelium, prior to morphologically identifiable changes.

776 Bladder Diverticula Demonstrate Limited Diffuse Smoothelin Expression within the Muscularis Mucosa (MM)

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Background: Smoothelin, a recently described marker of differentiated smooth muscle, is diffusely expressed by bladder muscularis propria (MP) and only weakly/focally expressed in the MM. This differential staining has potential utility in pathologic staging in bladder cancer. Diverticula in the bladder are usually acquired and are known to predispose urothelial cancer. In this setting, it is often difficult to differentiate hyperplastic MM from MP due to the altered histo-anatomy of the bladder wall. The aim of this study was to evaluate the utility of smoothelin immunoreactivity (IR) in diagnostic urologic pathology in the setting of diverticular disease.
Design: Diverticula from 40 patients (21 benign, 19 neoplastic diverticula) were included for study. Immunohistochemistry (IHC) using smoothelin antibody (clone R4A, Abcam) at a 1:150 dilution and was scored as 0 (no expression), 1+ (moderate expression <10% of cells), 2+ expression (moderate expression in >10% of cells), and 3+ (robust diffuse expression). Results were correlated with clinicopathologic features. All diverticula contained MM of varying caliber. Normal MP was included as a positive control. Staining in diverticular MM was compared with historic results in MM of bladder from cystectomy specimens.
Results: Mean patient age was 63 years, with a higher age in malignant (mean 65 years) versus benign (mean 43 years) diverticula (Student's t-test, $p=0.002$). There was overlap in morphology between hypertropic MM of diverticulae with MP muscle bundles. Smoothelin IR in the diverticular MM included 0 (16/40;40%), 1+ (11/40;27.5%), 2+ (12/40;30%) and 3+ (1/40;2.5%). Control cystectomy specimens demonstrated 3+ MP IR, whereas MM staining was 0(55%), 1+(12%), 2+(13%) and 3+(0%). Comparison between neoplastic and benign diverticula showed no difference between 2-3+ IR (Fisher's exact test $p=0.17$). No correlation was evident with smoothelin IHC and muscle caliber.
Conclusions: Bladder diverticula with/without cancer show frequent hypertropic MM that may morphologically mimic MP. Smoothelin confirms the paucity of classic MP and predominance of atypical MM patterns in diverticula resected for cancer. Criteria for pathologic staging of urothelial carcinoma in cystectomy specimens are not applicable in cancers arising in diverticula due to the altered structure of the bladder wall. Caution is warranted in interpreting smoothelin staining in diverticular specimens due to increased IR in MM.

777 Expression of N-Cadherin in Yolk Sac Tumor of the Testis

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Background: More than half of adult testicular germ cell tumors consist of more than one cell type. Appropriate medical management depends on accurate pathologic diagnosis and tumor classification. Yolk sac tumor (YST) is present in approximately 40 % of mixed germ cell tumors but may be difficult to recognize because it displays several microscopic patterns. In about 75 to 90% of cases α-fetoprotein can be detected in the serum or by positive staining in the tumor. The focal positivity can make it difficult to identify and differentiate the YST component. As such, additional markers of endoderm differentiation would be beneficial for the diagnosis and classification of these tumors. N-cadherin is a member of the cadherin gene family that encodes the N-cadherin protein, which mediates cell adhesion. Aberrant expression of N-cadherin by cancer cells, e.g. prostate cancer, can contribute to local invasion and metastasis. The purpose of this study was to determine the presence and distribution of this protein in germ cell tumors.
Design: Thirteen mixed germ cell tumors containing YST components were stained for N-cadherin utilizing immunohistochemistry. The sections were incubated with anti-N-Cadherin mouse monoclonal antibody (Dako North America, Inc., CA, USA) at a dilution of 1:120 for 1 hour, followed by 30 minutes in biotinylated horse antimouse (Vecto, Burlingham, CA) at a dilution of 1:400, and ABC (Vector, Burlingham, CA) Vector VIP was used as chromogen.
Results: All YST components stained positive for N-cadherin. Ten of the 13 were extensively positive, 2 were 3+ positive and 1 tumor had only weak positivity. In contrast, of the 12 tumors containing teratomas, 11 were weakly positive for N-cadherin and 1 was negative. Twelve tumors contained embryonal carcinomas none of which expressed N-cadherin. Eight of the mixed germ cell tumors contained syncytiotrophoblasts cells, none of which stained positive for N-cadherin. Similarly, none of the 6 areas of seminoma expressed N-cadherin.
Conclusions: We conclude that N-cadherin is a useful marker for YST and provides a novel method of identifying the presence of YST component within mixed germ cell tumors, allowing differentiation from seminomas, embryonal carcinomas, and teratoma.

778 Can Apical Prostatic Adenocarcinoma at Biopsy Stratify Patients for Ablative Therapies?

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Background: Ablative therapies that rely on urethral protective methods have been used as alternatives to surgical treatment of prostatic adenocarcinoma. These protective methods may result in inadequate treatment of tumor located in the periurethral region. It is known that the apex is the most common location of recurrence. This study was designed to identify if the presence of apical involvement on prostatic biopsies can be used to predict tumor in the periurethral area.
Design: Eighty-nine consecutive radical prostatectomy specimens with corresponding data of pre-resection biopsies from February 2007 to August 2008 were studied. Right and left central sagitally-sectioned whole mount slides from each case, including the entire length of the prostatic urethra from apex to base, were reviewed to determine the distance from the urethra to the nearest cancer. The urethra-cancer distance was correlated with tumor location and percentage of core involvement in the corresponding biopsies.
Results: The majority of the cases (69.7%, 62/89) showed apical involvement in the biopsy, which correlated significantly with the prostatectomy findings ($p<0.001$). The presence of apical tumor alone, when compared to non-apical involvement in the biopsy, did not correlate with periurethral tumor (urethra-cancer distance ≤ 3 mm) in the prostatectomies. However, the amount of tumor at the apex in the biopsy (10% or greater of the core) did correlate with the frequency of periurethral tumor when compared with those cases demonstrating less than 10% and non-apical involvement (65.9% vs. 37.5%; $p=0.007$). The presence of bilateral apical tumor involvement also correlates with the frequency of periurethral tumor in the prostatectomy specimens (76.9% vs. 46.1%; $p<0.05$). In addition, the highest frequency of periurethral tumor was obtained in those biopsy cases that demonstrated bilateral apical involvement with tumor extent of 10% or greater (90.9% vs. 44.9%; $p=0.004$).
Conclusions: 1. Prostate biopsy information can be used to predict periurethral tumor location in the prostatectomy specimen. 2. Apical involvement, when combined with tumor extent and bilaterality, exhibits the highest frequency of periurethral involvement. 3. Patients with above characteristics on prostate biopsies may not benefit from ablative therapy.

779 The Potential Value of a Simple Two-Level Grading System for Renal Cell Carcinomas

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Background: The Fuhrman nuclear grade (FG) is the most widely used prognostic factor for renal cell carcinoma (RCC). The interobserver reproducibility of this system however, is relatively low particularly between grades II and III and occasionally between grades III and IV. In this study we investigated the potential value of a simplified 2-level grading system (2G), low and high-grade, in predicting survival and metastatic behavior in the two most common types of RCC, conventional and papillary.

Design: Archival nephrectomy specimen slides from 104 patients with the diagnosis of conventional and papillary RCC were reviewed by 2 pathologists independently. We reassessed Fuhrman nuclear grade (FG) and pathological stage. We grouped tumors of FG I and II as low-grade and FG III and IV as high. Clinical follow-up was available for all cases. Fisher's exact test was used to examine associations between variables. Overall survival was estimated by Kaplan-Meier method and the effect of prognostic factors was examined by log-rank test and Cox proportional hazards models.
Results: Of the 104 cases of RCC, 81 were classified as conventional and 23 as papillary type. Forty-five cases (43%) were stage 1, 26 (25%) stage 2, 21 (20%) stage 3 and 12 (12%) stage 4. Seventy-nine (75%) patients were alive after a median time of 4 years, and 25 (24%) died of disease, with a median time to event of 2.3 years. FG I tumors accounted for 24 (23%), II 49 (47%), III 25 (24%) and IV 6 (6%). After grouping the cases using the 2G system, low-grade tumors accounted for 70% (73) and high-grade neoplasms for 30% (31). Both, the classic FG and the 2G, provide similar significant information regarding metastatic behavior and overall survival ($p<0.05$). When using the 2G, patients with high-grade neoplasms had a 2.9 fold increased risk of death as compared with patients with low-nuclear grade tumors ($p=0.008$). The agreement between the two pathologists was 73% for FG and 96% for 2G system.
Conclusions: The 2G system in RCC is a predictor of metastatic behavior and overall survival similar to the classic FG system. The use of the 2G system, however, is simple and may result in a better interobserver reproducibility.

780 Lymphatic Space Invasion Predicts Metastatic Behavior in Patients with Renal Cell Carcinoma, and It May Be an Independent Prognostic Factor for Overall Survival

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Background: The biological behavior and clinical outcome of renal cell carcinoma (RCC) are difficult to predict. Detection of lymphovascular invasion (LVI) has been associated with tumor recurrence and disease progression. The aim of this study is to evaluate if LVI in nephrectomy specimens is an independent prognostic factor for overall survival in RCC.
Design: Archival nephrectomy specimens slides collected over six years from patients with available clinical follow-up and vital status were reviewed. Two pathologists assessed tumor type, Fuhrman nuclear grade (FG), pathologic stage and the presence or absence of LVI. Fisher's exact test was used to examine associations between variables. Overall survival was estimated by Kaplan-Meier method and the effect of prognostic factors was examined by log-rank test and Cox proportional hazards models.
Results: A total of 104 cases (81 conventional type RCC, and 23 papillary type RCC) were analyzed. Male to female ratio was 2:1. Median age was 61 years old. Forty-five cases (43%) were stage 1, 26 (25%) stage 2, 21 (20%) stage 3 and 12 (12%) stage 4. Seventy-nine (75%) patients were alive after a median time of 4 years, and 25 (24%) died of disease, with a median time to event of 2.3 years. LVI was present on 34 (32%) of all cases examined. Twenty-seven of these cases were conventional type RCC and 7 were papillary type RCC. LVI was associated to FG III and IV tumors ($p=0.001$). LVI was more frequently found in advanced stage tumors ($p= <0.0001$). Twenty-five patients (24%) had documented metastasis. Twenty-three (67 %) had histological evidence of LVI ($p<0.0001$). In univariate analysis, LVI increased risk of death by 2.85 fold and this effect was statistically significant ($p=0.009$). However LVI effect on survival was reduced and not statistically significant after adjustment for stage (HR=1.66; $p=0.238$).
Conclusions: LVI is an important predictor of metastatic behavior in patients with RCC and it may be an independent prognostic factor for overall survival. LVI was associated to high FG and advanced pathologic stage. There is no association between tumor type and presence of LVI.

781 Association and Significance of Myc, Estrogen Receptor, and Metastasis-Associated Gene 1 (MTA1) Expression in Advanced Prostate Cancer

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Background: Overexpression of Myc and MTA1 is observed in many cancers. Myc is amplified in advanced prostate cancer (PCa) but there is no association between amplification and expression. Recent reports describe that Myc expression may be regulated by estrogen receptor (ER) and that MTA1 is a downstream effector of Myc. In the current study we analyzed protein expression of ER, Myc, and MTA1 in 114 clinically localized and advanced PCa cases to determine their association with each other and its significance for PCa progression.
Design: ER, Myc and MTA1 expression levels were evaluated on a scale of 1-4 by immunohistochemistry (IHC) on TMAs. We also determined ERG rearrangement for a subset of cases with FISH to identify its effect on this pathway.
Results: Mean patient age was 65 yrs (48-77), mean pre-operative PSA level 29.3 ng/ml (1-248), max follow-up time 154 months (mean 31.4). PSA recurrence occurred in 42/114 patients (37%), 21/114 (18%) were stage pT2 and 83/114 pT3 (82%), 77/114 (68%) were pN+ and 37/114 (32%) pN0. Gleason scores of 4-6 was found in 15/114 (13%) patients, score of 7 in 35/114 (31%), score of 8-10 in 64/114 (56%). Myc and MTA1 expression were significantly elevated in lymph node metastases compared to primary PCa ($p<0.000$ each, Chi-Square test), no difference was seen for ER. Expression levels of ER, Myc, and MTA1 were significantly associated with each other (ER-Myc ($r=0.35$, $p<0.000$), Myc-MTA1 ($r=0.411$, $p<0.000$), ER-MTA1 ($r=0.263$, $p=0.008$). Myc expression was also associated with Gleason score ($R=0.209$, $p=0.028$). Cox regression analysis revealed both Myc and MTA1 expression to be predictors of PSA recurrence (Myc: HR 1.51 (1.04-2.19), $p=0.032$; MTA1: HR 1.39 (1.02-1.89), $p=0.036$). No difference in Myc and MTA1 expression depending on ERG rearrangement was observed, but high Myc expression was a greater risk factor for PSA recurrence in ERG rearranged PCa (HR: 2.65 (1.1-6.4), $p=0.031$).
Conclusions: The association between ER, Myc and MTA1 expression suggest that these proteins are components of one progression pathway in prostate cancer. Myc and MTA1 expression are significantly associated with PSA recurrence and development of lymph node metastases and this appears to be accentuated in ERG rearranged cancers. We are currently expanding our analysis to functional assays in cell culture.

782 Differential Expression of Dicer in Oncocytomas, Papillary, Clear Cell and Chromophobe Renal Cell Carcinomas

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Background: MicroRNAs (miRNAs) are short 20–22 nucleotides noncoding RNA molecules that negatively regulate gene expression at the posttranscriptional level. Dicer is a cytoplasmic RNAse III endonuclease involved in the production of mature miRNAs from longer primary transcripts. Dicer was shown to be up regulated in noninvasive precursor lesions of the lung and down regulated in the advanced adenocarcinoma of the lung. Although in prostate adenocarcinoma up regulation of Dicer was seen. In the present study, we evaluated the immunohistochemical expression of Dicer in tissue microarrays (TMAs) of the various kidney tumors.
Design: The TMA set consisted of 163 kidney tissue specimens arrayed onto slides in quadruplicate. Formalin-fixed paraffin-embedded blocks of renal clear cell carcinoma (56 cases), papillary carcinoma (25 cases), chromophobe carcinoma (22 cases) and oncocytoma (60 cases) were selected and marked after microscopic examination of the corresponding hematoxylin and eosin stained slides. One-millimeter cores were extracted from the marked areas and arrayed onto four recipient blocks. Immunohistochemistry was performed using anti-DICER1 antibodies. The non-neoplastic kidney adjacent to the areas of tumor served as internal positive control. The intensity of staining was scored from 0-3 (0= no staining, 1=weak, 2=moderate and 3=strong).
Results: A total number of 163 cases of kidney tissue were analyzed for immunoreactivity. Among these 82 cases of non-neoplastic kidney adjacent to the tumor showed an average immunoreactivity of 1.86 (range of 0-3) and 60 cases of oncocytomas showed an average intensity of 1.58 (range of 0-3). The different types of renal cell carcinomas showed down regulation of Dicer. The highest intensity was noted in papillary carcinomas (25 cases) with average intensity of 1.2 (range of 0-3), followed by chromophobe (22 cases) with an average intensity of 0.38 (range of 0-2) and clear cell carcinoma (56 cases) with an average intensity of 0.34 (range of 0-2).
Conclusions: Down regulation of Dicer was seen in malignant tumors of kidney compared to the nonneoplastic kidney and oncocytoma which is a benign tumor. It suggests that Dicer/miRNA might act as tumor suppressor. To our knowledge this is the first study analyzing the expression of Dicer in the kidney tumors. The Dicer/miRNA expression might be used to classify various renal tumors, to predict the prognosis and may form targets for future miRNA based cancer therapeutics.

783 Increased Lef1 Expression in Metastatic Prostate Cancer

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Background: The identification of reliable biomarkers of prognosis in prostate cancer is a crucial issue to optimize the rational use of treatment modalities. Recent studies indicated that lymphoid enhancer-binding factor 1 (Lef1) regulated the expression of androgen receptor which played an important role in enhancing the growth and invasion abilities of the prostate cancer cells. In this study, we assessed Lef1 expression in human metastatic prostate cancer and its role in prostate cancer invasion.
Design: Immunohistochemical expression of Lef1 was examined in 20 cases of benign prostatic tissue, 56 cases of primary androgen-dependent prostate cancer and 10 cases of metastatic prostate cancer. The percentage of cells stained (0-100%) was recorded. Statistic analysis was performed using Fisher Exact Test. The invasion ability of LNCaP cells overexpressing Lef1 was determined by Matrigel invasion assays.
Results: No Lef1 expression was identified in all benign prostatic specimens except for some scattered nuclear staining in the basal cells in certain cases. Lef1 was expressed in 9 of 56 (16%) cases of primary androgen-dependent prostate cancer with percentage of positive tumor cells ranging from 20% to 60%. Lef1 was expressed in 5 of 10 (50%) cases of metastatic prostate cancer with percentage of positive tumor cells ranging up to 60%. The differences of Lef1 expression between metastatic prostate cancer and primary androgen-dependent prostate cancer, and between metastatic prostate cancer and benign prostatic tissue were statistically significant ($p=0.0289$). In parallel to its increased expression in metastatic cancer, Lef1 also increased LNCaP cell invasion up to 2.5 folds in Matrigel invasion assays.
Conclusions: Our data demonstrate for the first time that increased Lef1 expression is associated with metastatic prostate cancer and that Lef1 may be used as a potential biomarker for metastatic and advanced prostate carcinomas.

784 TMPRSS2 Protein Expression in Human Prostate Cancer: A Tissue Microarray and Automated Quantitative Analysis

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Background: TMPRSS2 is a type II transmembrane-bound serine protease that has gained interest owing to recent discovery of TMPRSS2-ERG gene fusions identified in the majority of prostate cancer and its highly localized expression in the prostate. Some studies suggest that TMPRSS2 protein is regulated by androgen and is overexpressed in prostate cancer. However, limited quantitative data are available on TMPRSS2 protein in human prostate tissue.
Design: To validate the role of TMPRSS2 in prostate cancer and its progression, a prostate cancer tissue microarray (TMA) with duplicate cores was constructed. The

TMA includes 41 localized prostate cancers (Pca_local), 18 aggressive prostate cancers (Pca_aggr), 18 metastatic prostate cancers (Met), 24 benign prostate hyperplasia (BPH), 18 high grade intraepithelial neoplasia (HGPIN) and 41 benign prostate tissues. The cellular expression level of TMPRSS2 was quantified using automated quantitative analysis (AQUA, HistoRx, New Haven, CT)). The comparisons of TMPRSS2 expression levels between groups were preformed using one-way ANOVA (SPSS 11.5, SPSS Inc; Chicago, IL). A two-sided significance level of 0.05 was used for each statistical test.

Results:

Table 1. AQUA scores of TMPRSS2

Dx	Age (y)	GS	N	AQUA Score (mean±SE)	Sig
Benign	61.3		80	185±18	
BPH	67.8		39	316±42	0.014*
HGPIN	62.9		36	249±25	0.68
PCa_local	58.2	6-9 (7)	72	302±25	0.006*
PCa_aggr	62.2	7-8 (8)	29	366±60	0.001*
Met	67.6		24	242±40	0.879

GS, Gleason score; N, core number analyzed; Sig, significance level

Conclusions: The expression levels of TMPRSS2 protein were higher in proliferative prostate diseases compared to benign prostatic tissue, and significantly higher in localized and aggressive prostate cancers. However, the elevation of TMPRSS2 protein expression in metastatic cancer was not significant compared to that in benign prostatic tissue.

785 Immunohistochemichal Analysis of Hypoxia-Inducible Protein 2 (HIG2), KSP-Cadherin and Carbonic Anhydrase IX (CAIX) Expression in Papillary, Clear Cell and Chromophobe Renal Cell Carcinoma

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Background: Owing to overlapping morphologic features subset of renal cell carcinomas (RCC) cannot be classified into the major histologic types. There is a need for additional markers that would allow more accurate characterization of such tumors. HIG2 is expressed in fetal kidney and renal cortical carcinomas, KSP-Cadherin is found in basolateral membrane of renal tubular epithelium and colleting ducts as well as in chromophobe RCC and a subset of oncocytomas whereas CAIX expression was noted in clear cell RCC.

Design: We have performed immunohistochemistry for HIG2 (Novocastra, UK), CAIX (Novocastra, UK) and KSP-Cadherin (Cell Marque, USA) on tissue microarrays of 37 chromophobe RCC, 44 papillary RCC and 42 clear cell RCC that also contained 17 different normal tissues. Staining was evaluated semiquantitativly using a scoring system of 0-3+ for intensity and 1+ - 3+ for extent (<25%, 25-50%, >50%).

Results: CAIX positivity was seen in 39 of 41 (95%) clear cell RCC, in 42 of 44 (95%) papillary RCC and 0 of 37 (0%) chromophobe RCC. HIG2 expression was present in 33 of 42 (79%) clear cell RCC, 34 of 44 (77%) papillary RCC, and 8 of 37 (22%) chromophobe RCC. KSP-cadherin positivity was detected in 7 of 41 (17%) clear cell RCC, 38 of 44 (86%) papillary RCC and 31 of 35 (89%) of chromophobe RCC. In normal tissues, KSP-cadherin was only seen in kidney (tubules and collecting ducts), whereas weak HIG2 positivity was seen in liver, placenta, panceas, and in gallbladder and jejunal mucosa. Membranous CAIX staining was noted in gastric, gallbladder and small bowel mucosa, bile duct epithelium and skin.

Conclusions: The KSP-cadherin positive, HIG2 and CAIX negative phenotype was only seen in chromophobe tumors. On the other hand, a CAIX and HIG2 positive but KSP-cadherin negative phenotype strongly favors clear cell RCC. In addition, KSP-cadherin and HIG2 appear to be candidate markers for renal cell origin based on their staining pattern of benign tissues and renal cortical carcinomas.

786 PAX8 Expression in Clear Cell, Papillary and Chromphobe RCC and Urothelial Carcinoma of Renal Pelvis

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Background: PAX8 is a novel cell lineage restricted transcription factor expressed by renal tubules. The current study evaluated PAX8 immunoexpression pattern in primary and metastatic renal cell carcinomas (RCC) as well as in upper urinary tract urothelial carcinomas primary to renal pelvis (UT-UCa).

Design: PAX8 IHC staining was performed on Tissue microarray (TMA) constructed from 144 primary and 40 metastatic archival RCC specimens (2004-2006). Primary RCC tumors included 68 clear cell (cRCC), 45 papillary (papRCC) and 31 chromophobe (chRCC) cases. All metastatic RCC were of clear cell type. PAX8 IHC was also performed on 11 primary renal pelvis urothelial carcinoma (UT-UCa) using routine sections. IHC was performed using Bond-Leica automated stainer and a monoclonal Ab (Protein tech group, Inc.; IL). Nuclear staining intensity was assigned an incremental 0, 1+, 2+, 3+ score. Extent of staining was categorized as focal (<25% of cells), multifocal (25-75%) or diffuse (>75%).

Results: Primary RCC: PAX8 was most frequently expressed in papRCC (43/45; 96%) followed by cRCC (44/68; 65%) then chRCC (17/31; 55%). The difference in expression rates among histologic types was statistically significant (p=0.0001). Variability in staining intensity and extent was encountered from case to case in each of the three histologic types. However, PAX8 staining was generally multifocal to diffuse in nature in all three types and was focal in extent (<25% of cells) in only a minority of cases: 4/44 (9%) of positive cRCC, 1/43 (2%) of positive papRCC and 6/31 (19%) of positive chRCC. We found no correlation between Fuhrman grade and rate of PAX8 positivity, extent or intensity of PAX8 positivity in either cRCC or papRCC (p:NS). **Metastatic cRCC:** PAX8 expression was detected in 33/40 (83%) of metastatic cRCC. Extent of expression was focal in only 2/40 (5%) of cases. The difference in rate of expression between primary and metastatic cRCC in our cohort was statistically significant (p: 0.05). **Renal Pelvis UCa:** None of the 11 UT-UCa were positive for PAX8.

Conclusions: PAX8 is expressed by the three most common types of RCC. Expression rate is significantly different among RCC types and is lowest in ChRCC and highest in papRCC and cRCC. In positive cases, PAX8 expression is multifocal to diffuse in the majority of cases potentially increasing its utility in needle bx setting. PAX8 was negative in UT-UCa cases suggesting its potential utility in distinguishing RCC from UT-UCa in difficult needle bx of a renal mass.

787 Neuroendocrine, Proliferative and Apoptotic Markers in Prostatic Adenocarcinoma and Small Cell Carcinoma of the Prostate: A Tissue Microarray Analysis of 64 Cases

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Background: Prostate cancer (PCa) is the most common non-cutaneous malignancy and third leading cause of cancer-related deaths in men. Selection of the best treatment protocol for the individual patient, is challenging and markers which may predict disease outcomes are not routinely utilized. We studied the expression of neuroendocrine, proliferative and apoptotic markers in prostatic adenocarcinoma and small cell carcinoma of the prostate.

Design: Sixty patients treated with radical prostatectomy or transurethral resection of prostate with or without neoadjuvant chemoradiation therapy from 1996 - 2007, for localized PCa, were studied. The patient population was equally divided in to quartiles based on Gleason scores (3+3=6, 3+4=7, 4+4 =8 and 4+5=9). Four additional patients with primary small cell carcinoma of the prostate were included in the study. Immunohistochemical stains were performed for synaptophysin (SYN), chromogranin (CHR), CD56, ki-67, p53 and activated caspase on tissue microarray.

Results:

	Gleason score 3+3=6 (15) 3+4=7 (15)	Gleason score 4+4=8 (15) 4+5=9 (15)	Small cell carcinoma (4)
Neuroendocrine markers SYN & CHR no(%)	0(0)	16(53)	4(100)
Ki-67 labeling index (mean %)	1	20	80
p-53 mutation (mean %)	3(10)	10(33)	4(100)
Activated caspase 3 no(%)	0(0)	0(0)	0(0)
Clinicopathologic characteristics in SYN & CHR +ve tumors	Number(%)	Number(%)	Number(%)
Lymph node metastases	0(0)	9(56)	4(100)
Angiolymphatic invasion	0(0)	9(56)	4(100)
Bone metastases	0(0)	2(13)	NA
Extra prostatic extension	0(0)	2(13)	NA
PSA/biochemical failure post chemoradiation	0(0)	3(19)	NA

Conclusions: This study demonstrates that the presence of SYN, CHR and CD56 expression along with p-53 mutation and high Ki-67 labeling indices correlates directly with high Gleason score, small cell histopathology and clinicopathologic parameters of progression. Neuroendocrine marker expression may be helpful in predicting treatment failure in PCa. Studies of aggressive therapy targeted at neuroendocrine differentiation in PCa are suggested.

788 PAX-8 Is a Specific Marker for Renal Neoplasms. Comparison with PAX-2, Renal Cell Carcinoma Marker Antigen (RCCM) and Kidney Specific Cadherin (KSP)

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Background: The current diagnostic immunomarkers for renal neoplasms (RNs) are limited. RCCM and KSP are terminally differentiated molecules expressed by proximal and distal tubules, respectively, and thus are markers only for subsets of RNs. PAX-2, a transcription factor promoting renal differentiation, is a sensitive RN marker. PAX-8 is another transcription factor that co-ordinates with PAX-2 in renal differentiation. Its role as a RN marker is not known.

Design: 51 RNs including 32 clear cell RCCs, 7 papillary RCCs, 7 chromophobe RCCs, and 5 oncocytomas were subjected to immunostain for PAX-8. Consecutive tumor tissue sections were also stained for PAX-2, RCCM, and KSP. The staining was quantified in terms of intensity and extent, and was compared among the four markers.

Results: PAX-8 expression with exclusive nuclear staining was successfully identified in formalin-fixed, paraffin-embedded tissue. In non-neoplastic kidney tissue, PAX-8 was identified a portion of normal collecting duct cells, and atrophic tubular cells regardless of origin. PAX-8 was also expressed in the vast majority of RNs (96%), regardless of histologic subtypes or differentiation, and was the most sensitive RN markers among those evaluated in table 1.

	PAX8 %	PAX2 (%)	RCCM (%)	KSC (%)
Clear cell (n=32)	100	91	81	34
Papillary (n=7)	100	100	100	0
Chromophobe (n=7)	86	86	0	100
Oncocytoma (n=5)	80	100	0	80
Total (n=51)	96	92	65	43

PAX-8, like PAX-2, was expressed by virtually all tumor cells in positive cases, in contrast to much smaller percentage of tumor cells expressed by RCCM or KSC.

Conclusions: PAX-8 can be reliably detected in archival tissue. Among the evaluated RN markers, it is the most sensitive. Since its staining is both widespread and independent of tumor grade and type of RNs, PAX-8 appears to be of important diagnostic utility for small tumor samples and determining renal origin of unknown primary tumors. Because PAX-8 constantly stains atrophic tubular cells regardless of origin, it may play an important pathogenic role in tubular atrophy.

789 Expression of the Notch Family of Proteins in Prostatic Adenocarcinomas (PACs): Notch2 Signaling Is Associated with High Tumor Grade, Advanced Stage and Biochemical Disease Recurrence

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Background: Notch signaling is associated with cell differentiation and proliferation in experimental cancer models. Aberrant Notch signaling has been documented in prostate cancer xenografts. However, the prognostic significance of Notch signaling in human PACs has not been elucidated.

Design: Formalin-fixed, paraffin embedded sections from 143 PACs were immunostained by automated methods (Ventana) with antibodies to Notch1, Notch2, Notch3 and Notch4 (Santa Cruz). Cytoplasmic immunoreactivity for each protein was scored based on intensity and percentage positive cells in both tumor and adjacent benign epithelium. A multiplicative index was calculated and cases assessed as tumor=benign (T=B), tumor>benign (T>B) and tumor<benign (T<B). Results were correlated with clinicopathologic variables.

Results: Variable cytoplasmic immunoreactivity was noted in adjacent benign glands for all 4 Notch proteins in all cases. Tumor immunoreactivity was predominantly cytoplasmic for all 4 proteins, while rare nuclear positivity was observed for Notch1 and Notch4. Notch protein expression was noted as: Notch1 [T=B 88%, T>B 11%, T<B 1%]; Notch2 [T=B 24%, T>B 76%]; Notch3 [T=B 13%, T>B 87%]; Notch 4 [T=B 38%, T>B 57%, T<B 5%]. Increased Notch1 expression correlated with high grade [16% HG vs 7% LG, p=0.05]; while nuclear expression was noted in 8 cases and correlated with HG [13% HG vs 0% LG, p=0.001]. Increased Notch2 expression correlated with HG [90% HG vs 67% LG, p=0.001], advanced stage [86% advanced vs 67% early, p=0.014], and biochemical disease recurrence [87% recur vs 68% non-recur, p=0.008]. While Notch3 immunoreactivity was increased in a majority of cases and basal cell expression was observed, there were no significant correlations. Overall Notch4 dysregulation [T>B + T<B] correlated with HG (p=0.028); while nuclear expression was noted in 5 cases and correlated with advanced stage (p=0.026). On multivariate analysis, advanced stage (p=0.001) and increased Notch2 expression (p=0.05) independently predicted biochemical disease recurrence.

Conclusions: Aberrant Notch signaling is associated with disease aggressiveness in PAC with Notch2 overexpression independently predicting disease recurrence. Further study of Notch protein expression in PAC as prognostic factors and targets of therapy appears warranted.

790 Signature Markers of Renal Epithelial Neoplasms by Hierarchical Clustering Analysis

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Background: Distinguishing different types of renal epithelial tumors is important for clinical management; however, sometimes it is difficult to discriminate by histology alone. Many immunohistochemical markers for renal epithelial tumors have been reported for tumor subclassification. A few have been reported to be differentially expressed in different subtypes of renal cell carcinoma (RCC), but a single marker is not sensitive or specific enough to discriminate each subtype. In this study, we hypothesized that a panel of antibodies would enhance the ability of pathologists to make more accurate diagnoses of renal tumors.

Design: Tissue microarray sections of renal tumors, including 32 clear cell RCCs (ccRCC), 25 papillary RCCs (papRCC), 15 chromophobe RCCs (chRCC), & 15 oncocytomas, & 6 normal kidneys, were stained with the following panel of markers that have been previously reported to show relative specificity for certain renal tumors: glutathione S-transferase α (GST-α), carbonic anhydrase 9 (CA9), alpha-methylacyl-CoA racemase (AMACR), vimentin, C-kit, & cytokeratin 7 (CK7). Hierarchical cluster analysis was performed on immunostained sections based on the staining intensity scanned from ChromoVision Automatic Cellular Imaging System. Pearson correlation and average linkage were used to generate protein and sample trees using software originally designed for analyzing gene microarray data. A dendrogram and Treeview were generated by an unsupervised cluster analysis to establish tumor signatures of selected markers. The proximity of the immunostaining levels in each tumor can be visualized.

Results: A Treeview illustrated three clusters: ccRCC, papRCC, & chRCC with oncocytomas. The majority of the ccRCCs clustered together (31/32); 12 of 25 papRCCs clustered together. The majority of the chRCCs clustered together (10/15), & the remaining ones clustered with oncocytomas. This is not unexpected as chRCCs & oncocytomas are genetically related tumors, & a good marker to differentiate them has not been fully characterized.

Conclusions: We illustrated that a panel of 6 immunohistochemical stains can be used to establish marker signatures to differentiate the most common subtypes of renal epithelial tumors. Using hierarchical clustering, we separated the distinct clusters of renal neoplasms. This method of using marker signatures provides an objective measure of a renal tumor and may assist surgical pathologists in making more accurate diagnoses. Further study is needed to better assess the clinical utility of this panel of markers.

791 Renal Angiomyolipoma in the First Two Decades of Life: A Clinicopathologic Study of 44 Cases

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Background: Angiomyolipoma (AML) is derived from the perivascular epithelioid cells (PEC) and part of the PEComa family. AML occur both sporadically and in association with tuberous sclerosis (TS). Although most AML are discovered and diagnosed in adults, this series reviews the clinicopathologic features of renal AML in the first two decades.

Design: 66 cases of PEComa family tumors in patients, <21 years of age, were pulled from our files. The study was confined to renal AML with available material.

Results: 44 pediatric renal AML were included. Patient ages ranged from 8-20 years (mean age 16). 42% were male, 58% female. Of patients with known clinical history, 38% had a definite or suspected diagnosis of TS. Four patients presented with retroperitoneal hemorrhage, three with flank pain. AML had a nearly equal predilection for kidney side. Tumor sizes ranged from 1.8 cm- 25 cm, mean 11.6 cm. Microscopically, the main mass separable from surrounding renal parenchyma, and predominantly (n=32) spindled smooth muscle rather than epithelioid . The smooth muscle component compared with fat ranged from 10% to nearly 100%, with more than half composed of >90% smooth muscle. Findings included necrosis (n=11), pleomorphism (n=15), regional lymph node involvement (n=3), coexisting oncocytic neoplasm (n=1), coexisting renal cell carcinoma (n=1), hemorrhage (n=15), prominent collagen (n=10), hemangiopericytoma-like vascular pattern (n=7), multinucleation (n=8), myxoid myointimal proliferation (n=8), prominent nucleoli (n=5), increased cellularity (n=6), and nuclear pseudoinclusions (n=2). None had easily identified mitotic activity. Multifocal angiomyolipoma (n=17) and microscopic cysts mostly corresponded to known TS patients. Concurrent extrarenal tumors included AML of adrenal gland (n=1), lymphangioleiomyomatosis of lung (n=1), and PEComa of unknown site (n=1). All cases studied were positive for HMB45, actin, and desmin33. There were no known metastases.

Conclusions: Pediatric myomelanocytic tumors are mostly renal AML that occur in teenagers with equal sex distribution, 38% associated with TS. The latter patients often have multifocal AML. Similar to adults, these tumors can be symptomatic with hemorrhage or flank pain or discovered incidentally. Pediatric renal AML are mostly composed of spindled smooth muscle, with little fat or epithelioid change. Necrosis, atypia, multifocality or lymph node involvement do not necessarily indicate malignancy.

792 Distribution of Smoothelin Expression in the Musculature of the Genitourinary Tract

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Background: Smoothelin is a novel smooth muscle (SM)-specific marker expressed strongly in terminally differentiated SM cells with absent to limited expression in non-contractile SM and myofibroblastic cells. It has been suggested that smoothelin expression may help distinguish bladder muscularis propria (MP) from muscularis mucosae (MM), with implications for cancer staging. However, smoothelin expression in various smooth muscle components of the GU tract has not been well studied.

Design: Normal bladder, ureter, bladder neck, prostate, urachal remnant, and bladder diverticulae tissues were identified and sections were stained with antibody to smoothelin (1:500; mAb, Abcam Inc, Cambridge, MA). Immunohistochemical staining was evaluated for staining intensity (0 to 3+) and % positive cells.

Results: Normal bladder [n=10]: all cases showed moderate (2+) to strong (3+) staining in 70 to 100% of cells of MP and no (0) to weak (1+), focal staining in MM. Ureter [n=5]: smoothelin staining did not identify a distinct MM. 2 to 3+ staining was observed throughout the MP, but with progressively weaker labeling from outer MP to MP closest to the lumen, corresponding to the thinning of the MP fibers on H&E. Bladder neck [n=5]: medium/large discrete SM bundles from the bladder base margin of radical prostatectomy specimens showed no labeling to 1 to 2+ staining in <25% of cells. Prostate [n=5]: although dispersed among glandular elements, the SM component of prostatic stroma consistently stained strongly (2 to 3+) and diffusely (nearly 100% of cells). Urachal remnant [n=3]: the entire musculature of the urachal remnant showed 0 to 1+ focal staining in contrast to the 3+ bladder MP, often seen in the same section. Bladder diverticulae [n=15]: two types of SM were observed in diverticular walls: thin wisps only [n=5], which were uniformly negative, and hypertrophic SM bundles accompanied by thin wisps [n=10], in which 1+ labeling was seen in 10-25% of cells in 5 cases.

Conclusions: Intensity and extent of smoothelin staining accurately distinguishes muscularis propria from muscularis mucosae in the intact urinary bladder. The immunostaining patern of the muscles in diverticular walls strongly supports their muscularis mucosae origin, even when appearing hypertrophic. Further investigation of patterns of smoothelin expression is warranted, as its variable positivity in GU tract smooth muscles may help define their functional status (ureter, bladder neck) and/or impact cancer staging (bladder, urachus).

793 Study of Useful Markers for Differential Diagnosis of Non-Clear Cell Type Renal Cell Carcinoma Including Papillary Renal Cell Carcinoma, Chromophobe Renal Cell Carcinoma, and Xp11 Translocation Carcinoma

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Background: Renal cell carcinoma represents over 90% of all malignancies of the adult kidney. This tumor is a morphologically and genetically heterogeneous tumor. Although clear cell tumor type is the most frequent and usually not difficult to make diagnosis, other histologic subtypes of renal cell carcinoma, such as papillary, chromophobe, and Xp11 translocation carcinoma, can be encountered in practice and sometimes differentiation between them is difficult. In this study we tried to do immunohistochemistry for the diagnosis of these tumors.

Design: 55 cases of renal cell carcinomas (30 of clear cell type, 12 of papillary type, 7 of chromophobe type, 6 of Xp translocation carcinoma) were obtained at Yeungnam University Hospital from Jan. 2005 to July 2008. Immunohistochemistry using E-caderin, beta-catenin, PTEN, EGFR, gaelctin-3, and TFE3 was done on tissue microarray slide. The staining reaction was classified according to a semi-quantitative reference scale ranging from 1 to 3+.

Results: Papillary subtype showed 90% positivity for beta-catenin and PTEN, while clear cell subtype revealed average 20% positivity for E-cadherin and PTEN. 6 and 7 cases out of 7 chromophobe subtype were significantly strong staining pattern in E-cadherin and galectin-3, respectively ($p<0.01$). All of 6 Xp11 translocation carcinoma showed overexpression of EGFR protein ($p<0.001$), even though TFE3 positivity was present in 3 cases. TFE3 expression was also noted in some of papillary and clear cell renal cell carcinoma.
Conclusions: Expression of galetin-3 was very useful in diagnosis of chromophobe renal cell carcinoma. Xp11 translocation carcinoma revealed more specific expression of EGFR than TFE3. It is likely that TFE3 expression may not specific to translocation carcinoma.

794 Role of Tissue Biomarker Expression in the Prognosis of Clear Cell Renal Cell Carcinoma

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Background: Conventional, or clear cell, renal cell carcinoma is the most common subtype of renal cell carcinoma, characterized by its diverse clinical course. Identification of clinicopathologic parameters and specific molecular markers predicting clinical outcome has become an important and controversial issue. The aim of this study is to analyze the expression of several biomarkers in clear cell renal cell carcinoma and to assess their prognostic significance. We also tried to correlate these molecular markers to clinical and pathologic indices, such as age, sex, tumor size, TNM stage, and Fuhrman nuclear grade.
Design: The study is composed of 111 clear cell renal cell carcinoma obtained from nephrectomy specimen at Yeungnam University Hospital between 2000 and 2007. The expression of biomarkers including EGFR, erbB2/neu, KIT, PDGFR-α, TGF-β receptor type II, galectin-3, PTEN and Ki-67 was immunohistochemically performed on tissue microarray slides contained a tissue core from normal renal tissue and tumor. Stained slides on each marker were evaluated for protein expression using 4-level scale (o = none, 1 = low, 2 = moderate, 3 = high). Level 2 and 3 was considered as positivity.
Results: Tumor stages were pT1 89 (80%), pT2 11 (10%), pT3 10 (9%), pT4 1(1%). Fuhrman nuclear grade 1/ 2/ 3/ 4 was 10 (9%)/ 43 (39%)/43/15 (13%), respectively. There was no significant difference between clinicopathologic features and patients' survival. 50 out of 111 cases of clear cell renal cell carcinoma (45.0%) showed overexpression of EGFR, while 74 cases of PTEN (76.6%), 89 of galectin-3 (80.9%), and 76 of TGF-β receptor type II (68.5%) revealed none to low expression. EGFR overexpression was significantly associated with higher nuclear grade ($p = 0.001$), larger tumor size ($p = 0.016$), and higher Ki-67 labeling index ($p = 0.034$). None/low TGF-β receptor type II expression was significantly correlated with decreased overall survival ($p = 0.023$) and higher nuclear grade ($p = 0.001$), and low PTEN expression ($p = 0.001$). Fuhrman nuclear grade was closely related to overexpression of EGFR, none/low TGF-β receptor type II expression, and loss of galectin-3 protein ($p = 0.014$).
Conclusions: This study showed that overexpression of EGFR and none to low expression of TGF-β receptor type II, and loss of galectin-3 are not only linked to poor clinical outcome but also well correlated with higher nuclear grade in clear cell renal cell carcinoma.

795 Immunohistochemical Stain for Cytokeratin 7, S100A1, and Claudin 8 Is Valuable in Differential Diagnosis of Chromophobe Renal Cell Carcinoma from Renal Oncocytoma

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Background: Some cases of chromophobe renal cell carcinomas (CRCC) have overlapping histologic features with renal oncocytoma. Although both CRCC and renal oncocytoma are originated from the intercalated cells of collecting ducts, each type has different biological behavior. We studied the difference of CK7, S100A1, and claudin 8 immunomarkers in classical chromophobe renal cell carcinoma (ChRC), renal oncocytoma (RO), and tumor of overlapping histology (TOH).
Design: We used archived specimens of 24 classical ChRCs, 25 RO, and 11 TOH. The author analyzed the immunohistochemical expressions of CK7, S100A1, and claudin-8 in differentiation of CRCC and renal oncocytoma. Based on this data we applied and analyzed immunohistochemical stain in tumors of overlapping histology.
Results: CK7 was positive in 21 out of 24 classical ChRCs, and was negative in 23 of 25 ROs. S100A1 was negative in all classical ChRCs, and was positive in 23 of 25 ROs. Claudin 8 was positive with membranous pattern in 14, and with cytoplasmic pattern in one of 24 classical ChRCs, respectively. However, it was positive with cytoplasmic pattern in 24 of 25 ROs. When the immunoreactivity of three immunomarkers are combined as a panel, classical ChRCs and ROs revealed different immunophenotypes. Accordingly, 11 TOHs could be classified as 6 ChRCs and 5 ROs. We examined 4 cases of TOH by electron microscopy, and found that the ultrastructural findings were concordant with the diagnoses based on immunophenotypes.
Conclusions: We demonstrate that classical ChRC and RO reveal very significant difference in CK7, S100A1, and claudin 8 immunoreactivity. And also suggest that a panel of three immunostains could be used to classify TOH into ChRC and RO.

796 Luteinizing Hormone-Releasing Hormone (LHRH) Expression in Male Meningiomas (MM)-Implications for Treatment of Coexisting Prostate Cancer with LHRH Agonists

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Background: Meningiomas are mostly benign tumors accounting for approximately 20% of intracranial neoplasms in adults and have a female predominance. Both MMs and female meningiomas (FM) are hormonally sensitive and express predominantly progesterone receptor (PR) therefore, PR-blocking agents have been used in their treatment. LHRH has also been reported to be associated with meningioma proliferation in vitro. There have been reports of patients with prostate cancer who had progression of their coexisting meningiomas while on LHRH agonist treatment. Our aim was to evaluate LHRH expression in MMs and correlate this with PR and Ki-67 staining. To our knowledge no such study has been performed thus far.
Design: Twenty-four MMs were retrieved from our pathology files from 1991-2008 and compared with 24 FMs. Normal pituitary was used as control. MM and FM of all grades and histologic subtypes were included. Sections from the paraffin blocks of study cases and controls were stained with mouse monoclonal GnRH/LHRH, Ki-67 and PR. LHRH staining was cytoplasmic and its distribution was interpreted as being either focal or diffuse and staining intensity was graded as weak (1+) or strong (2-3+). More than 10% staining with PR was considered positive and the percentage of Ki-67 positivity was recorded in all cases. All stains were reviewed and graded by 3 reference pathologists.
Results: The distribution and intensity of LHRH staining in MM and FM is summarized in the table below. LHRH was diffusely positive in 92% of MM and 88% of FM, with both showing strong intensity (67% and 79% respectively).

LHRH- Distribution and Intensity of Staining

	Diffuse	Focal	Strong Intensity	Weak Intensity
Male	22 (92%)	2 (8%)	16 (67%)	8 (33%)
Female	21 (88%)	3 (12%)	19 (79%)	5 (21%)

Ki-67 positivity ranged from 1- 20% in both MMs (mean 5.4%) and FMs (mean 6.4%). PR was positive in 20 of 24 (83%) MMs and 23 of 24 (96%) FMs.
Conclusions: Our study shows that the majority of MM and FM are strongly and diffusely positive for LHRH in addition to PR and have a low proliferation index. LHRH expression in MM may have significant implications for the treatment of coexisting prostate cancer with LHRH agonists. Therefore, newer LHRH antagonists need to be evaluated as an alternative for the treatment of prostate cancer in this unique group of patients.

797 Evaluation of Perivesical Lymph Nodes in Radical Cystectomy Specimens for Bladder Carcinoma and Their Impact on Tumor Staging

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Background: Lymphadenectomy has an important role in staging of bladder cancer and identifying patients who may benefit from adjuvant therapy. Approximately 20 percent of patients undergoing pelvic lymphadenectomy with radical cystectomy for bladder cancer have lymph node metastases. Previous studies have also shown that an increased number of lymph nodes retrieved from a cystectomy specimen is associated with improved overall survival for a given stage. In general, pathologic evaluation of lymph nodes in bladder cancer has come from formal pelvic lymph node dissections separate from the cystectomy specimen. To our knowledge, there have been no studies evaluating lymph nodes retrieved from the perivesical fat attached to the cystectomy specimen.
Design: A total of 33 radical cystectomies for bladder cancer from 2002 to 2008 were reviewed from our files. In all cases, formal lymph node dissections were sent separately by the surgeon, while perivesical lymph nodes were dissected from the perivesical fat by the pathologist. Thirteen cases had perivesical lymph nodes identified. These cases were reviewed for the presence of tumor metastasis to perivesical lymph nodes and were compared with the pelvic lymph node dissections sent with each specimen.
Results: Perivesical nodes were reported in 13 of 33 cases. Of these, 2 cases were pT1, 3 cases pT2, 6 cases pT3, and 2 cases pT4. The number of perivesical nodes retrieved ranged from 1 to 12, and the number of these with metastatic carcinoma ranged from 1 to 3. The size of these nodes ranged from 0.1 cm to 1.9 cm, and the size of the metastasis ranged from less than 0.1 cm to 1.9 cm (entirely replacing the node). The number of nodes in the formal lymph node dissections ranged from 1 to 20, and the number of these with metastases ranged from 0 to 3. In 6 cases, perivesical nodes were the only nodes with metastases, changing the pathologic stage from N0 to N1 in 4 cases and N0 to N2 in 2 cases. In 1 case, metastases to perivesical nodes as well as pelvic nodes changed the stage from N1 to N2.
Conclusions: In nearly half of the cases, perivesical lymph nodes were the only nodes in which metastases were identified. In more than half of cases, identification of metastases in perivesical nodes increased the pathologic stage by at least one level. These results emphasize the need for a liberal dissection of perivesical fat by the surgeon and for extensive sampling of the fat for lymph nodes by the pathologist.

798 The Utility of Immunohistochemical Markers in Differentiating between Recurrent Clear Cell Renal Cell Carcinoma, Retroperitoneal Paraganglioma and Adrenal Cortical Lesions with Limited Biopsy Material

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Background: Retroperitoneal recurrence of clear cell renal cell carcinoma (CCRCC) after surgical resection is usually investigated by needle biopsy. However, CCRCC can be difficult to distinguish from adrenal cortical lesions or paragangliomas based on morphologic examination of limited biopsy material. This study explored the

diagnostic utility of a panel of immunohistochemical markers in dealing with this differential diagnosis.

Design: To stimulate the scarcity of biopsy material, a tissue microarray (TMA) with 1 mm tissue cores was constructed to include 21 cases of CCRCC, 19 normal adrenal cortex, and 15 retroperitoneal or mediastinal paragangliomas. Triplicate cores were used for each case. The TMA was then immunostained with epithelial markers (AE1/3, CAM 5.2, EMA), RCC markers (CD10, CA9, RCC antigen), adrenal cortical markers (calretinin, melan-A), and neuroendocrine markers (synaptophysin, chromogranin, CD56, NSE). The staining intensity and % of positive cells were recorded for each case.

Results: The % of positive cases for each marker is shown in the table.

Immunohistochemical markers		Number (%) positive cases		
		CCRCC	Adrenal cortex	Paraganglioma
Epithelial	AE 1/3	12/21 (57.1%)	1/19 (5.3%)	0/15 (0%)
	CAM 5.2	17/21 (81.0%)	17/19 (89.5%)	1/15 (6.7%)
	EMA	14/21 (66.7%)	0/19 (0%)	0/15 (0%)
RCC	RCC antigen	16/21 (76.2%)	0/19 (0%)	0/15 (0%)
	CA9	18/21 (85.7%)	0/19 (0%)	1/15 (6.7%)
	CD10	21/21 (100%)	1/19 (5.3%)	0/15 (0%)
Adrenal cortical	Calretinin	0/21 (0%)	19/19 (100%)	5/15 (33.3%)
	melan-A	4/21 (19.0%)	18/19 (94.7%)	5/15 (33.3%)
Neuroendocrine	Synaptophysin	0/21 (0%)	17/19 (89.5%)	15/15 (100%)
	Chromogranin	4/21 (19.0%)	8/19 (42.1%)	6/15 (40%)
	CD56	1/21 (4.8%)	19/19 (94.7%)	15/15 (93.3%)
	NSE	21/21 (100%)	18/19 (94.7%)	15/15 (100%)

Conclusions: Although immunohistochemical stains, including epithelial and adrenal cortical markers, are extremely useful, and in some cases are necessary, to differentiate CCRCC from adrenal cortical lesions and retroperitoneal paraganglioma, CAM5.2 and melan-A should not be used for this purpose as there is significant overlap in the staining patterns of these 3 lesions. In addition, immunohistochemical stains are not useful in differentiation between adrenal cortical lesions and paragangliomas.

799 Estrogen and Progesterone-Receptor Expression in Renal Dysplasia: An Analysis of 14 Cases

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Background: ER/PR-positive stroma has recently been reported in non-tumorous proliferations of the kidney in association with urinary obstruction. These proliferations were morphologically similar to the ovarian-like stroma found in cystic nephroma, mixed epithelial-stromal tumor, and angiomyolipoma with epithelial lined cysts. However, to the best of our knowledge, ER/PR-positive stroma has not yet been described in renal dysplasia.

Design: A search of the surgical pathology files at our institution from April 1991 to April 2008 revealed 14 cases of renal dysplasia with sufficient tissue available to perform immunohistochemical stains for ER and PR. The percentage of positive-staining nuclei in foci of immature mesenchyme cuffing primitive-looking tubules was calculated by counting all the nuclei in 3 different foci.

Results: Patients ranged in age from three months to 44 years, with thirteen out of 14 aged less than 13 years old. Seven were males. Twelve patients had radical nephrectomy or nephroureterectomy specimens. Eleven had unequivocal gross abnormalities involving the ureter and the collecting system including hydronephrosis, ureteral stenosis, and ureteral duplication. All cases showed the histologic hallmarks of renal dysplasia. In 13 of 13 evaluable cases (100%), there was strong ER nuclear staining in the mesenchymal cells surrounding the immature and/or dilated tubules and ducts. Eleven cases (79%) showed strong nuclear staining in >50% of the mesenchymal cells. PR nuclear staining was much less frequent and less intense, and was observed in 6 (43%) cases. In three of these, <10% of the cells were positive. ER/PR positivity was restricted to the mesenchymal collars and interstitial cells between primitive tubules and ducts.

Conclusions: To the best of our knowledge, we are the first to report in the literature the ER/PR positivity of the mesenchymal collars characteristic of kidneys affected by renal dysplasia. Our data confirm the observations of Tickoo et al who reported the presence of ER/PR positive stromal cells in the non-tumoral obstructed human kidney. Our findings argue against the hypothesis that the ER/PR stroma in neoplastic lesions either originate from müllerian remnants misplaced during embryogenesis or is tumoral. ER/PR positive stroma may be related in part to urinary obstruction possibly occurring also at an early stage of nephrogenesis.

800 Necrotizing Vasculitis Involving the Prostate or Periprostatic Tissue: An Analysis of 12 Cases

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Background: Vasculitis involving the prostate and periprostatic tissue has been described in the literature but still remains a rare finding. It can occur in patients with systemic vasculitis (Wegener's granulomatosis, Polyarteritis nodosa, and Giant cell arteritis) or as a localized process. Isolated forms include infectious vasculitis, localized small-vessel vasculitis, localized giant-cell arteritis, lymphocytic vasculitis of the prostate tranzition zone, and polyarteritis nodosa-like vasculitis.

Design: A search of the surgical pathology files at our institution from 1992 to 2008 revealed 12 cases of necrotizing vasculitis involving the prostate or the seminal vesicle. 6 were found on a radical prostatectomy and 6 were found on a needle core biopsy. Histological findings were recorded and clinical data were collected for each case.

Results: Patients ranged in age from 48 to 76 years (mean of 62). 8 patients (67%) had prostatic adenocarcinoma concurrently to vasculitis. Of them, 4 had Gleason score 6, 3 had Gleason score 7, and one had Gleason score 9. PSA levels were available for 7 patients (6 with adenocarcinoma) and ranged from 1.6 to 40 ng/ml. All cases had fibrinoid necrosis. Transmural inflammation was granulomatous in 2, purely lymphocytic in 2, lymphoplasmacytic in 1, and composed of a variable mixture of neutrophils, lymphocytes, plasma cells, and histiocytes in 7. Eosinophils were predominant in 2 cases. Vasculitis involved small vessels in 4 cases (33%), small to medium vessels in 5 cases (42%), and medium vessels in 3 cases (25%). Tissue involvment was focal in 9 cases (75%) and multifocal in 3 cases (25%). The location of the vasculitis was intraprostatic in 7 cases (58%), periprostatic in 3 cases (25%), periseminal vesicle in 1, and both intraprostatic and periprostatic in 1. Clinical follow-up was available for 10 patients. No evidence of a systemic vasculitis was seen in 6 patients (mean follow-up of 52 months). Systemic disease was observed in 4 patients (33%) including a systemic granulomatous disease of unknown etiology, Rickettisia infection, monoclonal IgG paraproteinemia, and Hodgkin lymphoma.

Conclusions: Although rare, prostatic and periprostatic vasculitis may be the first manifestation of a systemic disease. However, isolated forms confined to the prostate have been described and are more frequent than previously thought.They can be a component of a paraneoplastic syndrome and may represent vascular changes secondary to a localized immune response.

801 Urothelial Carcinoma Involving Both Upper and Lower Urinary Tracts: Predictors of High Grade Tumor in Upper Urinary Tract

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Background: Urothelial carcinoma (UC) involving both upper and lower urinary tracts (UUT, LUT), either synchronous or metachronous, is relatively uncommon. Grade and stage of LUT UCs have been cited as significant prognostic indicators. However, little data is available regarding the predictive factors for high grade (HG) UUT tumors.

Design: The departmental surgical pathology files were searched from 1989-2007 for patients with UUT UC treated with nephrectomy and with prior or subsequent LUT UC, sampled either by biopsy or resection. UUT UC were evaluated for: age, tumor location, grade, stage, lymph node status, diagnosis interval between UUT and LUT UCs (synchronous within 2 months of each other, diagnosis 2-12 months apart, diagnosis >12 months apart) and grade of corresponding LUT UC.

Results: A total of 246 consecutive patients with UUT UC were identified. Among them 86 (35%) patients (mean age 78) had UCs also involving LUT: 23 cases with a synchronous diagnosis; 41 cases with UUT UC first, 22 cases with LUT UC first. 34 (40%) of cases had low grade (LG) UC in both sites, and 33 (38%) had HG UC in both sites. When the LUT UC was HG, it was more likely that the UUT UC was also HG (33/35 vs. 17/50, p = 0.0001).

UC Grade: UUT versus LUT

	Upper Tract LG (n)	Upper Tract HG (n)
Lower Tract LG (n)	34	17
Lower Tract HG (n)	2	33

p<0.0001

When both UUT UC and LUT UC presented synchronously or when they presented more than one year apart, the UUT UC was more likely to be HG (41/56 vs. 10/30, $p < 0.05$). Involvement of ureter with (32) or without (3) pelvis was associated with increased HG disease in comparison with UUT confined to pelvis (26/36 vs. 18/50, p<0.05). Similarly, involvement of the ureter (35) was associated with higher stage (III or IV) at presentation (54% vs. 27%), but this prediction was not statistically significant.\

UUT UC Site and Grade

	Low Grade	95% CI	High Grade	95% CI
Ureter +/- Pelvis	10/36	15.9,44.0	26/36	56.1,84.2
Pelvis Only	32/50	50.1,75.9	18/50	24.1,49.9

p<0.05

HG UUT UC was more often seen in high stage disease ($p < 0.05$). Other parameters, such as patient age or which carcinoma was diagnosed first, did not predict HG disease in the UUT.

Conclusions: There is a strong correlation with tumor grade between UUT UCs and LUT UCs. HG LUT UC was predictive of HG UC in the UUT. Additionally, UUT UC and LUT UC with a synchronous diagnosis or a diagnosis interval of greater than one year were strong predictive factors for HG UUT UCs. Ureter involvement was also correlated with HG UUT UC.

802 Clinicopathologic Features of Transurethral Resection of Bladder Tumor (TURBT) for Prediction of TNM Stage of Urothelial Carcinoma of Urinary Bladder

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Background: Urothelial carcinoma (UC) is the most common malignant tumor of the urinary bladder, accounting 84% and 79% in male and female patients, respectively. Tumor stage is the most important prognostic factor and presence of proper muscle invasion is critical for the selection of treatment. TURBT is adequate for diagnosis and treatment of superficial UC (≤T1). Patients with muscle-invasive deep UC (≥ T2) undergo additional treatment including radical cystectomy (RC). The aim of this study is to analyze tumor characteristics of TURBT and subsequent RC specimens and to find predictors of the final TNM stage of UC in TURBT specimens.

Design: We analyzed clinicopathologic features of 190 UC cases that underwent both TURBT and RC at Asan Medical Center from 1996 to 2006. The two-tier tumor staging system, superficial and deep UC, was applied in the last TURBT before RC and RC specimens. Because TURBT might remove most of UC and then subsequent RC might not represent the tumor stage accurately, the highest tumor stage from TURBT or RC was used to assign the final TNM stage. The TNM stage was divided as low and high stage groups: superficial non-metastatic UC as the low stage group (≤ stage I) and muscle-invasive or metastatic UC as the high stage group (≥ stage II).

Results: The TURBT and RC specimens revealed superficial UC in 109 and 64 cases and muscle-invasive UC in 76 and 126 cases, respectively. The depth of tumor invasion of TURBT specimen could not be determined in 5 cases. Among the 185 cases of known

tumor stage in TURBT specimens, the stage at RC was identical to TURBT in 140 cases and upstaged in the other 45 cases. Patients with the high TNM stage group, compared to those with the lower TNM stage group, were older and their TURBT specimens demonstrated higher tumor stage and grade and more proportion of invasive tumor and micropapillary components.

Conclusions: These results demonstrate that the tumor stage of TURBT upstaged in RC in 24% of UC cases. Besides older age population, pathologic features including higher tumor stage, grade, and more invasive components and micropapillary histology in TURBT specimens might be useful for the accurate prediction of the final TNM stage of UC.

803 Nuclear Expression of SOX9 Protein as a Prognostic Marker in Invasive Bladder Urothelial Carcinomas (UrCa): A Tissue Microarray Immunohistochemistry Study

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Background: SOX9 is a transcription factor that plays an important role in cellular differentiation during embryogenesis. Recently alteration in SOX9 expression have been described in several types of solid tumors. The specific imunohistochemical expression pattern of SOX9 in UrCa and its prognostic significance has not been studied.

Design: TMAs were constructed from archival tissue from 138 consecutive cystectomies performed in our institution between 1994-2002. Triplicate tumor samples, corresponding benign urothelium and, when available, corresponding flat CIS were spotted from each cystectomy. Immunohistochemistry of SOX9 using a standard immunohistochemistry protocol and appropriate controls Intensity of SOX9 nuclear staining was evaluated and assigned an incremental 0,1+,2+,3+ score. Extent of staining was categorized as focal (<25% of cells), multifocal (25-75%), or diffuse (>75%). SOX9 score was generated for each tumor/benign as a product of intensity and distribution.

Results: Mean patient age at cystectomy was 63 yrs and M:F ratio was 4:1 w/ mean follow-up of 28 mos (1-106 mos). 7 patients were lost to follow-up. A total of 84 of 138 cases with evaluable UrCa were scored for SOX9. The latter included 60 cases of UrCa with "favorable" histology (UrCa, UrCa w/ focal glandular or squamous features) and 24 cases of UrCa with "unfavorable" histology (micropapillary, lymphoepithelial-like Ca, sarcomatoid Ca, adenoCa, signet-ring Ca, poorly differentiated Ca, squamous cell Ca). 34 paired normal urothelium cases were also scored. Our tumors were staged as pT1 (n=4), pT2 (n=28), pT3 (n=40) and pT4 (n=12). A higher SOX9 expression score was encountered in UrCa compared to paired benign tissue (mean score: 0.35). SOX9 tumor scores were significantly higher in the group of UrCa with unfavorable histology (mean score: 6.7) compared to UrCa with favorable histology (mean score: 4.2); $p<0.005$. Higher SOX9 scores were also significantly associated with higher pathologic stage ($p<0.01$). However, SOX9 expression did not predict with presence of lymph node metastasis, disease-free survival or overall survival in our cohort. (p:NS).

Conclusions: We found a higher SOX9 expression in UrCa tumors compared to benign controls. Higher SOX9 expression was found in tumors with unfavorable histology and those with more advanced stage suggesting a potential prognostic role in UrCa.

804 Papillary Urothelial Neoplasm of Low Malignant Potential (PUNLMP): Outcome Analysis

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Background: Few long-term single center studies have addressed outcome in patients (pts) with PUNLMP. The present study evaluates behavior of de novo primary urinary bladder primary PUNLMP lesions (Primary-PUNLMP) as well as PUNLMP diagnosed during surveillance for a higher grade urothelial neoplasm in upper or lower urinary tract (Surveillance-PUNLMP).

Design: Surgical pathology records were searched for all bladder PUNLMP diagnosed between 1998-2008. Electronic medical records were retrospectively reviewed.

Results: We identified a total of 63 PUNLMP of the bladder; 34 (54%) were categorized as Primary-PUNLMP and remaining 29 (46%) as Surveillance-PUNLMP. **Primary-PUNLMP Group:** Pts age ranged from 42-93 yrs (mean 63 yrs) with M:F ratio of 2:1. During F/U (range: 3-108 mos), 19/34 (56%) pts developed no recurrences. Among the remaining pts, 5/34 (15%) developed PUNLMP recurrences (1-2 episodes in 1-6 yrs) and 10 (29%) progressed to a higher grade lesions within 1-4 yrs. Grade progression was to non invasive low grade urothelial carcinoma (LG-TCC) in 26% and non invasive high grade urothelial carcinoma (HG-TCC) in 3%. None of our "Primary-PUNLMP" pts developed invasive carcinoma or died of dz. Tumor size did not correlate with likeliehood for recurrence (p:NS). **Surveillance-PUNLMP Group :** 19/29 (66%) of PUNLMP lesions in this group occurred during surveillance for higher grade lesion of UB. These included: 14 (74%) within 7 yrs of prior LG-TCC; 4 (21%) within 4 yrs of prior HG-TCC and 1 PUNLMP found in cystectomy for invasive carcinoma (InvTCC). The remaining 10 (34%) bladder PUNLMP developed during surveillance (1-13 yrs) after a prior upper tract urothelial neoplasm (3 InvTCC, 5 LG-TCC and 2 PUNLMP). Five pts in this group were lost to F/U (including 1 upper tract InvTCC) and 1 died of unrelated cause. Two pts died of urothelial carcinoma (both ureteral InvTCC; DOD: 7%), 11 are alive with disease (1 PUNLMP, 7 LG-TCC, 1 HG-TCC, and 2 InvTCC; AWD: 40%) and the remaining 10 are alive with no evidence of disease (NED: 34%).

Conclusions: Bladder PUNLMP can occur either as a de novo lesion or during surveillance for prior higher grade urothelial neoplasm of UB or UT. None of our Primary-PUNLMP pts developed invasive cracinoma or died of their disease despite a 54% recurrence and 29% grade progression rates. In contrast, Surveillance-PUNLMP were associated with worse outcome (45% grade/stage progression rate; 7% DOD and 40% AWD) most likely dictated by their initial higher grade/stage urothelial neoplasm.

805 The Status of Positive Surgical Margin at Radical Prostatectomy and Risk of Biochemical Recurrence: A Single Institution Experience

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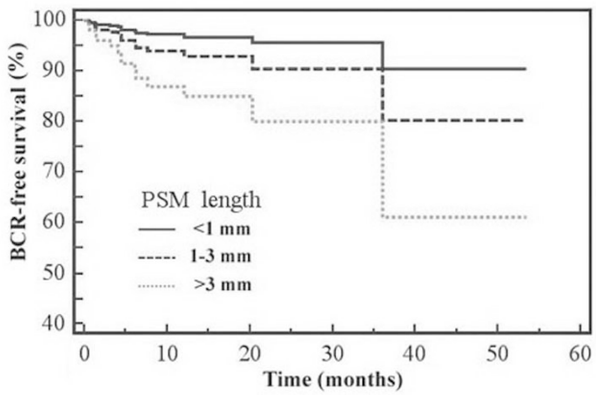
Background: After radical prostatectomy (RP), a positive surgical margin (PSM) is one factor that conveys an increased risk of biochemical recurrence (BCR). Higher risk may be associated with increased length of PSM. Stratifying PSM according to the relative risk of post RP recurrence may determine the potential benefit from adjuvant therapy after RP. Here we analyzed a large cohort of RP patients with PSM to determine risk factors associated with BCR.

Design: All RP cases from 2005 to 2008 at our institution were selected. PSM was defined as the presence of tumor cells at the inked margin. Specimens were assessed for the number, location and length of foci of PSM. For cases with multiple PSM foci, the total length was used in analysis. BCR was defined as a rise in the serum PSA level of ≥0.05 ng/mL. Follow-up data was obtained from a prospectively maintained database.

Results: Of 1398 RP, 243 (17%) had PSM, 25% with multiple foci. The location of PSM was most often posterolateral (45%) followed by apical (29%), bladder neck (6%) and anterior (2%). BCR was significantly higher in patients with PSM than those without PSM (11% vs 3%, $p < 0.0001$); and the median of BCR-free intervals was significantly shorter (8 vs 12 months, $p = 0.01$). The length of PSM was <1 mm in 44%, 1-3 mm in 33% and >3 mm in 23% of the cases. The difference in BCR-free survival of these groups during the follow-up was statistically significant (91%, 84%, and 60%, respectively, $P<0.05$, Fig. 1). The location and number of PSM foci did not correlate with BCR. By multivariate analysis, a PSM length of >3 mm, a Gleason score of ≥8 and tumor stage were independent predictors of BCR.

Figure 1. Kaplan-Meier BCR-free survival by the length of PSM



Conclusions: The length of PSM is a significant and independent prognostic factor for BCR in patients following RP, but not the number of PSM foci or their location. Including the length of PSM may help to stratify patients' risk of BCR after RP. Longer follow-up could further define the exact effect of PSM on the long-term oncologic outcome after RP.

806 Rhabdomyosarcoma of the Urinary Bladder and Prostate in Adults: A Clinicopathologic Study of 11 Cases

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Background: Rhabdomyosarcoma (RMS) of the urinary bladder and prostate is a rare tumor which occurs predominantly in children. There is limited information about this tumor in adults.

Design: We retrospectively searched our pathology file from 1980-2008 and identified 11 adult patients with rhabdomyosarcoma of the urinary bladder and prostate. Patients with previous history of carcinoma of the bladder or prostate were excluded from the study. The histology slides including immunohistochemical staining slides were reviewed. The clinical information was collected from patients' medical records.

Results: The average age of patients was 35 years (range, 18-60 years). The most common presenting symptom was urinary outlet obstruction (n=6), followed by pelvic pain (n=2), hematuria (n=2) and dysuria (n=1). All 11 patients had biopsies of the bladder or prostate. The biopsies showed embryonal type RMS in 8 cases, and non-classified RMS in 3 cases. The immunohistochemical stains showed that the tumor cells were positive for desmin in all cases. In addition, the tumor cells were also positive for myogenin (2 of 4 cases) and myoD1 (1 of 3 cases). Four patients received cystoprostatectomy and 1 patient had radical prostatectomy. In these resection specimens, the average size of tumor was 6.5 cm (range 4.5 to 10.0 cm), and the tumor extensively involved the bladder and prostate with positive resection margin in 2 cases. Followup information was available for 8 patients with an average period of 17 months (range, 3-34 months). The patients had received chemotherapy (n=7), radiation therapy (n=6), and surgery (n=5). Six patients died of disease at an average of 20 months (range, 3-34 months) and 2 patients were alive with disease at 7 and 10 month, respectively.

Conclusions: RMS of the urinary bladder and prostate is a highly aggressive disease in adults with extensive involvement of the bladder and prostate. Embryonal RMS is the most common histologic type in adults. This disease carries a poor prognosis despite multiple-modality therapy.

807 Urothelial Carcinoma with Divergent Villoglandular Differentiation: A Study of 14 Cases

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Background: Tumors of the urinary bladder may have a variety of histologic patterns. Tumors with either glandular or villous features, such as villous adenomas, in situ adenocarcinomas, invasive adenocarcinomas, and variants of urothelial carcinoma (UC) such as micropapillary carcinomas have been described. However, UC with both villous and glandular features have not been well characterized.

Design: We identified 14 cases of UC with divergent villoglandular differentiation (UCDVD). These cases were defined as having villoglandular features if they contained superficial finger-like processes lined by epithelium having true glandular lumina. The cases were evaluated for the following features; presence of non-invasive and invasive UC, variants of UC, adenocarcinoma component, cystitis cystica et glandularis, depth of invasion and angiolymphatic invasion.

Results: Mean patient age at presentation was 70 years (range 46 -84 years) with a male predominance (5:1). 5 cases (36%) had lamina propria invasion, 5 cases (36%) had muscularis propria invasion and 1 case (7%) had extravesicular extension. A concurrent high grade papillary UC component was identified in 11 cases (79%), superficial micropapillary component in 5 cases (36%), in-situ UC component in 3 cases (21%), plasmacytoid component in 3 cases (21%), invasive adenocarcinoma in 2 cases, sarcomatoid carcinoma component in one case (14%) and small cell carcinoma component in 1 case (7%). Cystitis glandularis was present in 3 cases (21%). Angiolymphatic invasion was identified in 3 cases (21%). Histologically, the villoglandular components were composed of finger-like processes lined by glands intimately admixed with high grade UC. Many of the glands had cribriform features lined by non-mucin producing cuboidal to columnar cells. However, the size and shape of the glands were variable, with some being small and slit-like and others being large and adenoma-like. In one case, the glands were lined by goblet cells. Variable quantities of intraluminal necrosis, apoptotic bodies and eosinophilic secretions were present in all cases. Luminal mucin was present in two cases.

Conclusions: UCDVD are high grade tumors typically seen in elderly males, characterized by superficial filliform processes lined by glands intimately admixed with high grade UC (in situ or invasive) and other aggressive variants of UC. These relatively rare tumors should be recognized as a divergent variant of UC.

808 Insulin-Like Growth Factor Binding Protein 3 (IGFBP-3) Is Up-Regulated in Prostatic Adenocarcinoma (PAC) and Correlates with Gleason Grade

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Background: Insulin-like growth factor (IGFs) signaling pathway components and its docking molecules are associated with regulation of cell growth, differentiation, establishment of neoplastic phenotype and apoptosis. Carrier proteins such as IGF binding protein 3 (IGFBP-3) are modulators of IGF actions and are known to demonstrate proapoptotic and inhibitory effects. Abnormalities of the IGF axis have been reported in breast and lung cancers. However, published data are contradictory regarding its association with PAC with some studies showing a positive correlation. To clarify this we studied its expression in PAC.

Design: Formalin-fixed paraffin-embedded whole-mount radical prostatectomy tissue sections from a representative block from 199 patients with PAC were evaluated for IGFBP3 expression by immunohistochemistry. Hematoxylin and Eosin stained slides were reviewed and tumors graded based on the Gleason grading system in all cases. Sections were stained by automated methods (Ventana Medical Systems, Tucson, AZ) using mouse anti-human IGFBP-3 antibody (MAB305; R&D Systems, Minneapolis, MN). Tumors were scored semi-quantitatively based on staining intensity (weak, moderate, strong) and distribution (focal, regional, diffuse). These results were tested for correlations with Gleason grade and tumor stage.

Results: IGFBP-3 immunoreactivity was cytoplasmic in the positive cases. Cytoplasmic IGFBP-3 over expression was observed in 119/199 (60%) of all tumors. 144/199 (72%) patients received no therapy prior to radical prostatectomy. Within the non-treated subgroup, cytoplasmic over expression correlated with high tumor Gleason grade (Gleason score of 7 or more) [65% high grade versus 48% low grade, p=0.042]. There was a trend towards advanced stage, with 66% advanced stage tumors over expressing IGFBP3 protein versus 51% organ confined tumors, p=0.10).

Conclusions: IGF-BP3 is up-regulated in PAC and correlates with high tumor grade suggesting its role in prostate cancer pathogenesis. Further studies are warranted to investigate prostate cancer management through manipulation of the IGF axis.

809 Renal Medullary Carcinoma: Molecular, IHC, and Pathologic Correlation

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Background: Renal medullary carcinoma is a highly aggressive tumor occurring in patients with sickle cell hemoglobinopathy. It is characterized by advanced stage at the time of presentation and lack of response to therapy. The molecular alterations of these tumors are not well-known. Mutations/deletions of SNF5/INI1 gene and absence of expression of INI 1 have been demonstrated in a variety of tumors, such as renal rhabdoid tumors, and CNS atypical teratoid/rhabdoid tumors. In animal models, loss of hSNF5/INI1 gene predisposes to aggressive cancers. In the present study, we investigated the genetic, immunohistochemical, and pathological changes of renal medullary carcinoma and their role in diagnosis.

Design: Fifteen renal medullary carcinoma cases were collected from NCI pathology files. All cases had characteristic histologic features of renal medullary carcinoma. Immunohistochemistry of CK7, CD20, AE1/3, Vimentin, CAM5.2, CEA and INI1 were performed. Tumor and normal tissue from paraffin-embedded, histology sections were manually microdissected, and PCR-based microsatellite analysis of LOH for INI1 was performed using 5 pairs of primer sets from D22S303, D22S257, D22S345, TOP1P2, D22S310 regions on chromosome 22q.

Results: Ten patients were males and 5 females ranging age from 8-49 years (mean 26). History of sickle cell trait was obtained in 8 cases, one case had sickled RBCs in the specimen, and the sickle cell status of the remaining cases was unknown. Tumors involved predominantly the right kidney (right,11 cases; left, 4 cases). The average size of the tumor was 6 cm (1.1-15 cm); 11 cases had lymphovascular invasion; 9 cases had perirenal fat invasion; 9 cases had lymph node metastasis, 3 cases had renal vein invasion. IHC showed 9/9 cases positive for CEA; positive staining was also seen with CK20, CK7, CAM5.2, AE1/3, and Vimentin. INI1 immunohistochemistry staining was negative in all 6 cases studied similarly to 3 renal rhabdoid tumor cases and in contrast to a renal papillary carcinoma that were used as controls. Among the 9 cases tested by LOH analysis, 8 cases were found to have LOH of hSNF5/INI1 gene with varying degree of deletion.

Conclusions: Our study shows LOH of hSNF5/INI gene in the tumors, suggesting that LOH of hSNF5/INI may be involved in the pathogenesis of renal medullary carcinoma. The immunohistochemical profile reported here maybe helpful in confirming the diagnosis of these aggressive tumors.

810 The Expression of Tyrosin-Phosphatase SHP-1 Is a Novel Prognostic Marker in Prostate Cancer

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Background: Tyrosin phosphatase SHP-1 has been recently described in human and murine prostate, and also in established prostate cancer cells. We hypothesize that this tyrosin-phosphatase may act as a suppressor gene and evaluate the expression of SHP-1 in a clinical series of prostate cancers with adequate long-term follow-up.

Design: A series of 114 patients with prostate cancer of variable clinical stage (22 T1, 34 T2, 46 T3 and 12 T4) were retrospectively analyzed. Specimens were collected from radical prostatectomy and TUR samples. Clinicopathologic data were homogeneously evaluated. Immunohistochemistry was performed using SHP-1 commercial antibody. Total RNA was extracted after deparaffination of tumour tissue. Real time RT-PCR was performed using specific primers for SHP-1 and ribosomal 18S. The quatification value of SHP-1 mRNA was described as each value relative to 18S mRNA ((ΔΔCt method). Ki-67 index and the expression of neuroendocrine markers (chromogranin and synaptophysin) were also evaluated. Multivariate analysis regarding disease-specific mortality was performed to evaluate prediction of prognosis.

Results: A total of 55 patients (48%) revealed immunohistochemical expression of SHP-1, and this finding was not associated to T category, metastatic status or Gleason score. All patients were followed with emphasis on disease specific mortality (28% die of cancer during follow-up). Patients dying of other cause were censored. Mean follow-up was 68.06 months (range, 10-166). Multivariate analysis revealed metastatic disease at the time of diagnosis ($p<0.0001$), Ki-67 index ($p<0.0001$), absence of SHP-1 expression on IHQ ($p=0.003$) and high Gleason score ($p=0.05$) were independent predictors. Expression of SHP-1 on RT-PCR was 2.07 times higher in tumours staining positive for SHP-1 on IHQ and 4.7 times lower in hormone-resistant disease.

Conclusions: Loss of tyrosin-phosphatase SHP-1 expression is an independent variable of poor prognosis in prostate cancer. This finding is not associated to standard clinico-pathologic predictors. It is likely that this enzyme may act as the product of a suppressor gene, the role of which in prostate cancer needs further investigation.Supported by the grant ISCIII PI020964.

811 Light and Immunohistochemical Characterization of Renal Cell Carcinoma under 40. A Multicenter Study of 114 Cases

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Background: Renal cell carcinoma (RCC) is a rare event in young adults, very few large series being published so far. The aim of this study is to review the histological characteristics and the immunohistochemical profile of a large series of RCC under the age of 40.

Design: A total of 114 cases were collected from 7 hospitals in Spain. Five different categories were considered by light microscopy: Clear cell renal cell carcinoma (CCRCC), papillary renal cell carcinoma (PRCC), chromophobe renal cell carcinoma (ChRCC), clear cell papillary renal cell carcinoma (CCPRCC), biphasic renal cell carcinoma [carcinomas with two different, but clearly recognized, histologies (BRCC)] and unclassified renal cell carcinoma (URCC). Fuhrman's grade was evaluated only in CCRCC. CD10, EMA, CK7, CK20, CD15, CD117, AMACR, CAM 5.2, 34betaE12, e-cadherin, and Melan-A were systematically tested in all cases.

Results: Males predominated (83M/31F), average age of 32,9 years (range, 14-39). Organ confined disease (pT1/2) was observed in 91% of the cases. pN+ status was detected in 2,6% and pM+ in 0,9%. Fuhrman's grade in CCRCC group was G1: 15%, G2: 53%, G3: 23%, G4: 9%.

Histological typing and immunohistochemical profile

Histology (n)	CD10 (n/%)	EMA (n/%)	CK7 (n/%)	CD15 (n/%)	AMACR (n/%)	CAM 5.2 (n/%)	E-cadh (n/%)
CCRCC (58)	**53/91**	52/89	12/20	35/60	6/10	40/69	10/17
PRCC (9)	4/44	8/89	4/44	5/55	**9/100**	8/89	2/22
ChRCC (16)	5/31	16/100	**12/75**	0/0	3/19	15/94	**14/87**
[italic]CCPRCC (12)	*9/75*	*9/75*	*5/42*	*7/58*	*8/67*	*9/75*	*4/33*
BRCC (4)	4/100	4/100	2/50	4/100	0/0	4/100	3/75
URCC (15)	11/73	14/93	2/13	6/40	2/13	11/73	11/73

Most significant markers in CCRCC, PRCC and ChRCC in bold. CCPRCC results in italic. The results of CK20, CD117, 34bE12 and Melan-A were not contributory

Conclusions: CCRCC account for only 50% of RCC in this age group, with a significant number of ChRCC and URCC cases. URCC is an odd group with oncocytic changes and high grade features. CCPRCC, a newly proposed entity, represent a relevant proportion of cases and show mixed characteristics between CCRCC and PRCC.

812 Handling and Reporting of Transurethral Resection Specimens of the Bladder in Europe: A Web-Based Survey by the European Network of Uropathology (ENUP)

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Background: Most of the published data on handling and reporting of transurethral resection specimens of the bladder is from North America and little information is available about European practices.There has until now not been any accessible channel for distribution of professional information to or data collection from urological pathologists in Europe.

Design: A European Network of Uropathology (ENUP) was organized with the purpose of disseminating guidelines and consensus documents, carrying out survey studies and being a hub for research collaborations. Names and email addresses of uropathologists were collected from 336 pathology laboratories in 15 west European countries. The aim of this ENUP survey was to collect information about handling and reporting transurethral resection specimens of the bladder (TURB).

Results: A total of 177 (52.2%) of the ENUP members replied to a web-based questionnaire. Some routines were adopted by a majority, e.g. formalin fixative (92.5%), separate containers for tumor and resection base (72%), embedding the entire specimen (60%), muscularis mucosae based pT1 substaging (64.3%).Of importance in terms of clinical implication is the fact that 19.5% of respondents would report cancer along/in adipose tissue as pT3a, and the presence of non-invasive TCC in prostatic ducts/glands would be considered pT4a by 16.1%. As many as 72.6% of respondents recognize the entity PUNLMP but rarely report it. When diagnosing bladder cancer, IHC is rarely used by 91.7%, and markers used are CK20 (76.9%), CK7 (66.7%) and Ki67 (38.8%). Only 24.8% of respondents report prognostic markers for bladder cancer with Ki67 (84.4%) and p53 (64.4%) being the most common. Concerning how European uropathologists grade bladder tumors, only 50.9% report the ISUP 98/WHO 04 grading system followed by the WHO 73 (43.4%) and the WHO 99 (31.4%). When multiple grading systems are reported, the ISUP 98/WHO 04 and the WHO 73 are the most common.

Conclusions: Some routines are almost universally adopted in Europe, while other still need to be standardized.

813 TMPRSS2-ERG Gene Fusions Are Infrequent in Prostatic Ductal Adenocarcinomas

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Background: Ductal adenocarcinoma (DCa) of the prostate is a rare subtype generally associated with a more aggressive clinical course than typical (Gleason pattern 3) acinar adenocarcinoma (ACa). Given its frequent association with an ACa component, some have challenged the concept of prostatic DCa as a distinct variant. We studied the occurrence of TMPRSS2-ERG gene fusions in DCa cases and in their associated ACa component.

Design: Fluorescence in situ hybridization (FISH) using a break apart probe for 5' and 3' ERG was performed on two tissue microarrays (TMA) constructed from 40 radical prostatectomy specimens with DCa. In 20/38 evaluable DCa cases (53%), a concurrent ACa component was scored as well. An additional group of 38 pure ACa cases matched with the DCa group for pathological grade (69% Gleason 7, 31% Gleason 8-10) and stage (39% pT2, 57% pT3) was used as a control. DCa and ACa cases were scored for presence of TMPRSS2-ERG gene fusion through deletion or translocation as well as for polyploidy (≥3 copies) at the ERG locus. Each case was spotted in quadruplicate and at least 50 cells were scored.

Results: 11% (4/38) of DCa cases had the TMPRSS2-ERG gene fusion, with 75% (3/4) showing the deletion and 25% (1/4) showing the translocation. No cases of duplication of the fusion were observed in the DCa group. In 95% of DCa cases where a concurrent ACa component was available for analysis (19/20), there was concordance for presence/absence of TMPRSS2-ERG gene fusion between the different histologic subtypes. In the control group of grade and stage-matched pure ACa cases, 45% (17/38) had the gene fusion with 53% (9/17) showing the deletion, 6% (1/17) showing a duplicated deletion, and 41% (7/17) showing the translocation. Compared to the matched ACa cases, the TMPRSS2-ERG gene fusion was significantly less frequently observed in DCa (p= 0.002, Fisher's exact test).

Conclusions: The presence of TMPRSS2-ERG gene fusions in some cases of DCa as well as the high concordance of genotype between ductal and acinar components in the same tumor support the concept that prostatic DCa and ACa are closely related histologic variants. However, the significantly lower rate of the gene fusion in DCa cases when compared to grade and stage matched pure ACa cases underscores the fact that enough genetic and potential biologic differences exist between these two histologic subtypes to warrant separate classification for the current time.

814 Prostate Adenocarcinomas Aberrantly Expressing p63 Have a Luminal Cell Phenotype

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Background: We have recently described a group of prostatic adenocarcinomas (PCa) that show diffuse aberrant expression of p63. Although these tumors lack high molecular weight cytokeratin expression as evidenced by absent 34βE12 immunostaining, they are strongly positive for bcl-2, a marker typically seen in basal cells. Given these conflicting results, we hypothesized that much like basal-like breast carcinomas (BLBCs), these prostate tumors may be arising from a stem cell with a partially retained basal cell immunophenotype. Thus, we examined their expression of additional basal cell, luminal cell and "stemness" markers.

Design: p63-expressing tumors from 11 radical prostatectomy specimens and 2 needle biopsies were immunostained for luminal cell markers (CK18, androgen receptor, prostein/p501s), additional basal cell markers (CK5/6) and pluripotency/progenitor markers (β-catenin, Oct4, c-kit).

Results: All 13 p63-expressing cases showed strong diffuse positivity for CK18, a LMWCK expressed in benign prostatic luminal cells, but not in basal cells. Similarly, 10/10 of evaluable cases also expressed the androgen receptor, with 90% (9/10) of these cases showing strong nuclear staining of equal or greater intensity than surrounding benign luminal cells. In contrast, basal cells in the surrounding benign glands showed only weak, focal positivity for the androgen receptor. 92% (12/13) of evaluable cases strongly expressed prostein (p501s), an additional marker of luminal prostatic epithelial cells. Conversely, basal cytokeratins (CK5/6) were predominantly negative in the p63-expressing tumors, with only 36% (4/11) of evaluable cases showing weak, focal expression in tumor cells at a much lower level than benign basal cells. Pluripotency/pluripotential markers including β-catenin, Oct4 and c-kit were uniformly negative in p63-expressing tumors, additional support for their luminal phenotype.

Conclusions: PCa with aberrant p63-expression belong to a small group of human carcinomas expressing p63 with luminal-type cytokeratins. BLBCs show a similar immunophenotype, with expression of p63 and CK8/18, however, unlike p63-expressing prostate adenocarcinomas, BLBCs invariably express HMWCK (CK5/6, 34βE12) and are typically negative for hormone receptors (ER/PR). Our results suggest that p63-expressing prostate carcinomas show a predominantly luminal cell immunophenotype and raise the possibility that the p63 transcription factor may be expressed but inactive in these tumors.

815 Antiproliferative B Cell Translocation Gene 2 (BTG2) Protein Is Down-Regulated in Prostate Adenocarcinoma (PAC)

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Background: BTG2 is a p53 inducible antiproliferative gene that, in response to DNA damage or other cell stresses, regulates the G_1/S transition of the cell cycle. Expression of BTG2 has been found in benign prostate glands, and increased in atrophic glands. Its role in PAC has not been extensively studied.

Design: Formalin-fixed paraffin-embedded whole-mount radical prostatectomy specimens from 225 patients with PAC were evaluated for BTG2 expression in tumorous, atrophic, and normal benign glands by immunohistochemistry. Slides were stained by automated methods (Ventana Medical Systems, Tucson, AZ) using rabbit anti-human BTG2 polyclonal antibody (sc-33775; Santa Cruz Biotechnology, Santa Cruz, CA). Staining intensity and percentage of positive cells were evaluated in benign, atrophic, and carcinomatous elements. Tumors were scored semi quantitatively based on staining intensity (weak, moderate, and strong) and distribution (focal <25%, regional 25-50%, and diffuse>50%). These results were correlated with Gleason grade and tumor stage.

Results: Most of 225 prostatectomy specimens showed patchy moderate cytoplasmic staining of BTG2 in the benign basal cell layer as well as luminal glandular epithelium. Of the 225 cases, 145 (64.4%) PAC expressed less BTG2 when comparing with adjacent benign prostate acini, 59 (26.2%) with similar expression, while 21 (9.3%) cases had elevated expression in PAC. There was no statistically significant correlation of BTG2 down-regulation among Gleason grade (65.2% in Gleason score 6 or less, vs. 64.8% in Gleason score 7 or more, p = 0.82) or tumor stage (64.8% in T1/T2 vs. 61.1% in T3/T4, p=0.58). Of the atrophic glands, 20 of 49 cases (40.8%) had elevated expression comparing to normal benign glands, 18 (36.7%) with similar intensity, while 11 (22.4%) had decreased expression. PACs expressed significantly less BTG2 when compared with atrophic glands (p<0.0001).

Conclusions: BTG2 expression is significantly less in PAC when compared with atrophic and benign prostate acini. BTG2 down-regulation appears to be an important step in PAC pathogenesis.

816 Patients with Recurrent Prostate Adenocarcinoma Show Higher Levels of Somatostatin Receptor 2 Expression

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Background: Numerous studies have explored the role neuroendocrine differentiation plays in prostate adenocarcinoma (PA) prognosis, with mixed results as to its clinical significance. Because at least some studies have suggested a worse prognosis in PA with neuroendocrine differentiation, there has been an interest in determining somatostatin receptor expression in those tumors showing neuroendocrine differentiation stemming from the possibility of employing somatostatin analogues as treatment. To date, however,

no study has examined the relationship between neuroendocrine differentiation, somatostatin expression and outcome in PA.
Design: 26 patients with recurrent prostatic adenocarcinoma and 25 patients who, after 10 years of follow-up, were free of recurrent prostatic adenocarcinoma were matched according to Gleason score and margin status at prostatectomy. Immunohistochemical staining utilizing standard technique with formalin fixed paraffin embedded tissue from each patient's prostatectomy specimen was performed with primary antibodies to chromogranin (DAKO, 1:200 dilution), synaptophysin (DAKO, 1:50 dilution) and a novel antibody to somatostatin receptor 2 which has been previously characterized in the literature. Percent of tumor cells with staining for chromogranin, synaptophysin and somatostatin receptor 2 was recorded.
Results: Neuroendocrine differentiation as defined by either chromogranin or synaptophysin staining of ≥5% tumor cells was seen in 7 patients with recurrent PA and 4 without recurrence. In patients with recurrent PA, a higher percentage of tumor cells expressing somatostatin receptor 2 was observed when compared to the tumors from patients without recurrence ($p = 0.058$).
Conclusions: Patients who exhibited recurrence of their prostatic adenocarcinoma showed increased levels of somatostatin receptor 2 expression. While the underlying pathobiology requires further investigation, the findings may provide some rationale for the use of somatostatin analogue treatment for patients with recurrent or advanced prostate adenocarcinoma.

817 Tissue Factor (TF) Expression in Prostate Cancer (PCa) Using a Matched Case-Control Outcome-Based TMA Design Set

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Background: Tissue factor (TF), a 47 kDa transmembrane protein, acts as a receptor for activated FVII and plays a major role in the extrinsic coagulation pathway. It is thought to be involved in cancer metastasis and progression in several malignancies, including PCa. Our study evaluates the immunohistochemical (IHC) expression of TF in PCa recurrence after prostatectomy.
Design: PCa tumor samples were obtained from an outcome-based TMA set (5 tissue microarray blocks with 200 paired recurrence (R) and non-recurrence (NR) cases, 400 subjects total, each with quadruplicate 0.6 mm in diameter cores) from the Cooperative Prostate Cancer Tissue Resource (CPCTR). Each case was obtained from a PSA-recurrence subject matched to a non-recurrence subject based on age; race; interval since prostatectomy; Gleason sum-score and pTNM stage. All had at least 5 years follow up, and cases with known metastases were excluded. TMA slides were incubated with anti-TF monoclonal antibody 4509 (American Diagnostica), and immunoreactivity was graded by a single pathologist using three staining intensity levels (0–3 for negative, weakly, moderately and strongly positive, respectively). The percent of immunostained tumor cells at each intensity level was recorded. Average and index score for TF expression were assessed in each core as follows: for average intensity, stain level was summed and divided by the number of cores with tumor; for index score, percent of immunostained tumor cells was summed and divided by the total of cores with tumor for each case. The Wilcoxon signed-rank test was used for the statistical analysis of the pair-matched dataset to assess the 2 groups (R vs. NR) for differences in TF expression.
Results: The Wilcoxon signed rank test showed no statistically significant differences between the two groups (R vs. NR) for either average intensity (median=1.00 [range 0-3] vs. median=0.33 [range 0-3], p=0.1278) or the index score (median=9.00 [range 0-295.00] vs. median=1.25 [range 0-277.50], p=0.4190).
Conclusions: To our knowledge, this is the first large series of arrayed PCa with ≥ 5 years clinical follow up to evaluate the expression of TF among two different groups (case-control matched pairs). Even though TF expression has been associated with high Gleason score and stage of the disease, this study showed that TF is not a prognostic marker for PCa recurrence after prostatectomy.

818 Cancer to Prostatic Urethra Distance in Radical Prostatectomy: Implications for Urethral Thermoprotection in Prostate Cryoablation Surgery

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Background: Cryoablation is a viable option for prostate cancer (PCa) patients who are not candidates for prostatectomy. A heating coil is placed in and portects the prostatic urethra from the cryo injury. The tissue within certain depth beneath the urethral mucosa, including PCa in that zone, is therefore not cryoablated. The cancer to prostatic urethra distance and the preoperative predictors of such distance are important for urologists and patients to decide if to undergo cryoablation.
Design: PCa was mapped in radical prostatectomy (RP) speciments and the shortest distance between the prostatice urethra and PCa was measured. The distance was then correlated with the preoperative serum PSA levels as well as prostate biopsy parameters, including Gleason score (GS), number of positive cores, highest % of positive cores, cancer in apex and bilateral disease.
Results: 100 RPs were studied. Patients' mean age was 58.6 (range 39-75) years. Preoperative serum PSA was 6.4 (range 1.6-23) ng/ml. Cancer to urethra distance was 0 (cancer at the urethra), 0.1-1, 1-2, 2.1-3, 3.1-4, 4.1-5 and >5 mm in 4, 19, 30, 14, 15, 7 and 11 cases, respectively. Bilateral PCa was marginally associated with shorter distance to the urethra (2.4 mm vs. 3.2 mm in bilateral and unilateral PCa, respectively; p=0.07). There was also a marginal association between PSA level and distance to the urethra. For one unit of PSA increment, the distance to the urethra decreased 0.12 mm (Pearsoncorrelation=0.19, p=0.06). No association was found between the distance and biopsy GS, number of positive cores, highest % of positive cores and cancer in apex.
Conclusions: In 100 RPs, PCa was at the urethra in 4% cases, and within 5 mm of the urethra in 91% cases. Most of the preoperative biopsy parameters, including GS, number of positive cores, highest % of positive cores and cancer in apex, did not correlate with the cancer to urethra distance. Only bilateral PCa and serum PSA levels marginally correlated with such distance. Our study indicates that the preoperative estimation of the cancer to urethra distance still have to rely on the imagining study; serum PSA and bilaterality of PSA may be helpful.

819 Kidney Injury Molecule-1 (KIM-1) Expression in Sarcomatoid Differentiation in Renal Cell Carcinoma (RCC): Implications for Differential Diagnosis of Malignant Spindle Cell Lesions of the Kidney

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Background: KIM-1 is a transmembranous protein involved in phagocytosis of injured proximal tubules in the kidney. Recent studies have shown it to be a sensitive and specific marker for RCC particularly those derived from proximal tubules. In this study, we investigate the utility of this marker in distinguishing sarcomatoid differentiation in RCC from potential diagnostic mimics.
Design: 13 cases of sarcomatoid differentiation in RCC (11 clear cell and 2 papillary RCC), 6 dedifferentiated liposarcomas primarily involving the kidney and presenting clinically as RCC, 15 cases of renal angiomyolipomas and one malignant PEComa of the kidney were stained with antibody directed against KIM-1 molecule (clone: AKG7; dilution1:20). Controls for this study included 10 cases of renal parenchyma and 4 cases each of clear cell type and papillary RCC. Staining results were recorded for both intensity (0 - 3+) and extent (negative, focal, moderate, diffuse).
Results: KIM-1 expression was noted in 12 out of 13 cases of sarcomatoid RCC. Staining was diffuse in sarcomatoid areas in 6 cases, with staining intensity ranging from 1+ to 3+. One case showed focal moderate staining. Staining was focal and weak in additional 5 cases and one case was negative. None of the other cases - dedifferentiated liposarcomas, renal angiomyolipomas or PEComa - stained with KIM-1.
Conclusions: KIM-1, a relatively specific marker for proximal tubule associated renal neoplasia, is expressed in a large majority of sarcomatoid RCCs. This finding has potential diagnostic utility in distinguishing between malignant spindle cell lesions involving the kidney, particularly in needle biopsies where the epithelial component of the RCC may not be represented. This immunostaining has a potential role in metastatic settings where accurate diagnosis may have therapeutic ramifications.

820 Cathepsin-K Expression in Both Classic and Epithelioid Angiomyolipoma of the Kidney

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Background: Angiomyolipoma is the most common mesenchymal tumor of the kidney. It can occur in patients with and without tuberous sclerosis, an inherited syndrome due to losses of *TSC1* or *TSC2* genes having an important role in the regulation of mTOR pathway which is suppressed by Sirolimus and analogous drugs. Epithelioid angiomyolipoma is a variant of angiomyolipoma composed of cells which closely resemble high grade or sarcomatoid renal cell carcinoma. Unlike classic angiomyolipoma, it can recur locally, metastasize, and cause death. Both the usual angiomyolipoma and epithelioid angiomyolipoma are frequently coexpress myogenic (actin) and melanocytic markers including MiTF. Recently overexpression of MiTF has been related to the expression of the papain-like cysteine protease cathepsin-K in osteoclasts where it has been inhibited by the mTOR inhibitor Everolimus. The aim of this study is to evaluate cathepsin-K immunoexpression in usual and epithelioid angiomyolipomas.
Design: We studied the immunoexpression of cathepsin-K in 20 usual and 4 epithelioid angiomyolipomas occurring in patients with (3) and without (21) tuberous sclerosis. As controls we analyzed the immunoexpression of cathepsin-K also in 140 clear cell renal cell carcinomas, 20 papillary renal cell carcinomas, 8 chromophobe renal cell carcinomas and 8 renal oncocytomas.
Results: In all of the usual renal angiomyolipomas strong cathepsin-K expression was demonstrated both in adipocyte-like cells and in smooth muscle cells. Similarly, all of the epithelioid angimyolipomas showed a strong and diffuse positive reaction with antibody to cathepsin-K. None of the clear cell renal cell carcinomas, papillary renal cell carcinomas, chromophobe renal cell carcinomas or renal oncocytomas showed a positive reaction with antibody to cathepsin-K.
Conclusions: We demonstrated that: 1) cathepsin-K is constantly and strongly expressed in both usual and epithelioid angiomyolipomas and can be a robust marker for their identification; 2) the expression in epithelioid angiomyolipoma of cathepsin-K, which has been demonstrated to be modulated by mTOR inhibitors such as Sirolimus, may suggest a rational base to test these drugs in patients with these tumors.

821 MiTF/TFE Family Renal Translocation Carcinomas Immunoexpress Cathepsin-K

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Background: MiTF/TFE family renal translocation carcinomas (tC) bear specific chromosome translocations that result in overexpression of TFE3 fusion gene products or native TFEB. TFE3 fusion gene product overexpression is due to different translocations involving chromosome Xp11.2 whereas TFEB overexpression is the result of the specific t(6;11). Both TFE3 and TFEB are closely related members of the MiTF/TFE transcription factor family which also includes TFEC and MiTF. All these

transcription factors bind specific target DNA both as homodimers or heterodimers, and have overlapping transcriptional targets. Recently overexpression of MiTF has been related to the expression of cathepsin-K in osteoclasts. The aim of this study is to evaluate cathepsin-K immunoexpression in MiTF/TFE family renal tC and its usefulness in their differential diagnosis.

Design: We studied the immunohistochemical expression of cathepsin-K in 9 cytogenetically confirmed MiTF/TFE family tC. Three cases demonstrated a t(6;11), and 6 cases had translocations involving Xp11.2, two cases t(X;1) PRCC-TFE3; three cases t(X;1) PSF-TFE3 and one t(X;3). As control we analyzed the immunoexpression of cathepsin-K in 140 clear cell renal cell carcinomas (RCC), 20 papillary RCC, 8 chromophobe RCC and 8 oncocytomas.

Results: All TFEB tC showed strong and diffuse cytoplasmic labeling for cathepsin-K. Among the cytogenetically confirmed TFE3 tC, 3 out of 6 were positive (two PRCC-TFE3 and one PSF-TFE3 tC). None of the clear cell RCC, papillary RCC, chromophobe RCC or oncocytoma labelled for cathepsin-K.

Conclusions: 1) Cathepsin-K is consistently and strongly expressed in TFEB tCs. 2) Cathepsin-K is expressed in 50% of the TFE3 tCs. It is possible that whether or not there is expression may be related to the specific TFE3 fusion harbored by the neoplasm. 3) Cathepsin-K immunoexpression in both TFE3 and TFEB tCs distinguishes these neoplasms from the more common adult RCCs 4) These results suggest that overexpression of TFE3 fusion proteins or native TFEB in these renal neoplasms activates expression of genes normally regulated by MiTF in different cell types.

822 Comparison of Putative Renal Cell Carcinoma (RCC) Immunohistochemical (IHC) Markers in Primary Adrenal Cortical Lesions (ACL) and Metastatic RCC: A Tissue Microarray Study of 246 Cases

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Background: Given their morphologic overlap, differentiating primary ACL from metastatic clear cell RCC (CC-RCC) can be challenging, particularly on a small biopsy sample. A formal IHC study comparing the sensitivity and specificity of newer, novel RCC markers in this distinction has not been previously reported.

Design: IHC analysis was performed on 61 ACL (41 cortical adenomas, 4 cortical adenomas of uncertain malignant potential, 7 cortical carcinomas, 6 cortical hyperplasias, 3 cortical rests) and compared with 185 metastatic CC-RCC using tissue microarray technology with antibodies recently described in RCC (CAIX, HNF-1beta, and hKIM-1). CC-RCCs were classified as well differentiated (WD) in cases where morphologic features strongly suggested CC-RCC or poorly differentiated (PD) when RCC would not have been suspected by morphology alone. Both staining intensity and extent were semiquantitatively scored on duplicate sections (0-3+).

Results: IHC findings are summarized in Table 1. Specificity for metastatic CC-RCC versus ACL is listed in Table 2. The staining differences among the various ACL studied were not statistically significant, but both lesions with immunoreactivity to CAIX were adrenal cortical carcinomas.

Immunoreactivity in ACL and CC-RCC

	CAIX	HNF-1beta	hKIM-1
CC-RCC (overall)	162/184 (88%)	156/184 (85%)	170/185 (92%)
CC-RCC (WD)	121/133 (91%)	113/133 (85%)	122/133 (92%)
CC-RCC (PD)	41/51 (80%)	43/51 (84%)	48/52 (92%)
ACL	2/61 (3%)	0/61 (0%)	0/61 (0%)

	Specificity for CC-RCC
CAIX	97%
HNF-1beta	100%
hKIM-1	100%

Conclusions: The recently described antibodies CAIX, HNF-1beta, and hKIM-1 are sensitive and specific immunohistochemical markers of CC-RCC in the distinction from ACL. HNF-1beta and hKIM-1 appear to be more specific than CAIX in the setting of an adrenal cortical carcinoma, and hKIM-1 has the highest sensitivity in metastatic poorly differentiated RCC.

823 A Cautionary Note Regarding the Use of PAX-2 Immunohistochemistry (IHC) in Differentiating Metastatic Clear Cell Renal Cell Carcinoma (CC-RCC) from Adrenal Cortical Lesions (ACL): A Tissue Microarray Study of 245 Cases

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Background: PAX-2, a transcription factor expressed during renal development, is a useful marker for distinguishing metastatic CC-RCC from potential mimics. We recently noted PAX-2 nuclear reactivity in normal adrenal cortex suggesting a lack of specificity. In this study, we evaluate PAX-2 IHC in a large number of ACL and metastatic CC-RCC, including well-differentiated (WD) and poorly differentiated (PD) tumors.

Design: IHC analysis for PAX-2 was performed on 61 ACL (41 cortical adenomas, 4 cortical adenomas of uncertain malignant potential, 7 cortical carcinomas, 6 cortical hyperplasias, 3 cortical rests) and compared with 184 metastatic CC-RCC using tissue microarray technology. CC-RCCs were classified as WD when morphologic features strongly suggested CC-RCC or PD when RCC would not have been suspected by morphology. Staining intensity and extent were semiquantitatively scored (0-3+) on duplicate sections using two dilutions (1:50 and 1:100), and a mean intensity (MI) and mean extent (ME) were calculated.

Results: IHC sensitivities are in Table 1. Specificities for metastatic CC-RCC (overall) versus ACL by PAX-2 dilution are listed in Table 2. Results did not vary among the various ACL studied.

PAX-2 Reactivity by Titer

	PAX-2 (1:50)	**PAX-2 (1:100)**
ACL	35/61 (57%)	0/61 (0%)
	MI=1.1; ME=1.5	MI=0; ME=0
CC-RCC (overall)	148/184 (80%)	111/184 (60%)
	MI=2.5; ME=2.5	MI=2.4; ME=2.6
CC-RCC (WD)	115/133 (86%)	88/133 (66%)
	MI=2.5; ME=2.7	MI=2.4; ME=2.8
CC-RCC (PD)	33/51 (65%)	23/51 (45%)
	MI=2.4; ME=2.3	MI=2.4; ME=2.4

Specificity for metastatic CC-RCC

PAX-2 (1:50 dilution)	43%
PAX-2 (1:100 dilution)	100%

Conclusions: ACL may show faint, usually 1+ nuclear PAX-2 expression (57%), which can be eliminated by antibody dilution. Since not all ACL have this expression, negative control tissues must be chosen carefully. Antibody dilution significantly decreased the sensitivity of PAX-2 for CC-RCC. The alternative, a requirement of 3+ staining intensity with more concentrated antibody, also lowered the sensitivity. PAX-2 did show immunoreactivity in the subset of PD tumors, but with decreased sensitivity (65 and 45%). This study highlights problems with the use of PAX-2 in the distinction of adrenal cortex from CC-RCC, and underscores the importance of using a panel of antibodies in this diagnostic setting.

824 Assessment of Utility of TMPRSS2: ERG Gene Aberrations To Predict Salvage Radiotherapy Prostate Cancer Outcomes

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Background: Radiotherapy for biochemical recurrence after radical prostatectomy offers durable salvage rates in about 40-50% patients, and therefore better biomarkers are needed to identify patients unlikely to benefit from this therapy. Gene fusions between androgen regulated TMPRSS2 and ETS transcription factor gene ERG has been proposed as potentially aggressive form of prostate cancer. We examined the relationship between ERG gene fusions and outcomes after salvage radiotherapy for patients with biochemical recurrence after radical prostatectomy.

Design: A tissue microarray representing 61 patients who received salvage radiotherapy for biochemical failure after prostatectomy was analyzed for TMPRSS2 and ERG fusions by fluorescence in situ hybridization assay using 5' and 3' split probes flanking ERG gene. The relationship between ERG rearrangement (translocation and deletion), other risk factors and biochemical failure after salvage radiotherapy with or without associated hormonal treatment were analyzed.

Results: Of 37 analyzable case, 49% demonstrated ERG rearrangement with 45 % rearranged through intronic deletion of 5' ERG. Rearrangement of ERG through 5' deletion is previously associated with androgen independent metastatic PCA and poor outcome in clinically localized prostate cancer. In the present cohort, there was a trend for ERG rearrangement through deletion with greater risk of PSA recurrence after initial response to salvage radiotherapy (P= 0.07).

Conclusions: In this study there is a trend for ERG fusion through deletion and association with increased biochemical failure rates after salvage radiotherapy. This molecular subtype may provide a value in the risk assessment for patients likely to harbor subclinical metastasis for biochemical failure after radical prostatectomy and of need for early systemic therapy.

825 Thyroid Transcription Factor-1 Expression in Urothelial Carcinomas

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Background: Thyroid transcription factor-1 (TTF-1) has been considered to be a sensitive and relatively specific marker of tumors originating in the thyroid and lung. It is widely used in surgical pathology practice to discriminate tumors of pulmonary and thyroid origin from tumors originating in other organs. TTF-1 however, is not 100% specific and has been reported in tumors originating in other organs. For instance, a significant proportion of small cell carcinomas of urinary bladder show TTF-1 positivity. We recently encountered a rare case of metastatic urothelial carcinoma showing TTF-1 expression, a finding not described in the literature so far. This led us to investigate the expression of TTF-1 in a series of primary and metastatic urothelial carcinomas.

Design: We studied invasive urothelial carcinoma in TURP specimens (n=13), cystectomy specimens (n=6), kidney/ureter specimens (n=6) and metastatic urothelial carcinoma from multiple sites (n=21). Representative paraffin blocks were selected and three immunostains performed, TTF-1 (clone 8G7G3/1, 1:100, DAKO), p63 (clone 4A4, 1:8000, Labvysion) and Cytokeratin 20 (Ks20.8, 1:200, DAKO). TTF-1 expression was evaluated for intensity and extent in the both invasive carcinoma and non-invasive components.

Results: Eight cases (17%) of primary invasive urothelial carcinomas showed TTF-1 expression ranging from isolated positive cells (n=6), to focal areas of diffuse positive staining (n=2). Additionally, 3 cases (6.5%) showed TTF-1 expression only in the areas of non-invasive carcinoma. Staining intensity ranged from weak to strong. TTF-1 expression was noticed in two cases of metastatic urothelial carcinomas, in form of isolated positive staining. P63 nuclear expression was noted in 43 (94%) cases, and was generally strong and diffuse. CK20 expression was positive in 33 (72%) cases.

Conclusions: Although TTF-1 is considered a relatively specific marker for lung and thyroid neoplasms, it can occasionally be expressed in tumors from other sites. The current study demonstrates TTF-1 expression in both invasive and non-invasive urothelial carcinomas. Most of these neoplasms exhibit focal staining, but occasionally

there is diffuse immunoreactivity. Interpretation of TTF-1 stain in isolation can lead to misinterpretation. TTF-1 should be interpreted in the context of the clinical settings, radiologic findings, morphologic features, and the results of other immunostains.

826 TMPRSS2-ERG Gene Rearrangement Is Associated with C-MYC Protein Over-Expression in Prostate Cancer

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Background: TMPRSS2-ERG aberration in prostate cancer constitutes one of the most common chromosomal rearrangements known in human malignancy. However, the mechanism of gene fusion mediated prostate cancer is still under investigation. MYC is an established regulator gene in prostate cancers and over expression of C-MYC protein is known to reflect deregulation of MYC gene. We sought to identify any potential association between ERG rearrangements and MYC expression in prostate cancer.

Design: A tissue microarray (TMA) representing 96 patients with clinically localized prostate cancers who underwent radical prostatectomy as the primary monotherapy was analyzed for gene fusions between TMPRSS2 and ERG by fluorescence in situ hybridization (FISH) assay using 5 and 3 split probes flanking ERG gene and C-MYC protein expression. FISH signals were scored manually (100x oil immersion) in morphologically intact, non-overlapping nuclei and a minimum of 50 cancer cells from each site. The same TMA was assessed for C-MYC protein expression using standard immunohistochemical protocol (Mouse monoclonal antibody MS-139-P0, CA 94539) and evaluated using a previously validated semi- quantitative assessment system.

Results: Rearrangement for ERG was seen in 58 % of the cases in this cohort, out of which 43% cases demonstrated ERG fusion through deletion. Intronic loss of genomic DNA between TMPRSS2 and ERG has been associated with androgen independent metastatic prostate cancer and poor outcome in clinically localized prostate cancer. Fitting a logistic regression model with MYC staining intensity (strong versus weak) and ERG status (negative, split, and deletion) as a categorical predictor, we found that ERG gene fusion (through split or deletion) is significantly associated with increased MYC expression with the effect of ERG deletion on MYC expression being stronger (P= 0.01 for split; P= 0.003 for deletion).

Conclusions: Our results indicate that TMPRSS2: ERG gene fusions are associated with significant over-expression of C-MYC in clinically localized PCA, thus suggesting an important role of C-MYC (an oncogene strongly implicated in prostate tumorigenesis) in gene fusion positive prostate cancers.

827 Atypical Small Acinar Proliferation, When Combined with Prostatic Intraepithelial Neoplasia on Biopsy, Has a Higher Rate of Cancer Detection on Subsequent Biopsies Than When Diagnosed Alone

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Background: Atypical small acinar proliferation (ASAP) can occur in three different situations in the prostatic needle biopsy (PNB): ASAP alone, or associated with high grade prostatic intraepithelial neoplasia (HPIN); either discontinuous (ASAP+PIN) or contiguous (PIN-ASAP). Using a large data set, we assess whether these different subgroups of ASAP denote a differing risk for detection of subsequent PCa.

Design: We reviewed the pathologic findings from 12304 men who underwent initial PNB during an 8 year period (May 1999- June 2007). PNBs were obtained from 28 community-based urologists in a setting without population based screening, typically for abnormalities in serum PSA or digital rectal examination. The PNBs were read by 3 urologic pathologists. Patients were included in the study if their initial diagnosis was ASAP, either alone or combined with HPIN, or a benign (BN) diagnosis and if at least one follow-up PNB was performed.

Results: Of the 12304 patients, 4938 (40.1%) and 329 (2.7%) had BN and ASAP diagnoses on initial biopsy. 1033 patients (845 BN; 189 ASG) were included in the study. The average number of PNBs was 2.3 and 2.4, the mean follow-up time was 2.4 and 1.2yr, and the time to second PNB was 2.1 and 0.7yr in the BN and ASAP groups, respectively. 187 (22%) and 91 (49%) developed PCa in the BN and ASAP groups, respectively ($p<0.0005$). These groups differed by age (61 vs 64yr, $p<0.00005$) but not by PSA, or extent of sampling (at first PNB). Only initial PSA predicted subsequent PCa detection ($p=0.03$, stratified by group). Within the ASAP group, Kaplan-Meier plots and Cox regression analyses reveal differing patterns in PCa detection. In all subgroups, there is a high rate of PCa detection within 0.7 yr, however, after this point the ASAP+PIN and PIN-ASAP subgroups separate from the ASAP alone subgroup and show a higher rate of PCa detection. This separation reaches statistical significance ($p=0.05$) at 3yr after initial PNB, at which point 45% of the ASAP alone patients are PCa free and only 29% of ASAP with HPIN patients are PCa free.

Conclusions: ASAP on initial PNB carries a high risk for subsequent detection of PCa compared to patients with a BN diagnosis. The presence of ASAP combined with HPIN, in any pattern, is associated with a greater risk of PCa detection in the long term (>3 yrs).

828 Comparison between Robotic Radical Prostatectomy and Open Radical Prostatectomy: Surgical Margin Status Against TNM Stage, Gleason's Score, and Tumor Volume

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Background: Robotic-assisted laparoscopic prostatectomy (RALP) is a recently developed minimally invasive alternative for prostate cancer. Its advantage over open procedure is still under evaluation, particularly in terms of surgical margin status, which is considered one of the key elements affecting patient's long-term outcome.

Design: To assess the degree of completion in excision of tumor between these two procedures, surgical margin was evaluated against the following parameters: pathologic stage, Gleason's score, and tumor volume. Intragroup assessment was also performed. A total of 584 consecutive RALPs performed over the last 1-year period and 218 open radical prostatectomies (ORPs) over the last 2-year period were retrospectively reviewed. Tumor TNM pathologic stage was divided into pT2 and pT3, Gleason's score (GS) into GS 6, GS 7, and GS 8-10, and tumor volume into <40% and ≥40%. Margin was assessed under each parameter.

Results: Results are summarized in table 1.

Table 1: Surgical margin status against TNM stage, Gleason's score and tumor volume

	RALP margin status		**ORP margin status**	
	Positive	Negative	Positive	Negative
pT2	60 (12%)	438	43 (22%)	151
pT3	54 (63%)	32	19 (79%)	5
GS 6	11 (6%)	190	13 (17%)	64
GS 7	86 (24%)	267	44 (34%)	85
GS 8-10	17 (57%)	13	5 (41%)	7
<40%	35 (11%)	278	14 (16%)	82
≥40%	79 (29%)	192	48 (39%)	74

Conclusions: With the only exception of Gleason's score 8-10, when compared to ORP stage for stage, Gleason's scores for the same Gleason's scores and tumor volume for tumor volume, the percentages of positive margin incidences in RALP were significantly lower. For the Gleason's score 8-10, however, a conclusion as to the difference of positive margin incidences between ORP and RALP cannot be drawn justifiably in this study owing to the small size of samples for both RALP and ORP (in particular ORP). On intragroup comparison for both RALP and ORP, with increase of stage, Gleason's scores and tumor volume, the percentages of positive margin incidences increased significantly. In conclusion, in addition to its advantage of short hospitalization, the results of this study supported a robotic approach which provided a significantly higher rate of tumor free surgical margin compared to ORP.

829 In Situ Adenocarcinoma of the Bladder in the Absence of Infiltrating Carcinoma: Report of 24 Cases

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Background: In situ adenocarcinoma of the bladder in the absence of infiltrating carcinoma is rare and the clinical outcomes for these patients are not well-characterized.

Design: 24 cases of in situ bladder adenocarcinoma on biopsy (n=20) or TUR (n=4) without an associated invasive component were retrieved from the consult files of one of the authors. The following cases were excluded: 1) CIS demonstrating 'gland-like' or 'pseudoglandular' components; 2) villous adenoma with in situ adenocarcinoma; and 3) intestinal metaplasia with in situ adenocarcinoma.

Results: Mean patient age at diagnosis was 70 years (range 48 to 87) and 75% were male. Half of the cases were pure in situ adenocarcinoma; half were associated with either CIS or high-grade noninvasive papillary carcinoma. The in situ adenocarcinoma component consisted of one or more patterns: papillary (46% of cases), glandular (42%), cribriform (33%), and flat (25%). Mitoses, apoptosis, and necrosis were identified in 83%, 67%, and 17% of the biopsies, respectively. One case was a recent diagnosis, and 5 either refused treatment or were lost to follow-up. Of the 18 patients with available follow-up information, 9 (50%) were treated with BCG and/or cystectomy and did not develop invasive carcinoma; the remaining 9 (50%) eventually developed an invasive bladder tumor. Of these, 2 were small cell carcinoma, 3 were poorly-differentiated urothelial carcinoma (2 of these developed widespread metastases), and 4 were urothelial carcinoma, not otherwise specified. In both instances of eventual small cell carcinoma, and in 2 of the 3 cases of poorly-differentiated urothelial carcinoma, the initial biopsy consisted of pure in situ adenocarcinoma without CIS or noninvasive papillary carcinoma. Of note, none of the patients in the study developed invasive adenocarcinoma.

Conclusions: Pure in situ adenocarcinoma is associated with an increased risk of developing invasive urothelial carcinoma. Furthermore, there seems to be a correlation between these in situ lesions and eventual high-grade, prognostically-poor invasive bladder carcinomas such as small cell carcinoma and poorly-differentiated urothelial carcinoma. This is particularly notable in cases with an initial biopsy that reveals in situ adenocarcinoma alone, without associated CIS or noninvasive papillary carcinoma. While in situ and invasive adenocarcinomas commonly coexist, the finding of purely in situ adenocarcinoma does not seem to have an association with the development of eventual invasive adenocarcinoma of the bladder.

830 High-Grade Non Invasive Papillary Urothelial Carcinoma (HG-TCC) of the Urinary Bladder: Clinical Outcome in a Single Academic Center Cohort

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Background: Few long-term single academic center large cohort studies have addressed biologic outcomes in patients with primary HG-TCC following the implementation of the WHO/ISUP classification. Accurately defining outcome is crucial to assessing the merit of a recent trend in more aggressively treating this class of noninvasive neoplasms.

Design: Our surgical pathology records were searched for all primary urinary bladder non invasive HG-TCC diagnosed between 1998-2004. Electronic medical records were retrospectively reviewed.

Results: Mean patient age at diagnosis was 68 years (range 18-86) with 3:1 Male/Female ratio. With a median Follow up of 26 months (range: 1-105), an overall 56% rate of recurrence and/or progression was documented in our cohort of 80 patients. Noninvasive HG-TCC recurrences developed in 25% (20/80) of patients with 30%

of recurrences being of 2 or more episodes. Overall disease progression rate was 31% (25/80). 11/80 (14%) patients developed invasive disease (pT1-T2) with additional 14/80 (17%) patients developing widespread metastases. Overall mortality rate was 16% (13/80) with a calculated high mortality rate of 52% (13/25) among progressors occurring on average 3.6 yrs (range:1-9) following initial diagnosis. Both initial tumor size and number of initial lesions significantly predicted tumor progression in our cohort (p=0.03 and p=0.01 respectively). Neither the presence of recurrence nor the frequency of recurrence episodes were predictive of progression (p: NS).
Conclusions: Almost one third of our non invasive HG-TCC patients (31%) eventually developed invasive or metastatic disease with an ensuing 16% overall moratlity rate. The high rate of mortality among progressors (52%) would support the recent trend for adopting more aggressive treatment strategies in noninvasive HG-TCC compared to lower grade urothelial neoplasms. Identifying clinicopathologic and biologic markers to better predict progression are needed to stratify treatment strategies.

831 The Utility of Microscopic Findings and Immunohistochemistry in the Classification of Necrotic Testicular Tumors: A Study of 11 Cases

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Background: Necrotic testicular tumors are relatively frequent and can present a significant diagnostic challenge. Because of the differing treatment modalities for seminomas versus non-seminomas, accurate histologic diagnosis is critical.
Design: Eleven totally (9) or almost totally (2) necrotic testicular tumors were retrieved from our consult files. They were evaluated for histologic features and, when material was available, by immunostaining with 7 antibodies: keratin (AE1/AE3), OCT4, PLAP, AFP, CD117, CD30, and S100. A stain was scored as positive when there was distinct reactivity in a cellular distribution in the necrotic zone; only nuclear reactivity was scored for OCT4 and membrane reactivity for CD117 and CD30.
Results: Patients averaged 34 years old (range, 16-63). Mean tumor size was 16 mm (range, 7-30). All patients presented with unilateral testicular masses (6 right, 5 left); 2 also had acute pain. The combination of histological features, immunostains and, in 1 case, serum AFP permitted classification of 8 tumors (4 seminomas, 3 embryonal carcinomas, 1 yolk sac tumor). Three were not classifiable. The necrotic seminomas lacked associated coarse intratubular calcifications and were positive for OCT4 (4/4) and CD117 (3/3) but negative for keratin (4/4) and CD30 (4/4). The necrotic embryonal carcinomas had associated coarse intratubular calcifications and were positive for keratin (2/3), OCT4 (2/2) and CD30 (3/3). In 1 unclassifiable tumor, only OCT4 was positive in the necrotic tumor. We did not find PLAP, AFP and S100 stains useful, although S100 did highlight tumor "ghosts" in 1 case. Other features present in most cases included IGCNU (6/10), tubular atrophy/hyalinization (10/10), tumor "ghost" cells (9/10), scar (9/10) and inflammation (9/10). Of the 5 patients with available follow-up, 3 were free of disease at 1, 5 and 8 yrs after orchiectomy (all necrotic seminomas). One patient with yolk sac tumor (age 63 years) developed multiple widespread metastases after 15 months and died of disease. The final case was initially misinterpreted as "testicular infarction [with] no malignancy" and 16 months later the patient developed a large retroperitoneal seminoma.
Conclusions: Most totally necrotic testicular tumors can be placed into clinically important groups by assessment for coarse intratubular calcifications and staining reactions for keratin, OCT4, CD117 and CD30.

832 Small Cell Carcinoma of the Renal Pelvis and Ureter: Clinicopathologic and Immunohistochemical Features

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Background: Small cell carcinoma (SCC) arising in the renal pelvis and ureter is rare, with only case reports published in the literature. These tumors may be difficult to identify especially when mixed with a high-grade urothelial carcinoma. Further, the immunohistochemical staining pattern of these tumors arising in the renal pelvis and ureter has not been well characterized in the literature. We report the clinicopathologic and immunohistochemical findings in the largest series of such cases to date.
Design: A review of the regional cancer registry identified 10 cases diagnosed as SCC from 858 patients with renal pelvic or ureteral cancer from 1971 to 1998. The original slides, demographic data, treatment, and clinical outcome were reviewed. The original blocks were recut and stained with hematoxylin and eosin and immunostained for AE1/AE3, CK7, CK20, CD56, synaptophysin, chromogranin and TTF1 using commercially available antibodies. The cases were reclassified using previously published morphologic criteria and the immunostaining pattern of each histologic subtype was assessed semi-quantitatively.
Results: On review of the 10 cases, 5 were pure SCC and 2 had a mixed pattern [urothelial carcinoma (UC) and SCC]. The others were reclassified based on histologic features as poorly differentiated squamous carcinoma (2) and high-grade UC (1) . The 7 SCCs (pure and mixed) had an age range of 50 to 80 years (median: 67 years) with a female:male ratio of 2.5:1. The pathologic stages were IV (6/7) and III (1/7). None of the cases received neoadjuvant therapy. Of the 7 cases, 6 died of disease (median survival: 8 months) and one patient died of unrelated causes. The SCC cases (pure and SCC foci in mixed tumors) revealed positive staining as follows: AE1/AE3 (7/7), CK7 (1/7), CD56 (7/7), synaptophysin (6/7), chromogranin (4/7) and TTF-1 (5/7). Negative staining was noted for CK20 (7/7). In the mixed tumors, foci of non-SCC were positive for CD56 (2/2), focally and weakly positive for chromogranin (1/2), and negative for synaptophysin (0/2) and TTF-1 (0/2). The pure non-SCC were negative for all neuroendocrine markers.
Conclusions: SCC of the renal pelvis and ureter usually presents at an advanced stage and is clinically aggressive with poor survival. It appears to be twice as common in females than in males. Immunostaining, especially for CD56, synaptophysin, TTF-1, CK7 and CK20, may help in distinguishing these tumors from their mimics.

833 Pathology of "Organ-Confined (OC), Gleason Score (GS) 6" Prostate Carcinoma with Post-Radical Prostatectomy (RP) Biochemical Progression

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Background: Prior studies of RPs have reported a small percentage of men with biochemical progression after RP showing OC, GS6. One might predict that this should virtually never occur.
Design: We identified 2,551 (1983-2005) RPs coded by the urologists as pathologically OC, GS6 cancer with >1 year follow-up. We reexamined histopathogically the serially sectioned and completely embedded RP specimens of 37 men who developed biochemical recurrence defined as a single PSA level of ≥0.2 ng/mL.
Results: In 26/37 (70%) of cases, pathology re-review showed higher grade or stage than coded by the urologists. In 10/26 there was OC with Gleason pattern 4 (3 + 4 = 7 in 7 cases; 4 + 3 = 7 in 2 cases; 2 + 4 = 6 in 1 case). There were another 8/26 cases with OC GS 3+3=6 with tertiary pattern 4; in 4 cases, tertiary pattern 4 was described in the initial pathology report but urologists considered the tumor GS 6. 1/26 cases had GS 3+3=6 with focal extraprostatic extension (EPE). 5/26 cases had GS 3+4=7 plus EPE (4 with focal and 1 with non-focal EPE). 2/26 cases had positive margins with capsular incision listed in the report, but were considered by urologists as OC. Undergraded Gleason patterns 4 included glands that were: poorly formed-fused (8 cases); large glomeruloid (2 cases); large irregular cribriform (2 cases); small/medium irregular cribriform (8 cases); and small/medium regular cribriform (4 cases). Undergraded pattern 4 tumor occupied on average 11% of the tumor area. Mean tumor volume of the 11 true OC, GS 6 tumors were 0.33 cm^3 (range: 0.02 – 1.24). Tumor volume was <0.5 cm^3 in 9 cases. 6/11 had biochemical progression ≥8 years following RP, which is unusual. 8/11(5 with radiation) had ≤0.4 ng/mL as their maximum post-RP PSA value. 2 of these men had maximum post-RP PSA of 0.2 ng/mL with tumor volumes of 0.02 cm^3 and 0.07 cm^3. 0/11 developed systemic disease.
Conclusions: Most prior reports of OC, GS6 with progression are undergraded (upgrading with revision of Gleason system), understaged (difficulty recognizing focal EPE), or suffer from situations with ambiguous staging (capsular incision) or grading (tertiary pattern 4 or 2+4=6). Even for the rare true OC, GS6 with no pattern 4 tumor with supposed biochemical progression, some may be false positive progression based on low post-RP PSA levels and minute tumors that seem highly improbable to progress. With accurate pathologic evaluation, men with OS, GS6 (no pattern 4) prostate cancer can be told that their risk of progression is exceptionally rare.

834 Low-Grade Papillary Urothelial Carcinoma (LG-TCC) of the Urinary Bladder: Clinicopathologic Outcome Analysis in a Post WHO/ISUP Classification Single Academic Center Cohort

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Background: Few long-term single academic center large cohort studies have addressed outcome in patients with primary non invasive LG-TCC following implementation of the WHO/ISUP consensus classification.
Design: Our surgical pathology records were searched for all primary urinary bladder LG-TCCs diagnosed between 1998-2008. All histologic sections initially diagnosed by non-specialized urologic pathologists were reevaluated for accuracy of classification. Electronic medical records were retrospectively reviewed.
Results: A total of 99 cases initially diagnosed as LG-TCC were identified. Of 42 cases initially diagnosed by non-urologic pathologists, 6 (14%) were reclassified as non invasive high-grade papillary urothelial carcinoma (HG-TCC) and were excluded from outcome analysis. Our cohort of 93 primary LG-TCC patients included 68 males and 25 females (mean age: 64.7 years). On follow-up (mean: 37.6; range: 2-107 months), none of our patients died of bladder cancer. Forty-eight (51.6%) patients developed recurrent tumor, including 31 (33.3%) recurrences of LG-TCC or lower grade tumors and 17 (18.3%) recurrences of HG-TCC. 5/17 (29%) patients with grade progression also developed stage progression (3 invasive and 2 metastatic TCC) resulting in 5.3% stage progression rate for the entire cohort. Radical cystectomy was performed in 6 patients. The mean number of recurrence episodes was 2.98 (range: 1-16), mean time to first recurrence was13.9 months (range: 2-72) and mean time to progression to HG-TCC was 26.7 months (range: 2-74). The mean LG-TCC tumor size was 1.68 cm. There was no significant correlation between tumor size and likelihood for recurrence (1.84 cm vs 1.54 cm in cases with or without recurrences respectively) or grade progression (1.58 cm vs 1.47 cm in cases with or without progression to HG-TCC). A trend for higher rate of recurrence was seen in patients with multiple LG-TCC tumors at the time of their initial diagnosis compared to those with a single tumor (Odds ratio = 3.82; p = 0.08).
Conclusions: None of our non-invasive LG-TCC patients died of carcinoma. More than half developed recurrent tumor with 18.3% grade and 5.3% stage progression. Tumor size, time to first recurrence, and number of recurrences did not predict recurrence or progression. Patients with multiple lesions at initial diagnosis tended to have a higher risk for recurrence. A tendency to undercall HG-TCC as LG-TCC continues to exist even in an academic setting.

835 Anastomosing Hemangioma of the Genitourinary Tract: A Previously Undescribed Lesion Mimicking Angiosarcoma

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Background: We describe six cases of a previously unrecognized vascular neoplasm that can simulate angiosarcoma.
Design: Cases of a novel rare vascular tumor with a proclivity for the genitourinary tract encountered in our consultation material were prospectively collected between 1999 and 2008. Follow-up information was obtained when possible.
Results: There were six tumors from 4 men (66%) and 2 women, ranging in age from 49-75 years (median, 59.5) involving the kidney and renal hilum (4, 66%) and testis (2). Tumors ranged from 1.3 - 1.7 cm (median 1.6 cm) and were grossly well-marginated with a hemorrhagic mahogany spongy appearance. Microscopically, at low power they had

a loosely lobulated architecture and were associated with a medium-caliber vessel (5/6, 83%). Most kidney (3/4, 75%) tumors showed minor extensions into adjacent adipose tissue. At higher magnification, the tumors consisted of interanastomosing sinusoidal capillary-sized vessels with scattered hobnail endothelial cells within a framework of non-endothelial supporting cells. There was a minimal inflammatory backdrop consisting of lymphocytes but not plasma cells or acute inflammation. Mitoses were absent (5/6, 83%) or rare (1 case; in supporting cells). There was mild cytologic atypia in one of the cases but no multilayering of endothelial cells in any case. Vascular thrombi were typical (5/6, 83%) and the lesions had zones of central infarction with variable sclerosis (5/6, 83%). Two (33%) tumors featured prominent extra-medullary hematopoiesis and two (33%) had striking hyaline globules reminiscent of those seen in Kaposi's sarcoma. Imunohistochemistry was available on some cases and the lesions stained with CD34, CD31, and FVIII but not keratin AE1/3, EMA, HMB45, PLAP, or HCG. In all but one submitted consultation, the possibility of angiosarcoma had been raised based on the anastomosing vascular pattern. On follow-up, there were no recurrences or metastases in 4 cases (range 5 - 36 months; median 12 months), one patient was lost to follow-up and the last was a recent case.

Conclusions: Anastomosing hemangioma of the genitouninary tract is a rare neoplasm displaying some overlapping features of both sinusoidal hemangioma and hobnail hemangioma of soft tissue and skin. However, in our opinion, it is a unique neoplasm with a proclivity for the kidney. Its anastomosing appearance can lead to concern for angiosarcoma but, despite small numbers and limited follow-up in our series, evidence to date supports that the lesion is benign.

836 RAP1 Signaling in Prostate Cancer Progression

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Background: RAP1 proteins are small G-protein regulators of E-cadherin cell-cell junctions and integrin-mediated cell-matrix adhesions. The role of RAP1 in prostate cancer progression is not well understood, but it has been linked to its regulation of integrins and cellular proliferation. RAP1 GTPase-activation Proteins (GAP) or RAP1GAP facilitates hydrolysis of GTP to GDP and inactivation of RAP1 proteins.

Design: Tissue microarrays (TMA) were constructed from 73 patients who were treated for prostate cancer (PCa) with radical prostatectomy as monotherapy. Their demographic, treatment and long-term clinical outcome data were obtained. TMA sections were stained with H&E and RAP1GAP antibody. Staining was scored on a four level scale (0 = no staining, 1+ = weak staining, 2+ = moderate staining, 3+ = intense staining). The findings were correlated with pathologic features. Significance of correlation with Gleason score was determined using a two-tailed Spearman's rho rank correlation test.

Results: RAP1GAP antibody staining was more intense in the PCa tissues compared to normal tissues, and the staining intensity was significantly correlated with Gleason score ($p = 0.0004$).

Conclusions: Changes in RAP1GAP expression are significantly correlated with Gleason score, suggesting that reduced RAP1 signals may play a role in metastatic progression of prostate cancer.

837 Renal Tumors Are yet Another Potential Pitfall in the Interpretation of Positive Urovysion™ Fluorescence In-Situ Hybridization (UV FISH) Results

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Background: UV FISH is an FDA approved test for the diagnosis and surveillance of urothelial carcinomas (UC). Renal tumor (RT) cells may also be shed in urine and we encountered such a case with positive UV FISH. We sought to determine if RTs express chr. abnormalities similar to those seen in UC and if so could cause a false positive FISH result in urine cytology.

Design: UV FISH probes were applied to paraffin blocks from 14 low-grade (LG, Furhman 1&2) and 12 high-grade (HG, Furhman 3&4) clear cell renal cell carcinoma (CCC), 8 papillary, (PRCC) (type 1&2), 3 sarcomatoid (SRCC), 2 chromophobe (CRCC), and 5 oncocytomas. Tumors were evaluated for polysomy 3, 7 and 17 and loss of both 9p21 alleles in > or =10% of cells.

Results: The results of hybridization are shown in tables 1 and 2.

Distribution of UV FISH Results

Diagnosis	Positive FISH	Negative FISH	Failed FISH	Total (%)
LG CCC	3 (21%)	11 (79%)	0	14 (32%)
HG CCC	6 (50%)	5 (42%)	1 (8%)	12 (27%)
PRCC 1	2 (40%)	3 (60%)	0	5 (11.3%)
PRCC 2	0	3 (100%)	0	3 (7%)
CRCC	1 (50%)	0	1 (50%)	2(4.4%)
SRCC	2 (67%)	1 (33%)	0	3 (7%)
Oncocytoma	2 (40%)	3 (60%)	0	5 (11.3%)
Total	16 (36%)	26 (59%)	2 (5%)	44(100%)

Polysomy Distribution by Tumor Type

Diagnosis	Polysomy 3	Polysomy 7	Polysomy 17	Polysomy 9p21
LG CCC	3	0	0	0
HG CCC	4	4	1	2
PRCC 1	1	2	2	1
PRCC 2	0	0	0	0
CRCC	1	0	1	0
SRCC	2	1	2	0
Oncocytoma	2	2	0	0
Total	13	9	6	3

Logistic regression analysis revealed that poly chr. 17 had a statistically significantly association with PRCC but not with the other renal tumors (p-value 0.0027). No other chr. abnormality including poly chr. 17 had a statistically significant association with grade/ stage of malignant tumors.

Conclusions: We found that the chr. abnormalities tested for by UV FISH are also seen in benign and malignant RTs. Poly chr. 17 has a statistically significant association with PRCC but not other tumor types. UV FISH is therefore not specific for UC and false positive results may occur in the urine of patients with RTs. It is therefore prudent to correlate UV FISH results with clinical and radiologic findings both at initial diagnosis and follow up.

838 Positive Surgical Margins in Otherwise Organ-Confined Disease: Reassessment of Extraprostatic Extension Versus Capsular Incision in 568 Robot-Assisted Laparoscopic Prostatectomies

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Background: Positive surgical margins (PSM) in prostatectomy specimens result from either a capsular incision (CI) into the tumor or extraprostatic extension (EPE). The distinction between the two patterns, although challenging by histology, has significantly different clinical implications. PSM due to CI is related to surgical techniques, whereas EPE denotes the biologic behavior of the tumor. Recently, more attention is being paid to address the difficulties in the histologic assessment of CI versus EPE, focusing on the unique characteristics at different anatomic locations (Chuang and Epstein 2008, Epstein et al 2005).

Design: Between 2004 and 2007, 568 robot-assisted laparoscopic radical prostatectomy procedures were performed at our institution. Two hundred fourteen cases had either EPE, PSM or both. Among this group, seventy-six cases were originally classified as organ-confined disease with PSM at various sites (pT2). PSM was noted at a single site in fifty-five cases and at multiple sites in twenty-one cases. Applying the published revised criteria to identify EPE at various anatomic locations, these cases were re-classified as shown in the table below.

Results: Our results are summarized in Table 1.

Location	Original PSM - No EPE	Upgraded to PSM @ EPE
Anterior	1	0
Anterolateral	4	0
Posterior	3	1
Posterolateral	14	6
Apex	23	10
Bladder Neck	10	0
Multiple Sites	21	8

Applying the revised criteria resulted in upstaging from pT2 to pT3 in 33% (25/76) of our cases. In re-examination of sites with PSM, the upgrades were mostly seen at the posterolateral and apical locations with or without other sites. As shown in the table, five cases of isolated anterior or anterolateral PSM were due to CI. In only one case with multiple sites of PSM, both anterior and anterolateral locations were reclassified as EPE in addition to the posterolateral location.

Conclusions: Our data re-emphasizes that by applying the revised criteria, tailored to specific anatomic locations, EPE will be recognized more effectively, especially in posterolateral and apical locations. As a prognostically significant finding, this will impact clinical staging and patient outcome. Anteriorly and anterolaterally, using the revised criteria did not change the stage, since the presence of tumor in adipose tissue would have likely been originally classified as EPE.

839 Identification of Extraprostatic Extension at Apical Margin: A Retrospective Review of 568 Robot-Assisted Radical Laparoscopic Prostatectomies

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Background: The boundary between the prostate and the surrounding tissue is ambiguous and causes difficulty in identifying extraprostatic extension (EPE) in many cases. This problem is further confounded in the apex of the prostate where the benign glands are intermingled with skeletal muscle, creating more difficulty and controversy in recognizing EPE. Even more problematic is to determine whether positive surgical margins (PSM) are due to capsular incision (CI) into the tumor or EPE. Recent revisions to address these issues have been published (Chuang and Epstein, 2008 and Epstein, et al, 2005).

Design: A total of 568 robot-assisted laparoscopic radical prostatectomy procedures were performed between 2004 and 2007 at our institution. Two hundred fourteen patients had either EPE, PSM or both. Among this group, forty-six cases were originally labeled as PSM at apical margin, either as a sole finding or along with other sites of EPE and PSM. Applying the revised criteria to identify EPE at apex, these cases were re-classified as shown below in Table 1.

Results: The results are shown in the table below:

Original Diagnosis	Revised Diagnosis	
	Apical CI (n=19)	Apical PSM@EPE (n=27)
Apical PSM only (23)	13	10
Apical PSM + PSM @ other sites (8)	5	3
Apical PSM + EPE @ other sites (15)	1	14

Using the revised criteria, 60% of our cases (27/46), originally diagnosed as apical PSM, regardless of additional findings, were due to EPE versus CI. Of more clinical significance are the two subsets of patients, initially classified as organ-confined disease (pT2), who are now upstaged to pT3, including 44% (10/23) of patients with apical PSM only and 38% (3/8) of patients with PSM at apex as well as other sites. Although a stage upgrade is not achieved in the subset of patients with EPE at other sites, the data clearly shows the probability of PSM due to EPE at apex is much higher (93% or 14/15) when compared to CI.

Conclusions: Our data supports that utilizing the revised criteria for recognizing EPE at apical margin prevents underestimation of EPE, a prognostically significant finding,

and more accurately reflects the clinical stage of the disease. The probability of apical EPE is markedly increased with EPE at other prostatic sites.

840 Renal Perivascular Epithelioid Cell Tumors [(PEComa), so Called Epithelioid Angiomyolipoma (EAML)]: Analysis of 61 Cases Including 44 with Pure/Predominant Epithelioid (P-PEComa) Morphology and Parameters Associated with Malignant Outcome

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Background: There are 109 published cases of renal "EAML" or "malignant AML" or "PEComas", mostly as case reports or small series. Metastasis or death is reported in 54% of cases. Criteria predicting malignancy are unknown.

Design: Clinicopathologic features of 61 cases (44 P-PEComas >80% epithelioid morphology (EM) and 17 mixed PEComas with 10 to 80% EM along with AML histology) were analyzed to identify features associated with malignancy.

Results: The results of the survival analysis for various clinicopathologic parameters in P-PEComas and mixed PEComa are tabulated below. The EM displayed 3 patterns: diffuse large discohesive epithelioid cells with eosinophilic cytoplasm and frequent multinucleation (46%); carcinoma like growth of cohesive cells with clear/eosinophilic cytoplasm or sarcomatous growth of atypical malignant spindle cells (31%) and; EM admixed with spindle cells (23%); multiple patterns were often seen in a case.

Clinicopathological Parameters	P-PEComa (n=44)	Mixed PEComa (n=17)	p value for survival
Mean Age	41	51	0.001 (favorable for age < 20)
Gender (M:F)	0.8:1	1:7	0.8
Presence of Tuberous Sclerosis	46.4%	60%	0.6
Associated AML	50%	38.5%	0.04
Mean T size	11.2 cm	6.3 cm	0.001 (for size>7 cm)
Carcinoma-like or sarcomatous growth	56.3%	11.1%	0.6
Perinephric spread	42%	15%	0.001
Necrosis	65%	40%	0.03
Mitosis	8.1%	0%	0.001 (for >5/10hpf)
Mean follow-up (mo)	50	45	
Disease progression	51.9%	18.2%	0.02 (for P vs. Mixed)

Conclusions: Unlike PEComas at other sites, renal tumors are often associated with triphasic AML histology. Almost 40% of renal PEComas have malignant potential; tumors with P-PEComa histology, those greater than 7 cm, with perinephric extension and with significant mitotic activity are associated with malignant outcome.

841 Loss of Heterozygosity on Chromosome 18 in Patients with Non-Muscle Invasive Bladder Cancer: Results from a Prospective Study

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Background: Somatic alterations on chromosome (Chr) 18q21-23, such as loss of heterozygosity (LOH), have been indicated as a critical step in bladder carcinogenesis. The aim of this study was to evaluate the prognostic role of LOH on Chr 18q21-23 in patients affected by low-grade, non-muscle invasive bladder cancer (NMIBC).

Design: A group of 108 consecutive patients (65 affected by low-risk NMIBC and 43 controls) were selected for this prospective study. LOH on Chr 18 was assessed on the blood/urine pair. The primers used were D18S51, MBP LW and MBP H. The data obtained were compared with follow-up information. Results were also analysed by using artificial neural networks (ANN).

Results: Out of the 65 patients with NMIBC, 38 (58.4%) showed at least one alteration on Chr 18, while 27 (41.6%) showed no alteration. In the control group, only 2 out of 43 subjects showed LOH on Chr 18. At the end of follow-up, 29 patients were alive without recurrence, while 36 had at least one recurrence. A significant correlation between LOH on Chr 18 and status at follow-up was found (p=0.022). The Kaplan-Meier curves demonstrated a significant correlation between recurrence-free status and LOH on Chr 18 (p=0.0003). At multivariate analysis, LOH on Chr 18 (p=0.002) and the number of lesions (p=0.03) were identified as independent predictors of recurrence-free probability. ANN analysis confirmed the results from multivariate analysis.

Conclusions: This study highlights the role of LOH analysis on Chr 18 in improving recurrence prediction in patients affected by low-grade NMIBC.

842 Persistence of Expression of the TMPRSS2:ERG Fusion Gene after Pre-Surgery Androgen Ablation Is Associated with Early PSA Relapse of Prostate Cancer

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Background: The TMPRSS2:ERG fusion is a common recurrent chromosomal aberration in prostate cancer. This gene fusion has been found to be associated with moderate to poorly differentiated prostate tumours, higher stage disease with pelvic lymph node metastasis and early biochemical recurrence following radical prostatectomy. However, there are a number of conflicting studies about the prognostic importance of the gene fusion; consequently the clinical significance of TMPRSS2:ERG fusion needs to be clarified.

Design: We assessed the TMPRSS2:ERG fusion status in tumour samples from 84 patients undergoing radical prostatectomy from 1998 to 2000. Sixty patients had surgery alone (group A), while 24 patients received androgen ablation therapy for 3 months before surgery (group B).

Results: The presence of the gene fusion product was demonstrated by reverse transcription polymerase chain reaction (PCR) in 84% of group A patients and in 54% of group B (p=0.01). The levels of TMPRSS2:ERG and ERG, measured by means of real-time quantitative PCR, did not show significant association with clinico-pathological characteristics, except for a negative correlation of ERG overexpression with Gleason score (R=0.457; p=0.0001) observed in group A. The lower proportion of group B patients harbouring TMPRSS2:ERG fusion suggests that androgen ablation inhibits the expression of TMPRSS2:ERG, underscoring the key role of androgen-mediated transcription control of the gene fusion. Furthermore, group B patients expressing the gene fusion experienced earlier biochemical (PSA) recurrence than group A patients (p=0.007).

Conclusions: In this specimen set, we observed that tumours in which androgen ablation prior to surgery fails to suppress expression of the gene fusion have a greater risk of recurrence. Further studies in larger tissue cohorts are under way to confirm these findings.

843 Interobserver Variability in Histologic Evaluation of Radical Prostatectomy (RP) between Central Pathologists (CP) and Local Pathologists (LP): Findings of a Multinational Clinical Trial

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Background: TAX 3501 is a randomized phase III multinational trial comparing outcome of post RP adjuvant androgen deprivation vs. docetaxel in high-risk patients. Eligibility is determined by a predicted biochemical progression free survival of 60% based on Kattan's nomogram following CP review.

Design: All RP sections were blindly rereviewed by one of two urologic CP on the trial. Data on Gleason Score (GSc), organ confined vs focal vs extensive extraprostatic extension (OC vs FEPE vs EEPE), margin (MG), seminal vesicle (SV) and lymph node (LN) status was compared between CP and LP in a total of 257 consecutive RP.

Results: GSc: Agreement was found in 181/257 (70%) RP. Among 76 cases with GSc discrepant diagnoses: CP upgrade occurred in 57 (75%) and downgrade in 19 (25%) cases. Most frequent upgrade was from 7 to 8/9 and most frequent downgrade was from 8 to 7. 37% and 2% of upgrades were of 2 and 3 GSc increments, respectively. 21% of downgrades were of 2 GSc with all remaining GSc changes being of 1 increment. **OC/FEPE/EEPE:** Agreement was achieved in 179/256 (70%) of RP. Among the 77 (30%) cases with stage discrepant diagnoses: CP upstaging occurred in 70 (91%) and downstaging in 7(9%) cases. Most frequent upstage was from FEPE to EEPE (44%) followed by OC to EEPE (27%). 25% of RP that were staged as capsular invasion by LP, were restaged as EEPE. Reasons that led to understaging of EEPE included: ambiguity at apex, tumor with desmoplasia lacking surrounding fat, ambiguity of "capsular invasion" definition. **SV Status:** Agreement was encountered in 238/257 (93%) cases. Among the 7% RP with disagreement, almost an equal number of under/overcalling was observed in LP compared to CP evaluation. Overcalling was due to presence of tumor in peri-SV tissue (not in muscle) or in ejaculatory duct and prostate tissue adjacent to SV. **MG Status:** Agreement was present in 229/256 (89%) RP. Among the 27 (11%) with discrepant diagnoses: CP review lead to reclassification from (-) to (+) in 17 (62%) and (+) to (-) in 10 (38%) cases. Overcalling due to artifactual tissue tear and undercalling due to missed cauterized/crushed tumor cells was observed. **LN status:** Positive LN missed by the LP was identified on CP review in 2/208 (1%) RP with agreement observed in all remaining cases.

Conclusions: Significant interobserver variation exists between CP and LP interpretations that could affect patient accrual to clinical trials and impact prognostication and management.

844 PAX8 (+)/p63 (-) Immunostaining Pattern in Renal Collecting Duct Carcinoma (CDC): A Useful Immunoprofile in the Differential Diagnosis of CDC vs Urothelial Carcinoma of Upper Urinary Tract

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Background: Collecting duct carcinomas (CDC) are rare aggressive tumors with variable morphologic features, at time making for a difficult dx. One of the WHO diagnostic criteria for CDC is the exclusion of urothelial carcinoma of renal pelvis (UrCa) from the differential diagnosis (DDx). PAX8 is a novel lineage restricted transcription factor expressed in renal tubules. We investigated the expression pattern of PAX8 in CDC and its utility, in combination with p63, in resolving the DDx of CDC vs upper tract UrCa.

Design: Archival tissues from 10 CDCs and 11 UrCa were retrieved from our institutional files. Immunohistochemistry of PAX8 (Protein tech group, Inc. IL.) and p63 (NeoMarkers) were performed on routine sections with Bond-Leica automated stainer using a standard immunohistochemistry protocol. Intensity of PAX8 and p63 nuclear staining was evaluated and assigned an incremental 0,1+,2+,3+ score. Extent of staining was categorized as focal (<25%), multifocal (25-75%) or diffuse (>75%).

Results: CDC: All 10 (100%) CDC were positive for PAX8. Furthermore, staining for PAX8 was moderate in intensity (2+) and diffuse in extent (>75% of cells) in 9/10 (90%) cases. p63 was positive in 2/10 (20%) CDC cases (PAX8 +/p63+). p63 staining was multifocal in both cases. **UrCa:** The 11 upper tract UrCa included 2 pTa, 3 pT2, 4 pT3 and 2 pT4 tumors. All were negative for PAX8 while 9/11 (82%) were p63 positive. The p63 positivity was generally of moderate intensity and multifocal in extent. The 2 p63 negative UrCa were low grade pTa tumors and were also negative for PAX8 (PAX8 -/p63-).

Conclusions: We suggest a panel including PAX8 and p63 in the differential diagnosis of poorly differentiated renal sinus epithelial neoplasms where the DDx includes CDC vs UrCa. The immuoprofile PAX8+/p63- supports the diagnosis of CDC with a sensitivity of 80% and a specificity of 100%. On the other hand, a (PAX8-/p63+) profile supports the diagnosis of UrCa with a sensitivity of 82% and a specificity of 100%. The inverse PAX8/p63 profiles seen in CDC and UrCa in our study further

support a renal tubular rather than a urothelial differentiation in renal CDC given the lineage restriction of PAX8.

845 Differential Steroid Hormone Expression in Renal Cell Carcinoma Subtypes

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Background: Recent studies have shown steroid hormone receptor positivity in renal cell carcinomas (RCC) specifically progesterone (PR) reactivity has been identified in renal oncocytoma and chromophobe renal cell carcinoma (CHR RCC), and estrogen (ER) and PR reactivity has been described in non-neoplastic renal tissue. Therefore, we have evaluated subsets of RCC to evaluate ER, PR and androgen receptor positivity and distribution, and whether these can provide diagnostic tools for renal cell carcinoma subtype differentiation.

Design: Archival formalin-fixed, paraffin-embedded renal tumor tissue from patients (n=95) who underwent nephrectomy over a 4- year period were combined into a tissue microarray and immunohistochemically stained for androgen (polyclonal rabbit antibody), ER (SP1) and PR (1E2). Immunoreactivity was scored semiquantitatively and results correlated with renal cell carcinoma subtype and clinicopathologic variables.

Results: The cohort included 73 cases of RCC (histological subtypes: 42 conventional clear cell type (CC RCC), 24 chromophobe,7 papillary), 15 oncocytoma, and 7 urothelial carcinoma. Immunoreactivity was: A) PR reactivity was found in 50% of CHR RCC, 100% of oncocytoma, 57% of urothelial cell carcinomas (staining was limited to non-neoplastic urothelium). B) None of the tumors or non-neoplastic components displayed ER positivity. C) Androgen positivity was found in 45% of CC RCC and 50%CHR RCC. Reactivity in CC RCC varied from weak to strong in 10-75% of nuclei. Androgen positivity in CHR RCC was weak to moderate in 10-20% of nuclei.

Conclusions: This study demonstrates a differential steroid hormone reactivity (PR and AR) in RCC subtypes. PR staining does not differentiate oncocytoma from CHR RCC however may be useful to differentiate them from other subtypes of RCC. Androgen receptor reactivity in CC and CHR RCC and in none of the oncocytomas or papillary RCC suggests inclusion in a panel of markers used to separate CHR RCC from oncocytoma. It also raises the possibility that androgen modulation may play some role in CC RCC and should be further studied for its therapeutic implications in metastatic disease.

846 Fluorescence In Situ Hybridization (FISH) Assay for *ERG* Gene Disruption in Acinar (PCA-A) and Ductal (PCA-D) Subtypes of Prostatic Adenocarcinoma

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Background: The *TMPRSS2-ERG* fusion (*TMP-ERG*) has been identified as a common chromosomal aberration in PCA-A. Literature has documented an *ERG* break-apart FISH assay utilized as indirect evidence of this *TMP-ERG* fusion. *TMP-ERG* has not been identified in benign prostate tissue (BPT). The aim of our study is to determine if 1) *ERG* disruption occurs in PCA-D and 2) if differing rearrangement patterns exist between PCA-A and PCA-D subtypes.

Design: Formalin-fixed, paraffin-embedded (FFPE) sections from 13 patients with PCA and 8 organ donor prostates (ODP) were obtained from the case files of the University of Pittsburgh Medical Center. Morphologic diagnosis was confirmed on H&E sections prior to *ERG* FISH. All patients included had both PCA-A and PCA-D on either needle biopsy (n=6) or resection (n=7) specimens. 6 patients had morphologically concurrent BPT on the section. The PCA-A and PCA-D (and if possible BPT) were targeted on the same slide. Dual-color FISH using a break-apart probe for *ERG* was performed on the targeted areas. Cases were positive if ≥20% of the cells showed balanced and/or unbalanced *ERG* disruption. This threshold was established by validation on ODP and BPT in patients with PCA. Gleason score was also assessed.

Results: Median (med) age of patients was 59 yrs. 77% (10/13) of the cases were *ERG* positive (PCA-A: 35%-med; PCA-D: 55%-med). 90% (9/10) *ERG*+ cases were positive for *ERG* disruption in both the PCA-A and PCA-D components. 46% (6/13) of the PCA-D had complex disruptions vs. 15% (2/13) of PCA-A. There was no association of *ERG* disruption with Gleason score. Low-level *ERG* disruption (below 20%) was identified in 30% of ODP (range 0-3.3%) and in all BPT of patients with PCA (15.4%-med; range 10.5-19%).

Conclusions: *ERG* disruption was identified in 77% of cases, considerably higher than previous published reports. The PCA-D component had a higher percentage of *ERG* disruption and had more complex disruptions than the PCA-A component. Although below threshold, concurrent BPT in patients with PCA had a level of *ERG* disruptions significantly higher than those of ODP. In contrast to previous reports, this suggests that *ERG* disruptions in the "BPT" of patients with PCA may precede morphologically recognizable PCA.

847 Expression of SOX2 and SOX17 in Testicular Germ Cell Tumors

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Background: Testicular germ cell tumors (GCTs) are conventionally divided into seminoma and non-seminomatous germ cell tumor (NSGCT), and the latter encompasses embryonal carcinoma, yolk sac tumor, teratoma and choriocarcinoma. Distinction between seminoma and NSGCT is crucial for the purpose of treatment and prognostification. Classification of GCTs in orchiectomy specimens is performed by histopathologic evaluation without much difficulty, however, it can be challenging in situations such as markedly necrotic tumor and biopsy specimens from metastatic tumor with crush artifact. There have been a handful of immunohistochemistry studies to differentiate between the two categories. We report on SOX2 and SOX17 in order to evaluate differential expression in a variety of GCTs.

Design: A total of 68 cases comprised of 42 pure GCTs (33 seminomas, 9 embryonal carcinomas) and 26 mixed GCTs (including 9 foci of seminoma, 18 embryonal carcinoma, 16 yolk sac tumor, 17 teratoma, 4 choriocarcinoma) were retrieved. SOX2 and SOX17 immunostains were performed. The extent of staining was graded as 1+, 1-25%; 2+, 25-50%; 3+, 50-75%; 4+, >75%.

Results: All but one of the seminoma components from pure seminoma and seminomatous areas of mixed GCTs showed 4+ SOX17 nuclear expression with the one exception showing 2+ reaction. The latter seminoma had little inflammation, contained numerous mitotic figures, and morphologically fits the so-called anaplastic type. All seminomas were negative for SOX2. All embryonal carcinomas showed diffuse (3+ and 4+) SOX2 nuclear expression whereas they were completely negative for SOX17. 15 of 16 yolk sac tumors (94%) showed variable SOX17 expression (1+ in 2, 2+ in 2, 3+ in 6, 4+ in 5). Teratomas showed variable SOX2 and SOX17 expression in epithelial elements of approximately half of cases. No Sox17 expression was seen in choriocarcinomas. SOX2 was negative in all yolk sac tumors and choriocarcinomas. Intratubular germ cell neoplasia was seen in 28 of 33 (85%) seminomas, and 31 of 35 (89%) NSGCTs, and was highlighted by SOX17 staining. Three embryonal carcinomas were associated with intratubular embryonal carcinoma, which showed SOX17(-)/SOX2(+) pattern. Additionally SOX17 expression was seen in rete testis in diffuse staining, and in gonocytes in focal and weak reaction.

Conclusions: SOX2 and SOX17 expression patterns can distinguish between seminoma and embryonal carcinoma, and this may be diagnostically useful. SOX17 expression in yolk sac tumor is in keeping with the known role of this transcription factor in the development of extraembryonic endoderm (yolk sac).

848 Squamous Hyperplasia (SH), Lichen Sclerosus (LS), Differentiated PeIN (PeIN) and Low Grade Variants of Squamous Cell Carcinoma (SCC): Distinctive Preputial Lesions in a Survey of 99 Circumcision Specimens from a Region of High Incidence of Penile Cancer

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Background: About half of penile cancers affect the foreskin and 20% originate at this site. It has been hypothesized that carcinomas of inner foreskin mucosa are histologically and perhaps etiologically different from those of the glans.

Design: To determine morphological features and prevalence of foreskin precancerous and cancerous lesions in circumcision specimens from phimotic patients at high risk to develop such lesions. 116 consecutive circumcision specimens (2-5 sections per case) were evaluated. Excluded were: condylomas (15 cases), verruciform xanthoma and an epidermoid cyst (1 case each). PeINs were classified in differentiated (HPV unrelated), 20 cases and undifferentiated (HPV related); no case found in the later group. Invasive SCCs were: usual (4), pseudohyperplastic (3), verrucous (1), papillary (1), basaloid (1).

Results:

	No lesion	SH	LS	Diff PeIN	SCC
# Cases	20	24	25	20*	10**
Average age	41	56	53	55	68

*13 cases with LS; ** 6 cases with LS

Conclusions: Preputial precancerous and cancerous lesions are distinctive and different from those of the glans. LS was found as an isolated lesion, associated with differentiated PeIN or SCC in 45% of the cases. Differentiated PeIN was present in 20 and SCC in 10% of the cases. HPV related lesions (undifferentiated PeIN and basaloid carcinoma), usually frequent in the glans, were absent or very unusual in the foreskin. Patients with SH, LS and differentiated PeIN were 12-15 years younger than those with carcinomas. Patients with phimosis in this endemic population for penile cancer are at risk for precancerous or cancerous lesions and we believe they should be circumcised.

849 Comparison of Gene Expression Profiles in Tubulocystic Carcinoma and Collecting Duct Carcinoma of the Kidney

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Background: The relationship between tubulocystic carcinoma (TCCa) and collecting duct carcinoma (CDC) of the kidney remains controversial. Some experts believe that the tumors are related, considering TCCa to be synonymous with low grade CDC. However, others maintain that the two are distinct, unrelated entities based on morphologic features and clinical outcome. In addition, recent reports suggest a distinct immunohistochemical (IHC) phenotype for TCCa, which may be useful for distinguishing this lesion from CDC and other renal tumor subtypes. To explore the relationship between TCCa and CDC, we compared the expression of several gene products at the mRNA level in cohorts of each tumor subtype.

Design: Seven cases of TCCa and eight cases of CDC were identified. Total RNA was isolated from formalin-fixed paraffin-embedded tissue from each case. Relative expression levels of vimentin, alpha methylacyl coA racemase (AMACR), E-cadherin, p53, CD10, cytokeratin 7 (CK7) and cytokeratin 19 (CK19) were assessed by quantitative RT-PCR. We selected these genes in light of recent immunohistochemical studies of TCCa, as well as previous differential expression data generated by our laboratory.

Results: TCCa were characterized by relative overexpression of vimentin, p53 and AMACR, compared to CDC ($p<0.05$ for each gene, T-Test). In general, TCCa expressed higher levels of E-cadherin and CD10, while CDC expressed higher levels of CK19; however, these trends did not reach statistical significance in this study cohort. TCCa and CDC did not express CK7 differentially. Case-to-case variability of gene expression limited the effectiveness of any one marker to distinguish the tumor types.

Conclusions: Our study demonstrates that TCCa and CDC have different expression profiles of selected genes, including vimentin, p53 and AMACR. Further analysis of additional cases, using quantitative RT-PCR and IHC, will be useful to test the reproducibility of these findings. In addition, larger studies may establish statistical differences in expression of other genes analyzed in this study. Overall, these findings support the view that TCCa and CDC should be considered as two distinct entities at the molecular level.

850 Immunohistochemical Analysis of Urachal Carcinoma (UC) with Emphasis on Its Morphologic Types and Their Differential Diagnosis with Metastatic Colonic Adenocarcinoma (CAC): Diagnostic Role of Traditional and Novel Markers

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Background: The vast majority of UC is urachal adenocarcinoma (UAC) which has several morphologic types including mucinous, colloid, enteric, not otherwise specified (NOS), mixed, and signet ring cell (SRC) types. Metastatic carcinomas from various sites can often mimic these different UAC types; such as the enteric and SRC types by those arising from the colon. However, the immunophenotypic data pertaining to UC and its morphologic types that may potentially aid in their distinction is very limited.

Design: We examined 24 bona fide UCs including 23 UACs (8 mucinous/colloid, 8 enteric, 1 NOS, 3 mixed and 3 SRC types) and 1 urachal urothelial carcinoma (uroCa) with glandular differentiation, and 5 bladder metastasis of CAC including 1 with SRC carcinoma (SRCC) differentiation. Non-urachal type bladder and equivocal UAC cases were excluded. Whole sections for all cases except for 1 from TMA were immunostained with the ff: p63, CK7, CK20, CDX2, β-catenin, claudin-18 and REG IV.

Results:

Histology	p63	CK7	CK20	CDX2	Nuclear β-catenin	Claudin-18	REG IV
UAC, overall	0/23	13/23	23/23	17/23	0/23	9/22	18/23
UAC, mucinous/colloid	0/8	5/8	8/8	7/8	0/8	2/7	7/8
UAC, enteric	0/8	6/8	8/8	6/8	0/8	2/8	5/8
UAC, NOS	0/1	1/1	1/1	0/1	0/1	0/1	0/1
UAC, mixed	0/3	0/3	3/3	3/3	0/3	2/3	3/3
UAC, SRC	0/3	1/3	3/3	1/3	0/3	3/3	3/3
Urachal UroCa	1/1	1/1	1/1	0/1	0/1	0/1	0/1
Metastatic mod diff CAC	0/5	1/5	5/5	5/5	4/5	0/5	0/5
Metastatic colonic SRCC	0/1	0/1	1/1	1/1	1/1	1/1	1/1

All UACs, urachal uroCa, and CACs showed membranous β-catenin positivity. p63 highlighted benign urachal remnants intermingled with neoplasm in 5 UCs.

Conclusions: 1) UC does not express nuclear β-catenin in sharp contrast to metastatic CAC. 2) Expression of CK7 and REG IV is higher in enteric type UAC compared to differentiated CAC. Thus, β-catenin, CK7 and REG IV may potentially aid in this morphologic differential diagnostic context. 3) In addition to the enteric type UAC, CDX2 can also be expressed with high frequency by UACs with no obvious enteric morphology. 4) The novel colonic SRCC selective markers claudin-18 and REG IV can also be expressed by SRC type UAC. 5) Although not expressed by UAC, p63 may highlight intermingled benign urachal remnants within the neoplasm.

851 Diagnostic Utility of Antibody to Smoothelin in the Recognition of Muscularis Propria (MP) in Transurethral Resection of Urinary Bladder Tumor (TURBT) Specimens

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Background: Accurate recognition of MP invasion by urothelial carcinoma is vital as it is a crossroad between conservative and aggressive management. Recently there has been attention to the hyperplastic pattern of MM which can mimic the MP. We have previously shown that smoothelin, a marker of terminally differentiated smooth muscle, is relatively specific for MP (positive staining) and is variably negative to weak in MM. The study was based on cystectomy specimen slides in which bladder cancer was not present. Pathologic staging in TURBT specimens is complicated by limited, unoriented, or highly cauterized samples. Herein, we test the capability of smoothelin in recognizing MP in TURBT specimens to substantiate its diagnostic applicability in routine practice.

Design: Representative sections from 70 TURBTs were immunostained with smoothelin (R4A; 1:150 dilution). MP was evaluated in H&E slides and the corresponding smoothelin immunohistochemistry (IHC) slides in a double blinded fashion. Smoothelin IHC staining was graded as 0 to +3.

Results: In 31/70 (44%) cases MP was involved by invasive carcinoma. Cautery artifact was present in 46/70 (66%) cases in variable amounts and IHC staining in MP was retained in its presence. 48/70 (69%) cases had MP by H&E microscopy and 48/70 (69%) cases had MP by smoothelin IHC based on 2+ or 3+ positivity in muscle bundles with round regular contour. Desmoplastic response to invasive carcinoma stained negatively for smoothelin. Retrospective analysis of morphology in 2 discrepant cases indicated the presence of cauterized MP in 1 case that was not originally identified. While in one case, MP recognizable on morphology was not detected by smoothelin. The sensitivity, specificity, positive predictive value and negative predictive value of smoothelin based on comparison with morphology in TURBT specimens was 97.9%, 95.2%, 97.9%, and 90.5%, respectively.

Conclusions: This study confirms the relatively high sensitivity and specificity for smoothelin in MP muscle including in TURBT specimens. Immunoreactivity is retained in the background of thermal tissue injury and involvement by carcinoma. Our data confirm the utility of smoothelin IHC in the accurate recognition of MP from MM or desmoplastic tumor reactions, thereby facilitating appropriate pathologic stage designation in often challenging TURBT specimens.

852 Oncocytic Papillary Renal Cell Carcinoma with Inverted Nuclear Pattern: A Distinct Subtype with an Indolent Clinical Course

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Background: Papillary renal cell carcinomas (PRCC) are the second most common renal cell carcinoma (RCC), accounting for approximately 10% of RCC. There are two types of PRCCs, which have different histologic features and clinical behavior with poorer prognosis in type 2. We present seven cases of a histologically distinct oncocytic papillary renal cell carcinoma (OPRCC) with an inverted nuclear pattern and histologic features overlapping with those of types 1 and 2 PRCC.

Design: Thirty three cases of pure PRCC were reviewed and seven cases of OPRCC with an inverted nuclear pattern were identified. We assessed their clinical and histologic features, immunohistochemical profile, and genomic aberration, in comparison with those of types 1 and type 2 PRCC. The oncocytic features was defined as abundant eosinophilic granular cytoplasm and inverted pattern as polarization of nuclei toward the surface of papillae as a single layer.

Results: The median age of the seven patients was 67 years. Grossly, tumors were well-circumscribed and small (1.2 cm ± 0.4 cm). Microscopically, the OPRCCs were composed of well-developed thin papillae, lined with a single layer of cuboidal-to-columnar oncocytic cells. The tumor cells had eosinophilic granular cytoplasm and round-to-oval nuclei. The nuclei were characteristically polarized toward the surface of the papillae and contained mostly small nucleoli. The tumors showed high expression of AMACR, CK7, MUC1, VEGF, E-cadherin, and CD117, low expression of CD10, and no expression of CK20. Genetically, we observed gains of chromosomes 3p, 11q, and 17q, and loss of chromosome 4q. All seven patients were alive with no recurrence or metastasis at a mean follow-up time of 37.1 ± 23.7 months.

Conclusions: OPRCCs show unique pathologic features with indolent clinical behavior and are more similar clinicopathologically to type 1 than to type 2 PRCC.

853 SLC45A3 Is a Common ETS Family Fusion Partner in Prostate Cancer

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Background: Recurrent gene fusions between TMPRSS2 and the ETS family transcription factors, most commonly ERG, have been identified in the majority of prostate cancers (PCa). The objective of this study was to characterize a novel gene fusion involving ERG.

Design: ERG and SLC45A3 gene rearrangements were assessed on 634 PCa samples using fluorescence in-situ hybridization (FISH). Gene fusion transcripts and ERG mRNA levels were characterized in 101 PCa samples using conventional and quantitative real-time reverse transcription-PCR.

Results: At the genomic and transcript level, we identified a novel gene fusion between the promoter of SLC45A3 and ERG. This fusion yielded ERG mRNA over-expression with levels comparable to those observed in samples harboring the TMPRSS2-ERG fusion. 553 samples could be assessed for ERG and SLC45A3 rearrangement. 37 samples (7%) showed ERG and SLC45A3 rearrangement, most likely resulting in a SLC45A3-ERG fusion, 17 samples (3%) showed SLC45A3 rearrangment only, and 267 samples (48%) showed ERG rearrangement only. Consistent with other studies, we found 55% samples with ERG rearrangement. To date, this is the largest PCa cohort assessed for ERG rearrangement. We found high levels of ERG mRNA overexpression in 90% (26/29) samples that demonstrated ERG rearrangement. We detected 7 TMPRSS2-ERG isoforms but did not observe a relationship between isoform expression and level of ERG over-expression.

Conclusions: Based on the characterization of 634 prostate cancer samples, we found that SLC45A3, a prostate-specific, androgen-regulated gene, represents the second most common 5' fusion partner of ETS genes in prostate cancer. Given the association between ERG gene rearrangement and prostate cancer progression, we hypothesize that tumors harboring the SLC45A3-ERG fusion may demonstrate the same clinical course.

854 TMPRSS2-ERG Gene Fusion Defines a Metastatic Phenotype of Prostate Cancer

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Background: Histologic and molecular heterogeneity is a hallmark of prostate cancer (PCa), suggesting that distinct cancer foci may arise independently. Previously work demonstrated that clonal intrafocal homogeneity and interfocal heterogeneity of TMPRSS2-ERG fusion status exists in multifocal PCa. This poses a significant limitation to the development of prognostic and predictive biomarkers as diagnostic needle biopsies may inaccurately represent the driving tumor(s). PCa progression is closely tied to the development of metastatic disease and previous work suggests that TMPRSS2-ERG fused PCa have a more aggressive natural history. The aim of this study was to interrogate multifocal PCa to determine if the TMPRSS2-ERG tumor foci were more apt to metastasize than fusion negative foci.

Design: We studied samples from 33 PCa patients who underwent radical prostatectomy and lymphadenectomy and that had at least two distinct cancer foci in the prostate and at least one lymph node (LN) metastasis. Each focus (PCa and LN) was assigned a

primary and secondary Gleason grade and assessed for fusion status by fluorescence in-situ hybridization (FISH). Fusion transcript was assessed by quantitative RT-PCR in a subset of cases.
Results: 15 out of the 26 cases (58%) exhibited interfocal homogeneity with regard to fusion status (9 cases being fusion negative in all foci and 6 cases being fusion positive in all foci). Two out of the 26 cases revealed interfocal heterogeneity with regard to fusion mechanism. 9 out of the 26 cases (42%) revealed interfocal heterogeneity with regard to fusion status. In all PCa cases that were fusion positive regardless of diameter we found that the corresponding LN metastasis were also fusion positive. The fusion status in the LN metastasis did not necessarily correspond to the fusion status of the largest PCa focus or to the fusion status of the focus with the highest Gleason score.
Conclusions: In this unique cohort, we found that tumor size and Gleason score was not the best predictor of pelvic LN metastasis. In this cohort, TMPRSS2-ERG fusion status was the strongest determinant of metastatic dissemination. These findings may have important clinical implications for the screening and diagnosis of PCa.

855 Vascular Expression of PDGFR-ß Isoform in Clear Cell Renal Cell Carcinoma: A Potential Therapeutic Marker?

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Background: Growing understanding of the molecular biology of Clear Cell Renal Cell Carcinoma (CRCC) has established Vascular Endothelial Growth Factor (VEGF) and Platelet-Derived Growth Factor (PDGF) mediated tumor angiogenesis as relevant therapeutic targets in this tumor type. PDGF receptor (PDGFR) is a transmembrane tyrosine kinase with α and β isoforms. Recent works underline the important role of PDGFRβ in angiogenesis promotion by pericyte recruitment. Inhibitors targeting this tyrosine kinase have been proved effective controlling tumor growth in preclinical models and are undergoing clinical testing in metastatic CRCC patients. Despite its role in pathogenesis and as a therapeutic target in CRCC, little is known about the tissular expression of PDGFRβ in this tumor type. The aim of this study is to characterize the immunohistochemical expression of PDGFRβ in tissue samples of CRCC.
Design: Three TMA with representative areas from 97 CRCC formalin-fixed paraffin-embedded specimens were constructed. Immunohistochemical study was performed using polyclonal antibody against PDGFRβ (Santa Cruz, dilution 1:200). Expression of PDGFRβ on tumor cells and vessels was assessed independently and semiquantitatively. Regarding the expression on tumor cells both the extent and intensity of staining were taken into account in a three grade score: negative, low and high staining. Expression of PDGFRβ in tumor vessels was categorized as negative, focal (positivity in ≤ 25%of tumor vessels) and diffuse (positivity in >25% of tumor vessels).
Results: While 83,5% (81) CRCC showed no expression of PDGFRβ in tumor cells; low staining was seen in 12,4% (12) of cases and high in 4,1% (4) of them. In contrast, vascular expression of PDGFRβ was detected in 51,6% (50) cases, 29 of them exhibiting diffuse and 21 focal expression.
Conclusions: Our results highlight low expression of PDGFRβ in tumor cells and document for the first time, vascular expression of this receptor in CRCC tissue samples. This data supports the studies that propose PDGFRβ as an mediator of angiogenesis in this tumor. Differential PDGFRβ vascular expression seen among CRCC cases poses if tumors with diffuse vascular staining could be more sensible to target therapy against this receptor. The consideration of vascular expression of PDGFRβ as a therapeutic marker deserves further investigation and contrast with clinical trials testing PDGFRβ inhibitors.

856 The Utility of PAX2 and Renal Cell Carcinoma Marker Immunohistochemistry in Distinguishing Papillary Renal Cell Carcinoma from Non-Renal Neoplasms with Papillary Features

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Background: PAX2 is a homeogene expressed during kidney development. It has been studied in primary and metastatic clear cell renal cell carcinoma (RCC). It has not been investigated in papillary (pap) neoplasms or in comparison to RCC marker (RCCma). We studied immunohistochemical (IHC) expression of PAX2 and RCCma in papillary RCC (P-RCC) and in a variety of non-renal papillary neoplasms (NRPN).
Design: 24 primary P-RCC (20 type I, 4 type II) and 66 NRPN {10 ovarian and 9 uterine serous pap (SP) carcinomas (Ca), 10 pap urothelial Ca, 10 cervical pap squamous cell Ca, 9 pap thyroid Ca, 5 pap squamous cell Ca of oral cavity, 2 pap intraductal mucinous pancreatic Ca, 3 choroid plexus papillomas, 2 myxopapillary ependymomas, 2 malignant meningiomas with pap features, 1 pituitary adenoma with pap features, 1 pap craniopharyngioma, 2 lung adenocarcinomas with pap features} were stained for PAX2 and RCCma. Nuclear staining for PAX2 and membranous staining for RCCma were scored semiquantitatively as: Negative: 0%, 1+: 1-25%, 2+: 26-50%, 3+: 51-100% of cells staining.
Results: 16/24 (66%) P-RCC were positive for PAX2, and 23/24 (95%) were positive for RCCma. The immunoprofiles of P-RCC were: PAX2+/RCCma+: 14/24 (58%), PAX2+/RCCma–: 1/24 (4%), PAX2–/RCCma+: 9/24 (38%), PAX2–/RCCma–: 0. Nine of 66 (13%) NRPN were positive for PAX2 {4/10 (40%) ovarian SPCa, 5/9 (68%) uterine SPCa}. RCCma was positive in 28/66 (42%), including 9/9 (100%) pap thyroid Ca, 8/10 (80%) ovarian SPCa, 4/9 (44%) uterine SPCa, 1/10 (10%) pap urothelial Ca, 1/2 (50%) pap intraductal mucinous pancreatic Ca, 3/3 (100%) choroid plexus papillomas, 1/1 (100%) pituitary adenoma with pap features, and 1/2 (50%) lung adenocarcinomas with pap features. The immunoprofiles of NRPN were: PAX2+/RCCma+: 7 of 66 (11%), PAX2+/RCCma–: 2 of 66 (3%), PAX2–/RCCma+: 21 of 66 (32%), PAX2–/RCCma–: 36 of 66 (54%). Positivity was variable between 1-3+.
Conclusions: PAX2 has lower sensitivity (66%), but higher specificity (86%) compared to RCCma (95 and 60%, respectively) for distinguishing P-RCC from NRPN. The sensitivity of PAX2+/RCCma+ immunophenotype was reduced to 58% with a slight increase in specificity (89%) for P-RCC. Therefore, PAX2 and RCCma IHC should be used and interpreted with caution in pap neoplasms, with particular attention to the possibility of ovarian and uterine SPCa, which can express both PAX2 and RCCma.

857 Immunohitochemical Study of Multilocular Cystic Renal Cell Carcinoma

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Background: Multilocular cystic clear cell renal cell carcinoma (MCCRCC) is a distinct subtype of renal cell carcinoma (RCC). The immunohistchemical properties of MCCRCC have not been studied.
Design: 21 MCCRCC were retrieved from the files at our institution. All cases were submitted for immunohistochemical studies. Five alveolar clear cell RCC (CRCC) measuring less than 2 cm in diameter and two cystic nephromas (CN) with focal clear epithelial cells were used as control.
Results: MCRCC were associated with female:male ratio of 1:4. The patient ages or tumor sizes ranged from 45 to 75 years (64±8) and 2.5 8 (4±2.5). Immunostaining for CK7 showed extensive reactivity in 3 tumors (group 1), focal immunostaining in 7 tumors (group 2) and negative in 11 cases (group 3). In group 1, the MCCRCC were predominantly cystic, with minor solid component. The tumor had focal areas of chromophil cells and displayed negative reactivity for RCC and weak reactivity for CD10. The intercystic septae were relatively cellular and showed positive reactivity for PR in a large number of cells. Group 3 showed large solid areas of clear cells. The tumor cells showed positive reactivity for RCC and CD10, and the intercystic stroma was sclerotic and showed negative reactivity for PR. Group 2 had intermediate histologic and immunhistopathological features of the other two groups. Immunostaining for CRCC and CN showed CK7-RCC+/CD10+/PR- and CK7+/focal CD+/focal RCC+/PR+ for CRCC and CN as previously described in literature. Focal areas with clear cells in CN showed weak CK7+.
Conclusions: Some MCRCC contained areas simulating CN. We propose that there is at least a subset of MCRCC that develop from CN. The difference in gender ratio between MCRCC and CN may be due to the fact that CRCC tend to develop more often in CN in male patients.

858 Prostate Biopsy Cannot Reliably Identify Patients for Focal Therapy: Correlations of Low-Risk Prostate Cancer on Biopsy with Radical Prostatectomy Findings

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Background: Growing evidence of overdetection and overtreatment in many men with low-risk tumors has resulted in the recognition that alternatives to conventional treatment strategies are needed. Organ-sparing focal ablation therapies—cryosurgery, high-intensity focused ultrasound (HIFU), photodynamic therapy and radiofrequency therapy—have emerged and are under development. At present, prostate biopsy remains the best means to evaluate patients who might be considered for a focal therapy. Can prostate biopsy reliably do so?
Design: We reviewed matching prostate biopsy and prostatectomy specimens from 142 patients at our institution from 2000 to 2008. The patients selected had low tumor volume (≤5%), low to intermediate Gleason score (GS≤6) on biopsy, and underwent subsequent prostatectomy. The pathologic parameters on biopsy (biopsy core number and positive core number) and prostatectomy (GS, tumor volume, laterality, extraprostatic extension, margin positivity and involvement of seminal vesicles and lymph node) were examined.
Results:

Table 1. Low-risk PCa on biopsy (tumor volume ≤ 5% and Gleason score ≤ 6) with 1 or ≥2 positive cores and pathologic parameters of matching radical prostatectomy

Bx Core#				Prostatectomy			
	GS ≥ 7	Vol ≥10%	Bilateral*	EPE (T3a)	SV+ (T3b)	margin+	LN+
6-12 (≥ 2+)	42/142 (30%)	64/142 (45%)	82/142 (58%)	9/142 (6%)	2/142 (1%)	29/142 (20%)	0
6-12 (1+)	22/44 (50%)	21/44 (48%)	35/44 (80%)	0	0	8/44 (18%)	0

Bilateral*, unilateral cancer in the biopsy and bilateral in prostatectomy; Vol, tumor volume

Conclusions: The majority of the patients with unilateral cancer on biopsy were found to have bilateral cancer in the prostatectomy specimen regardless of biopsy core numbers. Almost half of the patients had tumor volume ≥10% of the prostatectomy specimen, and up to 50% of the patients had GS ≥7. Moreover, a small number of patients with ≥ 2 positive cores had cancer extending beyond the prostate. We believe that prostate biopsy has limited value in predicting the pathologic parameters of PCa regardless of how many cores are positive and, therefore, cannot reliably identify patients for a focal therapy.

859 The Distribution of PAX-2 Immunoreactivity in the Prostate Gland, Seminal Vesicle, and Ejaculatory Ducts: Comparison to Prostatic Adenocarcinoma and Implications for Diagnostic Use

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Background: PAX-2 is a homeogene strongly expressed during development of the genitourinary tract. Expression of PAX-2 by immunohistochemistry has been studied mainly in renal epithelial and ovarian neoplasms with little attention to the male lower genitourinary tract. We studied PAX-2 expression in epithelium of normal seminal vesicle, normal ejaculatory duct, normal prostatic secretory epithelium, and prostatic adenocarcinoma to define its immunoreactivity pattern throughout the prostate gland and address its potential diagnostic role in the discrimination of seminal vesicle/ejaculatory duct from prostatic adenocarcinoma.
Design: Prostatectomy specimens from 12 patients were reviewed to identify seminal vesicle, ejaculatory duct, periurethral glands, benign prostatic glands, and prostatic acinar

adenocarcinoma. 2 tissue microarray (TMA) slides representing 15 seminal vesicles and 45 prostatic adenocarcinomas were also used. Immumnohistochemistry for PAX-2 (Z-RX2; Zymed, San Francisco, CA) was performed on each block.
Results: A total of 32 blocks from the 12 prostatectomies were evaluated. In these whole tissue sections, nuclear reactivity for PAX-2 was identified in 12/12 (100%) of the seminal vesicle epithelium, 9/10 (90%) ejaculatory duct epithelium, focally in 1/12 (8.3%) normal prostate secretory epithelium, 0/12 prostatic adenocarcinoma, and 0/6 high grade prostatic intraepithelial neoplasia. The intensity of nuclear PAX-2 reactivity was much stronger in seminal vesicle epithelium compared to the ejaculatory duct. The focal nuclear reactivity in normal prostatic secretory epithelium of one case was within the central zone. In the TMA sections, 15 additional seminal vesicles showed strong nuclear reactivity for PAX-2, while the 45 additional prostatic adenocarcinomas were all negative.
Conclusions: PAX-2 shows strong nuclear reactivity in seminal vesicle and ejaculatory duct epithelium, compatible with origin from the Wolffian duct. It appears to be a useful adjunctive immunohistochemical marker in the distinction of seminal vesicle/ejaculatory duct epithelium from prostatic adenocarcinoma that may be useful in difficult biopsies.

860 Glomeruloid Structures in Needle Prostatic Biopsies: Should They Be Assigned a Grade or Rather Just Grade the Surrounding Tumor?

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Background: The grading of glomeruloid structures (GS) is controversial. Some urological pathologists do not assign a grade to this pattern and just grade the surrounding tumor. Other experts in the field feel that all GS should be assigned a Gleason pattern 4. We studied the frequency and clinicopathological features of this lesion when present in prostatic needle biopsies (NB).
Design: The study was based on 264 NB and the correspondent radical prostatectomies (RP). Several clinicopathological variables were studied. Tumor extent in whole-mount processed RP was evaluated with a point-count semiquantitative method. Biochemical (PSA) progression was defined as PSA ≥ 0.2ng/mL. The data were analyzed using the Mann-Whitney test for comparison of independent samples and Fisher's exact test for comparing proportions. Time to progression-free outcome was studied using the Kaplan-Meier product-limit analysis; the comparison between the groups was done using the log-rank test.
Results: The frequency of NB showing GS was 28/264 (10.6%). From the 28 biopsies with this feature 9/28 (32.1%) biopsies showed low-grade surrounding tumor and 19/28 (67.9%) high-grade. Comparing patients without and with this feature in the needle biopsy for several clinicopathological variables, the findings were, respectively: mean age 62.9 yr and 65.6 yr (p=0.06); clinical stage T2 53.6% and 71.4% (p=0.11); mean preoperative PSA 9.6ng/mL and 9.7ng/mL (p=0.18); mean prostate weight 39.8g and 39.4g (p=0.78); positive surgical margins 42.3% and 57.1% (p=0.16); extraprostatic extension (pT3a) 24.7% and 32.1% (p=0.37); seminal vesicle invasion (pT3b) 11% and 21.4% (p=0.13); and tumor extent 34.2 positive points and 44.5 positive points (p=0.07). At 5 years, the PSA progression-free survival rates were 64% and 58% for patients without and with glomeruloid structures (log-rank, p=0.26).
Conclusions: The frequency of glomeruloid structures in needle biopsies is 10%. This feature is associated more frequently with Gleason high-grade surrounding tumor, however, the presence of this architectural pattern is not associated to any other adverse clinicopathologic finding. The result of this study seems that glomeruloid feature per se should not interfere in the grading of a tumor.

861 The Predictive Power of Prostate Cancer Extent Measurements in a Large Watchful Waiting Cohort

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Background: Decisions on radical or conservative treatment for prostate cancer are difficult. Cancer extent in prostate biopsies can be measured by a number of different methods. We wished to examine these methods in a large cohort of conservatively treated prostate cancers with considerable follow up information available, to determine the optimum method for predicting disease outcome.
Design: Patients diagnosed with gland confined prostate cancer between 1990 and 1996 and managed conservatively were identified from U.K cancer registries by the Transatlantic Prostate Group. The clinical end point was death from prostate cancer. Diagnostic specimens were reviewed and measurements of disease burden were made: fraction of positive chips in TURP specimens, fraction of positive cores in needle biopsies and the cumulative percentage of cancer in needle cores. The percentage of cancer in needle cores was measured. These assessments of disease burden were correlated with prostate cancer related deaths on univariate and multivariate analysis with Gleason score.
Results: 1656 patients had histological specimens available for review. Mean follow up was 117 months. Trans-urethral specimens accounted for 911 cases and 745 were needle core biopsies. On univariate analysis, the fraction of positive chips was the strongest indicator of outcome (X^2= 145.83). Biopsy cores showed a significant but lower association on univariate analysis: fraction of positive cores (X^2=26.24) and fractional length of tumour involvement (X^2=22.12). In multivariate analysis when compared with Gleason score, the extent of disease was still significant for the TURP specimens and also for the fraction of positive cores, but not for the fractional percentage of disease on biopsy.
Conclusions: Measurements of disease burden are helpful in predicting outcome in conservatively treated prostate cancers and could influence decision making for radical therapy. With long follow-up data available, volume of disease on prostatic chips was considerably more informative than that from biopsy material. However the fraction of positive biopsies was still a significant indicator of outcome on multivariate analysis and had better predictive ability than measurements of the percentage of core involvement.

862 YKL-40 as a Marker of Epithelial to Mesenchymal Transition in Primary and Metastatic Renal Cell Carcinoma

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Background: The process of epithelial mesenchymal transition (EMT), whereby epithelial cells acquire a more motile mesenchymal phenotype with the potential to break down extracellular matrix and invade into vessels, is now emerging as a common mechanism for invasion across many tumors. Renal cell carcinomas (RCC) are known to show a histologic correlate of EMT in the form of sarcomatoid RCC that shows a spindle cell, mesenchymal morphology and behaves very aggressively. YKL-40 is a secreted protein implicated in physiologic and neoplastic EMT and whose serum levels have been correlated to poorer prognosis in various solid tumors. We examined the tissue expression of this protein in primary, metastatic and sarcomatoid RCC.
Design: Tissue microarray sections were immunostained with antibody against YKL-40 on three distinct patient cohorts: A. Sarcomatoid RCC (n=25); B. High risk (pT3b, n=30), and low risk (pT1a, n=52) primary RCC; histology: 86% conventional/clear cell, 14% non-conventional; median follow up 77 months; C. Metastatic RCC(n=136) with a subset of matched primary(n=26) tumors; histology: 95% conventional, 5% non-conventional; median follow up 37 months. Immunohistochemistry on each core was scored as absent(0), weak(1+) or strong(2+).
Results: All sarcomatoid RCC showed strong (2+) immunoreactivity for YKL-40 confirming its mesenchymal phenotype. Primary RCC showed increased YKL-40 (mean:1.59, 63% 2+) compared to their matched metastases(mean: 1.33, 39% 2+), *P*=0.036. Among primary high and low risk RCC, YKL-40 expression did not show a significant association with either metastasis free survival or overall survival. However, a trend toward increased disease specific mortality was seen with increased YKL-40 expression in metastatic lesions, *P*=0.077.
Conclusions: YKL-40 expression is a marker of mesenchymal differentiation in RCC and a potential therapeutic target in this disease. Its downregulation at metastatic sites suggests that transition from an epithelial to a mesenchymal phenotype is largely operational at the site of the primary tumor. Tissue levels of YKL-40 do not appear to be prognostically significant in RCC.

863 IFI16 Protein: A Potential Molecular Marker in Prostate Cancer: From Molecular Lab to the Pathology Lab

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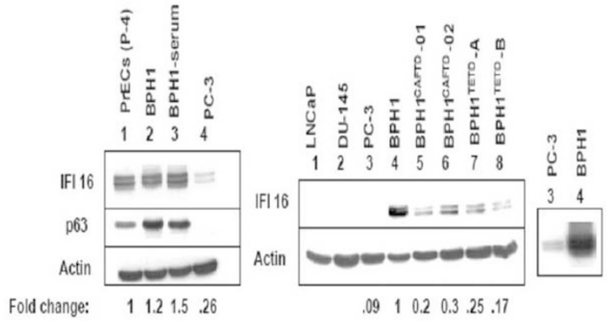
Background: Protein markers which predict progression of prostate cancer (PC) to a higher grade/stage such as overexpression of TMPRSS2 may help manage PC effectively. Interferon inducible IFI16 is a human member of the p200-protein family. Because increased expression of IFI16 protein in normal prostate epithelial cells (PrEC) inhibits cell proliferation, we investigated whether expression of IFI16 protein in the prostate could serve as a marker for low and high grade/stage PC.
Design: Expression of IFI16 protein was compared in total cell lysates from cultured normal (PrEC), a benign prostate hyperplasia cell line (BPH-1) and derivatives from the BPH1 cell line that have acquired the ability to form tumors in nude mice (aggressive lines) and PC cell lines (RWPE-1, DU-145, PC-3 and LNCaP). Human prostate tissue array slides (Imgenex) containing 98 prostate samples and 30 of our archival cases (total 45 BPH, 29 Gleason Score(GS)>6, 26 GS7, 24 GS<8, and 4 metastatic PC to bone marrow and lymph nodes) were stained with H&E and IHC for IFI16 (goat polyclonal sc-6050 Santa Cruz Biotech) and p63 (Ventana pre-dilute). All prostatic tissue arrays and our own cases were reviewed independently by two pathologists to agree on GS and rate IHC intensity and percentage of glands staining for IFI16 on a scale of 0(no stain) to 2(highest). Expression of p63 was used as a control in cell cultures and tissue IHC.
Results: Expression of IFI16 protein is detectable in normal PrECs and in a BPH cell line; however, IFI16 is reduced or lost in more aggressive BPH1 and most human PC cell lines tested. As a control p63 was lost in all PC cells and tissue.



Loss of IFI16 protein expression is associated with the development of an aggressive form of prostate cancer

Tissue expressed IFI16 in BPH and low grade PC; most high grade PC and metastasis lost staining (p=0.0003).

STAIN	BPH	GS<6	GS7	GS>8	METASTASIS
0	22(49%)	0	2(7%)	13(54%)	4(100%)
1	23(51%)	11(38%)	15(57%)	9(37%	
2	0	18(62%)	9(34%)	2(8%)	

IHC for IFI16 – #of cases (% of cases)

Conclusions: Loss of IFI16, a marker of cellular senescence, in human prostate tissue is associated with a higher grade/stage PC, including metastases to other organs. IFI16 may be implicated in the actual progression from a low to high grade/stage PC. Further studies are warranted.

864 Clear Cell Papillary Renal Cell Carcinoma (CCPAP): A Distinct Entity or a Variant of Clear Cell Renal Cell Carcinoma?

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Background: CCPAP, a recently described tumor, exhibits many differences from clear cell (CC) and papillary renal cell carcinoma (PAP). Utilizing fluorescence in situ hybridization (FISH), 3p deletion and trisomy 7 and/ or 17 were found to be lacking in CCPAP (Gobbo S. et. al., Am J Surg Pathol 2008). This cytogenetic data—in conjunction with the morphologic features and unique immunohistochemical (IHC) profile—has been put forth as the basis for recognizing CCPAP as a distinct entity in future classification systems. We evaluated IHC expression of several markers, including hypoxia inducible factor (HIF)/ von Hippel Lindau (VHL) related proteins, in a cohort of CCPAP, CC and PAP.

Design: IHC staining for CD10, α-methylacyl-coA racemase (AMACR), 34BE12, CK7, TFE3, HIF1-α, and carbonic anhydrase IX (CA9) was performed, using a tissue microarray of 32 cases of renal cell carcinoma including 9 of CCPAP, 10 of CC and 13 of PAP. The staining was graded from 0 to 3+ (0, no staining; 1+, 1-25% cells positive; 2+; 26-50%; and 3+, >50%). Cytoplasmic / membranous expression of CD10, AMACR, CK7, and CA9 were considered positive. Only nuclear expression of TFE3 and HIF1-α were considered positive.

Results: All 9 CCPAP cases showed strong co-expression (2-3+) of CK7, HIF-1-α and CA9 with no or low (0-1+) expression of CD10 and AMACR. 34BE12 was strongly expressed (2-3+) in 5/9 CCPAP. All ten CC cases showed strong co-expression (2-3+) of HIF-1-α, CA9 and CD10 with no or low (0-1+) expression of CK7, AMACR and 34BE12. The majority of PAP cases (11/13) showed strong (2-3+) co-expression of CK7 and AMACR. None of the PAP cases exhibited staining with HIF-1-α, CA9, or 34BE12. All thirty-two cases were negative (0) for TFE3.

Conclusions: The morphological features and immunophentotype (CA9+, CK7+, 34BE12±, CD10-, AMACR-) of CCPAP are sufficiently distinct to allow easy separation from CC and PAP. However, based on CA9 and HIF-1-α expression it appears that CC and CCPAP both have activated HIF pathways. The expression of these antigens could be related to any number of genetic events. However, given the known role of the *VHL* gene in the activation of the HIF pathway the possibility of a close relationship between CC and CCPAP, at least at tumorigenesis level, cannot be excluded. Further molecular studies of the VHL locus, that include mutational analysis and promoter methylation in addition to 3p deletion studies are warranted.

865 Oncocytic Renal Neoplasms (ORN): An Immunohistochemical (IHC) Study with an Emphasis on Mammalian Target of Rapamycin (mTOR) Related Proteins

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Background: Many patients with the Birt-Hogg-Dubé (BHD) syndrome develop renal neoplasms. Tumors with oncocytic features are over-represented in this setting. The mTOR pathway plays an important role in a subset of renal tumors. Recently, alterations of the *BHD* gene have been shown in various types of sporadic renal tumors and the protein product of the gene has been shown to interact with the mTOR pathway, resulting in overexpression of mTOR related proteins. In this study we evaluated the IHC expression of several markers—including mTOR related proteins (p-AKT, p-S6, p-4EBP1)—in a cohort of sporadic ORN.

Design: 54 cases of ORN, including 9 oncocytoma (RO), 26 chromophobe carcinoma (CHR) and 19 renal cell carcinoma, unclassified, low grade oncocytic type (ORNU). were examined The unclassified category included tumors with morphologic features that precluded their inclusion among RO or CHR. IHC was performed for CK7, CD117, carbonic anhydrase IX (CA9), TFE3, p-AKT, p-S6 and p-4EBP1. Staining was graded as absent/weak (0 or 1+, 0-25% cells positive) or strong (2+ or 3+, 26 - 100%). Cytoplasmic / membranous expression of CD117, CK7, CA9, and p-S6 were considered positive. Only nuclear expression of TFE3, p-AKT and p-4EBP1 were considered positive.

Results:

Staining Grade (%) by tumor type

	CHR (n=26)		RO (n=9)		ORNU (n=19)	
	Absent/Weak	Strong	Absent/Weak	Strong	Absent/Weak	Strong
CD117	4 (15)	22 (85)	1 (11)	8 (89)	4 (21)	15 (79)
CK7	8 (31)	18 (69)	9 (100)	-	19 (100)	-
p-AKT	16 (62)	10 (38)	5 (56)	4 (44)	12 (63)	7 (37)
p-4EBP1	26 (100)	-	6 (66)	3 (34)	16 (85)	3 (15)
p-S6	25 (96)	1 (4)	9 (100)	-	16 (85)	3 (15)

No RO or ORNU expressed CK7. None of the tumors studied expressed CA9 or TFE3. Of the mTOR related markers, p-AKT expression was observed most often, with strong expression in 37 to 44% of cases. Expression of p-4EBP1 was seen in RO (34%) and ORNU (15%) but not in CHR.

Conclusions: Most tumors studied expressed CD117 irrespective of histological classification. CK7 was strongly expressed in most cases of CHR, but none of the RO or ORNU. Strong expression of p-4EBP1 was only seen in RO and ORNU. Over one third of all ORN in this study express p-AKT. The staining patterns suggest that ORNU are more closely related to RO than CHR. Correlation of these IHC results with alterations in the *BHD* gene may provided a mechanistic explanation for mTOR related marker expression in these tumors.

866 Renal Cancer in Tuberous Sclerosis: An Underdiagnosed Disease? Molecular, IHC and Pathologic Correlation

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Background: Tuberous Sclerosis Complex (TSC) is an autosomal dominant disorder characterized by hamartomatous lesions involving various organs. Mutations in TSC1 (Hamartin) and TSC2 (Tuberin) are related to various phenotypic manifestations and risks of malignancy, such as an increased incidence of renal tumors. The most common renal tumors found in TSC are angiomyolipomas (AML). Renal cell carcinomas (RCC) account for about 5% of the kidney lesions. In this study we describe the morphological findings of renal cancer in patients with TSC along with correlation to molecular and IHC analysis.

Design: Renal tumors from eight female patients with ages between 42 and 64 years were reviewed. Five patients were known to have history of TSC. The remaining three patients were from the same family but had no known history of TSC. Morphological evaluation of the tumors and IHC analysis using antibodies against Tuberin, Hamartin, CK7, CK20, CD10, SMA and TFE3 was performed in all the cases. The tumors were also examined for loss of heterozygosity (LOH) at the TSC2 locus using chromosome 16p13 markers.

Results: All eight patients had RCCs with features of clear cell carcinoma. Three of the clear cell tumors had thick papillary structures containing muscle, lined by cells with abundant clear cytoplasm. Two patients from the same family had diffuse subcapsular and intra parenchymal nodules of smooth muscle proliferation. One patient had multiple tumors within the same kidney consisting of oncocytic lesions, chromophobe and clear cell RCC. Three patients had clear cell RCC with associated AMLs. By IHC the clear cell carcinomas of TSC patients stained positive with Tuberin in all cases and three showed diffuse loss of staining with Hamartin. The chromophobe tumor and one AML showed negative staining for Hamartin. The subcapsular smooth muscle proliferation had reduced staining with Tuberin and was negative for Hamartin. Analysis for loss of heterozygosity at the TSC2 locus demonstrated LOH in two clear cell RCCs and one chromophobe RCC. No LOH was seen in the smooth muscle lesions.

Conclusions: Although the most common TSC-associated renal carcinoma is clear cell with solid or papillary features, oncocytic neoplasms and chromophobe RCC can also occur. The presence of solid smooth muscle proliferation within the kidney should alert the pathologist of the possibility of TSC. Molecular and IHC data suggest that Tuberin and Hamartin may play specific pathogenic roles in these tumors.

867 Correlation of Conventional and Modified Gleason Grading of Prostatic Adenocarcinoma after Radical Prostatectomy

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Background: The modified Gleason system recommends assigning a score to each separate tumor nodule in radical prostatectomy (RP) specimens with the assumption that outcome will depend on the highest score. This is different to the practice with the conventional method in which a score is given for the entire tumor combined. It is not known how the modified Gleason system compares with conventional system when correlated with pathological parameters in RP specimens.

Design: Three hundred and sixty consecutive RP specimens were graded according to the conventional and the modified Gleason systems.

Results: A total of 29 cases (8%) were upgraded from Gleason score (GS) 7 with a tertiary pattern 5 to GS 9. Twenty of these cases (69%) had extraprostatic extension (pT3a) and 8 (27%) surgical margin (SM) positivity. When both systems were in agreement, cases with GS 7 with tertiary pattern 5 were pT3a in 36 of 63 cases (57%) and SM positive in 17 (26%) cases and GS 9 were pT3a in 15 of 20 (75%) and SM positive in 9 (45%) cases. Seminal vesicle involvement (pT3b) and lymph node positivity were seen in 24% and 3.4% in the upgraded cases, compared with 11% and 0% in the GS 7 with tertiary pattern 5 group and 40% and 15% in the GS 9 cases. The mean tumor

volume for the upgraded cases was 4.25 cc compared with 4.0 cc for the GS 7 with tertiary pattern 5 and 5.5 cc for the GS 9 cases. Cases upgraded to GS 9 in the modified system were more likely to present with high stage disease than GS 7 with tertiary pattern 5, but not as likely as GS 9 in the conventional system (Exact P value = 0.133 Trend P-value 0.04 17). Of 26 cases of GS 3+4 with tertiary pattern 5, 10 cases were upgraded to GS 4 + 3 with tertiary pattern 5. This significantly improved prediction of extraprostatic extension (Exact P = 0.04).
Conclusions: The modified Gleason system appears to improve prediction of pathological stage, when compared with the conventional Gleason system.

868 Decreased Expression of CD38 in High Grade Prostate Cancer

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Background: Prostatic adenocarcinoma (PCa) is the most frequently diagnosed cancer of men. Although most men with PCa have an indolent course, some tumors behave aggressively. Methods to identify potentially lethal PCa represent a major challenge, particularly based on small biopsy samples. Tumor grade is prognostically important, but substantial differences have been observed between grades assigned on biopsies versus subsequent prostatectomies. Grade-selective immunohistochemical markers may improve grading and allow identification of aggressive PCa. We used microarray data mining and tissue microarray (TMA) methods to identify and validate the grade-selective marker CD38.
Design: Using microarray RNA expression datasets, we identified genes whose RNA expression patterns distinguished Gleason pattern 3 from high grade (HG; Gleason patterns 4 and 5) patterns. CD38 was down-regulated in HG PCa, whereas prostate specific membrane antigen (PSMA) was up-regulated in HG PCa. TMAs were made from 48 prostatectomy specimens, with 40, 36, and 5 areas of pattern 3, 4, and 5, respectively. Commercial antibodies and 3,3-diaminobenzidine (DAB) were used to evaluate CD38 and PSMA expression. Immunostained slides were scanned and DAB staining intensity/area of PCa glands was determined using Aperio slide digitalization and computer-assisted techniques. P-values were derived from t-tests.
Results: CD38 expression was decreased in HG PCa. The mean expression was 23.3(standard error[SE]=5.2), 18.1(SE=4.9), and 4.5(SE=2.6) in patterns 3, 4 and 5, respectively. The expression in patterns 3 and 5 was significantly different(p=0.002). There was a trend toward higher PSMA expression in HG PCa. The mean expression was 35.2(SE=5.3), 45.9(SE=4.8), and 57.9(SE=5.4) in patterns 3, 4 and 5, respectively. CD38 has been shown to induce apoptosis in B-cell precursors. Decreased CD38 expression has been observed in proliferative prostate tissues including fetal prostate, benign prostatic hyperplasia and PCa. Our finding that CD38 expression is significantly decreased in HG PCa has not been reported previously to our knowledge. Together, these data suggest that HG PCa may have a proliferative advantage due to decreased CD38 expression and resultant decreased tumor cell apoptosis.
Conclusions: CD38 expression is significantly decreased in HG PCa. Diminished CD38 expression may result in decreased apoptotic activity and increased aggressiveness in HG PCa. Additional studies may address the potential utility of CD38 or other grade-selective markers in improving biopsy grading.

869 Lymphomas of the Genitourinary Tract: A Clinicopathologic Study of 40 Cases

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Background: Lymphomas of the genitourinary tract are rare, comprising only 3% of all primary extranodal lymphomas. Few reports have been published describing the predominant sites and subtypes of genitourinary tract lymphomas. Further characterization of these rare neoplasms may contribute to a more complete understanding of the pathogenesis of the disparate entities of this heterogeneous disease category.
Design: We identified 40 patients with lymphomas of the genitourinary tract. H&E slides and immunohistochemical stains were reviewed.
Results: Mean age was 56 years (range 4-86 years). Among renal, bladder, and ureter lymphomas, a male predominance was noted (1.6:1). In most cases (25, 63%), the genitourinary tract was the site of initial presentation of lymphoma. The lymphoma subtypes observed were diffuse large B-cell lymphoma (19 cases, 48%); Burkitt lymphoma (5 cases, 12.5%); extranodal marginal zone lymphoma (4 cases, 10%), with one case arising within a myelolipoma; SLL/CLL (4 cases, 10%); follicular lymphoma (4 cases, 10%); pre-B ALL (2 cases, 5%); plasmacytoma, and T-cell lymphoma NOS (1 case, or 2.5% each). The kidney (15 cases, 37.5%) was the most frequent site of disease. Other affected sites included testis (9 cases, 22.5%), prostate (8 cases, 20%), bladder (5 cases, 12.5%), penis (2 cases, 5%), and ureter (1 case, 2.5%). Two patients eventually had involvement of more than one genitourinary site.
Conclusions: Genitourinary tract lymphomas most commonly occurred in the kidney. B-cell non-Hodgkin lymphomas predominated, with diffuse large B-cell lymphoma being the most common subtype. Extranodal marginal zone lymphoma was seen only in the kidney, rather than the bladder, where it is typically thought to be more common. Burkitt lymphoma was the second most prevalent subtype, and occurred most often in the kidney. This may reflect overrepresentation of pediatric and HIV-positive individuals in our institution's patient and referral base population, as Burkitt lymphoma is more prevalent in these groups. While this study confirms the predominance of diffuse large B-cell lymphoma in extranodal sites, the findings also highlight the variety of lymphomas that may occur in the genitourinary tract. This diversity of subtypes affirms the importance of fully characterizing lymphomas by immunohistochemistry and other modalities, which are indispensable for accurate diagnosis.

870 Mutations in the Mucin Binding Domain of the Human Kidney Injury Molecule-1 (KIM-1) Gene Are Present in Renal Epithelial Neoplasms

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Background: KIM-1 is a sensitive and specific marker for identifying clear cell renal cell carcinoma (CRCC) and papillary renal cell carcinoma (PRCC). Over-expression of KIM-1 is only frequently observed in both CRCC and PRCC (Lin et al. AJSP 2007;31:371). Recent study showed KIM-1 to be a phosphatidylserine receptor with a phagocytic activity. But the role of over-expression of KIM-1 in RCC is not known. Elevated protein levels could arise as a result of mutations in the KIM-1 coding and/or promoter regions, or perhaps from an epigenetic basis. Alterations in the KIM-1 protein could also play a role in tumor pathophysiology. We therefore performed sequence analysis of the KIM-1 gene in four subtypes of renal epithelial neoplasms, i.e., CRCC, PRCC, ChRCC, and oncocytoma.
Design: Genomic DNA was prepared from paraffin-embedded tumor tissue containing four subtypes of renal tumors. Using the purified DNAs as the template, PCR was performed to amplify each of the nine major coding exons of the KIM-1 gene. Each PCR product then went through the cloning and sequencing process. Vector NTI software was used to compare exon DNA sequence to the published sequence of HAVCR-1 (NM_012206).
Results: No differences in DNA sequence for exons 2, 3, 5, 6, 7, 8, and 9 were encountered among the four subtypes of renal tumors. However, a number of mutations were observed for exon 4, a region of the protein thought to be in KIM-1's mucin-binding domain. The DNA sequence of exon 4 from PRCC agreed exactly with the published sequence of the protein. The reaming three subtypes of renal tumors showed a 15 base deletion in the exon 4. This would translate into a protein lacking a MTTVP stretch of protein. Additional mutations in exon 4 were observed: 1) a T to C mutation resulting in an amino acid change from Leu_{157} to Pro_{157}; 2) a T to G silent mutation in both ChRCC and CRCC (Thr_{207}); and 3) a double mutation within the same codon (T to G and A to G) in oncocytoma resulting in a change from Thr_{207} to Ala_{207}.
Conclusions: KIM-1 coding region mutations are present in CRCC but not in PRCC, even though over-expression of KIM-1 is observed in both conditions. Therefore, it seems unlikely that these mutations play a role in the regulation of KIM-1 expression. However, the finding of a 15 base deletion in Exon 4 in CRCC, ChRCC, and oncocytoma suggests that it may play a role in the development and/or progression of these tumors. Further investigation of this finding is of interest.

871 Evaluation and Significance of S100A4 Expression in Renal Neoplasms

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Background: S100 proteins are a family of calcium binding proteins, which have been implicated in various intracellular and extracellular functions ranging from the control of cell-cycle progression, cell differentiation, extracellular signaling, cell motility, signal transduction, and intercellular adhesion to invasion and metastasis. Overexpression of S100A4 has been associated with progression and poor clinical outcome in a variety of malignancies such as breast, pancreas, bladder and thyroid carcinomas. There have been conflicting reports on the expression of S100A4 in the kidney. In this study we evaluated the expression of S100A4 protein and mRNA in the normal kidney and renal neoplasms and correlated S100A4 expression with patient outcome.
Design: Tissue microarrays were created from archival paraffin-embedded tissue samples from 203 patients with renal cell neoplasms. The arrays included 155 clear cell (cRCC), 22 papillary (pRCC) and 13 chromophobe (chRCC) carcinomas as well as 13 oncocytomas (OC). The microarrays were immunohistochemically stained with an S100A4 polyclonal antibody (Dako). In addition, S100A4 mRNA was quantified by RT-PCR on frozen tissue samples of 20 cRCC, 4 pRCC, 3 OC and 3 chCC.
Results: *Results*: Nuclear and cytoplasmic S100A4 staining was detected in the glomerular epithelium and endothelium, distal tubules, collecting ducts and loops of Henle, with no or weak staining of the proximal tubules. A differential expression pattern was demonstrated between the various neoplasms. Positive staining was more common in pRCC (58%) than cRCC (11%) (P=0.01) whereas there was stronger S100A4 expression in chRCC (62%) than in OC (17%) (P = 0.04). The level of mRNA detected by RT-PCR were significantly higher in tumor as opposed to normal tissue in cRCC but not in the other neoplasms (15.4 vs 8.2 relative units, P= 0.03). Univariate and multivariate survival analysis revealed that S100A4 protein expression is an independent poor prognostic factor along with grade and stage in cRCC ($p<0.01$).
Conclusions: S100A4 expression is increased in all renal neoplasms and parallels the expression pattern seen in their histogenetic cells of origin from the normal kidney. Despite its relatively lower frequency of expression in cRCC, S100A4 was shown to be a poor prognostic factor in cRCC by both univariate and multivariate analysis.

872 Significant Inhibition of Tumor-Suppressor Genes in Renal Cell Carcinoma

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Background: One of the mechanisms involved in carcinogenesis is somatic mutation and subsequent inhibition of tumor suppressor genes enabling tumor cell growth in an uncontrolled manner. CDH1 or E-cadherin is an important gene that helps neighboring cells stick to one another (cell adhesion). It is believed that CDH1 acts as a tumor suppressor gene. Death-associated protein kinase (DAPK) is a calmodulin-regulated serine/threonine kinase and possesses apoptotic and tumor-suppressive functions. The Ras association domain family 1A gene (RASSF1A) is a recently discovered tumor

suppressor whose inactivation is implicated in the development of many human cancers. In this study, we analyze gene expression levels of 3 important tumor suppressor genes, CDH1, DAPK and RASSF1A in clear cell renal cell carcinoma (CC-RCC) as compared to matched normal renal parenchyma.
Design: Total RNA was extracted from 14 fresh CC-RCC samples and their matched benign renal parenchyma. Gene expression levels for CDH1, DAPK and RASSF1A genes were analyzed by real-time RT-PCR Quantitation with the ABI Fast 7500 Real-time PCR System using Taqman β-actin as an internal control for normalization.
Results: The mRNA expression levels are significantly lower in CC-RCC than those seen in adjacent normal parenchyma in 12 of 14 cases (85.7%) for the CDH1 gene, 11 of 14 cases (78.5%) for the DAPK gene and 12 of 14 cases (85.7%) for the RASSF1A gene. Overall, the mRNA expression levels in CC-RCC is about 32% for the CDH1 gene, 16% for the DAPK gene and 35% for the RASSF1A gene of those seen in normal renal tissues. Mean relative mRNA levels for CDH1, DAPK and RASSF1A in all 14 cases are1.0, 2.0 and 3.9 respectively as compared to a mean of 3.2, 12.9 and 11.0 for normal renal parenchyma. The differences in mRNA expression between CC-RCC and adjacent normal tissues for CDH1, DAPK and RASSF1A genes are statistically very significant ($p < 0.01$).
Conclusions: Significant inhibition of tumor suppressor genes occurs commonly in CC-RCC as seen in 85.7% cases for the CDH1 gene, 78.5% cases for the DAPK gene and 85.7% cases for the RASSF1A gene. These results support that the three tumor suppressor genes, CDH1, DAPK and RASSF1A, play significant roles in the development and/or progression of renal cell carcinoma.

873 Significance of Tertiary Pattern 5 in Prostate Needle Biopsies with Gleason Score of 3+4 or 4+3 Prostate Cancer: Pathologic Correlation Following Radical Prostatectomy

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Background: The International Society of Urologic Pathologists recommends biopsy Gleason score (GS) of 3+4 or 4+3 prostate cancer (PCA) with tertiary pattern 5 be classified as GS of 8 or 9, respectively so that it can be incorporated in the current PCA nomograms for management decisions. However, the scientific data supporting this recommendation and the overall significance of tertiary pattern 5 in prostate needle biopsy (NBX) is largely unknown.
Design: Prostate NBXs with Gleason score 7 (both 3+4 and 4+3) with tertiary Gleason pattern 5 (third least common pattern of any amount recognizable at low power) were identified to determine the prognostic significance and reporting recommendation of tertiary pattern 5. For control population NBXs with GS 7 without tertiary pattern 5 and GS 8-10 were included. For all groups only those patients who underwent subsequent radical prostatectomy as a definitive treatment were included for further analysis of pathologic outcomes as an end point.
Results: Total 30 cases met the criteria. Pretreatment serum PSA ranged from 1.7 to 42 ng/ml (median 7.4). The nmber of cores sampled ranged from 6 to 14 with one to three cores involved by tertiary pattern 5. Of 30 cases, 26 underwent radical prostatectomy (RP), 2 combined radiotherapy and androgen deprivation, 1 radiation, and 1 awaiting therapy. Of 26 patients with RP, 31% were pT2, 42% T3a, 23 % T3b, 4 % T4, 19 % with lymph node metastasis and 27% had positive surgical margins. Gleason 7 with tertiary 5 were significantly more likely to present with locally advanced caners (pT3/pT4) and lymph node metastasis, compared to Gleason 7 without tertiary 5 ($P \leq 0.0001$). At RP, pattern 5 was seen as primary, secondary and tertiary pattern in 4%, 42%, and 50% of biopsy GS 7 with tertiary 7 cases respectively, remaining 4% had no pattern 5.
Conclusions: Men with prostate cancer having biopsy GS 7 with a tertiary pattern 5 had a significantly high risk of adverse pathologic outcomes in comparison to biopsy GS 7 without tertiary 5. Our fundings justify the inclusion of tertiary pattern 5 in biopsy as a secondary pattern for prognostic and management decisions.

874 Incidence and Patterns of Prostatic Involvement by Urothelial Carcinoma in Patients with Bladder Cancer Examined by Sagittal Whole-Mount Section

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Background: Accurate assessment of prostatic involvement by urothelial carcinomas (UCa) in radical cystoprostatectomy specimens provides important information regarding tumor staging, assessment of the risk of local recurrence and prognosis in man with invasive bladder carcinoma. The reported incidences of prostatic involvement by UCa in the literature are quite variable ranging from 10-51%. In this study, we determined the prostatic involvement by UCa in a contemporary series of radical cystoprostatectomy using a sagittal whole mount sectioning protocol.
Design: Prostates from 67 radical cystoprostatectomy for bladder carcinoma performed between 2005 to 2008 were examined entirely by a protocol combining whole mount sagittal sections of the prostate along the prostatic urethra and transverse sections of remaining tissue. Prostatic involvement by in situ or invasive carcinoma, patterns of prostatic invasion, and the location of the protatic UCa are evaluated.
Results: Prostatic involvement by UCa was identified in 28 of 67 (41.8%) prostates from patients who underwent cystoprostatectomy. Half of the cases were stromal invasive prostatic UCa and the other half were in situ urothelial carcinoma. Three patterns of invasive carcinoma were identified: 1) 5 cases were direct penetrating invasion from the bladder neck; 2) 4 cases were direct invasion through the posterior perivesical tissue; and 3) the other 5 cases were invasive cancers arose from the prostatic urethra or from ductal-acinar structures. Fourteen (50%) had urothelial carcinoma in situ, with 7 of these involving the urethral mucosa only, and the other 7 involving both prostatic urethra and ductal-acinar structures. The site of involvement of the prostatic UCa was identified predominantly in the bladder neck or upper portion of the prostate (24 case), followed by entire prostate (3 cases), and lower portion of the prostate (1 case).
Conclusions: Sagittal sectioning of the prostate along the length of the urethra maximizes the detection of the prostatic UCa involvement. Using this protocol, prostatic UCa was identified in 41.8% of the prostates in patients who underwent cystoprostatectomy for invasive bladder carcinoma. Furthermore, we confirmed that both direct penetrating invasion of prostate through the bladder neck and mucosal spread are common pathways of prostatic invasion by UCa.

875 Diagnosis of Renal Mucinous Tubular and Spindle Cell Carcinoma by Virtual Karyotyping with SNP-Arrays Using Formalin-Fixed and Paraffin-Embedded Tissue

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Background: Mucinous tubular and spindle cell carcinoma (MTSCC) was listed as a histologic variant of renal cell carcinoma (RCC) in the 2004 WHO classification based on it unique morphologic and cytogenetic abberrations. Most studies have shown that MTSCC has an indolent behavior and favorable prognosis. However, there are significant problems in its differential diagnosis from RCC with sarcomatoid component or papillary RCC due to overlapping morphologic features or immunophenotypic profiles. This study evaluates the utility of virtual-karyotyping with single nucleotide polymorphism (SNP) arrays as a diagnostic modality for MTSCC.
Design: Six morphologically well-characterized MTSCCs, 3 sarcomatoid RCCs and 3 papillary RCCs with spindle cell areas were selected from three participating institutions for this study. The cases were reviewed by three genitourinary pathologists to verify the diagnosis and for selection of areas to be analyzed. DNA was obtained from 4–5 10-μm formalin-fixed paraffin-embedded (FFPE) sections and analyzed on GeneChip® Mapping 10 K 2.0 or 250K Nsp arrays (Affymetrix, Santa Clara, CA).
Results: Four of 6 MTSCCs were composed of typical tubular and spindle cell areas with mucinous stroma; 1 was predominantly made up of spindle cells with scant mucinous stroma; and other one was composed mainly of compressed tubules. Immunostains showed following positive results: RCC marker (6/6), AMACR (5/5), CD10 (2/6), and Ksp-cadherin (0/5). SNP array analysis showed characteristic chromosomal losses (-1, -14, -15) in all 6 cases and additional losses (-4, -6, -9) in 5 of 6 cases. Sarcomatoid and papillary RCCs showed chromosomal imbalances distinct from that of the MTSCC.
Conclusions: Our results show for the first time that chromosome copy number changes typical for MTSCC can be detected by virtual-karyotyping with SNP arrays using FFPE tissue. This approach appears to be highly reliable in the diagnosis of MTSCC and it has the potential to be a significant aid in the differential diagnosis from its morphological mimickers.

876 Oncofetal (IMP3) and Stem Cell Markers in Primary and Metastatic Renal Cell Carcinoma

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Background: Clinicopathologic parameters are the mainstay for prognostication of most epithelial tumors, including renal cell carcinoma (RCC). Recently, stem cell markers and the oncofetal protein IMP3 have been proposed as conferring metastasizing potential to various carcinomas. The RNA binding oncofetal protein IMP3 has shown promise as a biomarker in primary RCC. We evaluated the expression of IMP3 as well as stem cell markers CD44, OCT 3/4, and CD133 in a set of primary and metastatic RCC.
Design: Tissue microarray sections were immunostained with antibody against IMP3, CD44, OCT 3/4 and CD133 on two distinct patient cohorts: A. Locally advanced (n=30) and organ confined (n=52) primary RCC; histology: 86% conventional/clear cell, 14% non-conventional; median follow up of 77 months; B. Metastatic RCC(n=136) with a subset of matched primary(n=26) tumors; histology: 95% conventional, 5% non-conventional; median follow up of 37 months. Immunohistochemistry on each core was scored as absent(0), weak(1+) or strong(2+). For statistical purposes, IMP3 staining was dichotomized as absent(0) or present(1+) as well. Computerized image analysis was also applied to the IMP3 stained sections.
Results: Among primary RCC, only IMP3 expression correlated with decreased metastasis free survival and overall survival (P=0.005). CD44, OCT 3/4 and CD133 were not prognostic. On multivariate analysis, however, IMP3 lost its independent prognostic value with only pathologic stage being significant (P=0.0004), regardless of how IMP3 was quantitated. None of the markers studied among metastatic lesions--including IMP3--correlated with survival.
Conclusions: Oncofetal IMP3 appears to have some value as a tissue biomarker in primary RCC whereas stem cell markers CD44, OCT 3/4 and CD133 do not. However, depending on the patient population studied, IMP3 may not be a significant predictor of adverse outcome independent of pathologic stage. It is also not prognostically useful in metastatic disease.

877 What Are the Best Preoperative Predictors of Tumour Volume on Radical Prostatectomy?

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Background: Tumour volume (TV) is becoming an important parameter to assess on radical prostatectomy because it is a potential predictor of prostate cancer outcome. In the current practice of prostate specific antigen (PSA) driven biopsies using extended gland sampling, there is a need to reassess the preoperative variables that most reliably predict final TV on prostatectomy.

Design: We retrospectively collected the preoperative clinical and biopsy data in 1754 corresponding prostatectomies performed in our institution between 07/2000 and 06/2007. Prostate needle biopsies were performed using ten-core sampling. Preoperative clinical parameters included: age, gland volume, PSA, PSA density, digital rectal exam and ultrasound abnormalities. Biopsy parameters included: Gleason score, total percent core involvement (TPC), number of positive cores (NPC), high-grade prostatic intraepithelial neoplasia and perineural invasion. All prostatectomies were completely sampled. TV was routinely quantitated as a percentage of gland involvement by visual estimation. Given that TV was not distributed normally, it was transformed to the natural logarithmic scale, which resulted in better agreement with the linear regression assumptions. The relationship between TV and each of the pre-operative clinical and biopsy variables was assessed by the Spearman rank correlation coefficient. Univariate and multiple linear regression analyses were used to determine potential preoperative predictors of TV (natural logarithmic transformation) on prostatectomy.
Results: Highest Spearman correlation coefficient was found for TPC 0.55 ($p<0.001$), NPC 0.52 ($p<0.001$), PSA density (0.38 ($p<0.001$) and biopsy Gleason score 0.27 ($p<0.001$). Gland volume had a negative correlation with TV on prostatectomy (0.27; $p<0.001$). On univariate analysis, log TPC, NPC, gland volume, log PSA, PSAD, biopsy Gleason Score, perineural invasion and abnormal digital rectal exam were all statistically significant (all $p<0.001$). The final predictive multivariate model included only the following preoperative variables: log TPC, gland volume, log PSA and NPC (all $p<0.001$).
Conclusions: Although several preoperative variables were statistically significant on univariate analysis, only log TPC, gland volume, log PSA and NPC were identified as predictors of TV (natural log-transformation) on prostatectomy in the final multivariate analysis. TPC and NPC were the two strongest preoperative predictors of final TV on prostatectomy with Spearman correlation coefficients of 0.55 and 0.52, respectively.

878 Does Extensively Cystic Renal Cell Carcinoma Belong to the Same Spectrum of Tumors as Multilocular Cystic Renal Cell Carcinoma?

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Background: Multilocular cystic renal cell carcinoma (MCRCC) is a rare variant of clear cell RCC with excellent prognosis. Current WHO diagnostic criteria for MCRCC are restricted to tumors with no more than microscopic foci of clear cells in the septa. It is uncertain whether extensively cystic renal cell carcinomas (ECRCC) containing only microscopic nodules of clear cells have the same excellent outcome.
Design: We retrieved all cystic clear cell RCC from our institutional database containing 478 clear cell RCC, resected between 01/2000 and 08/2008. First, we excluded all cases with any solid component described grossly in the report. On microscopic review, the diagnosis of MCRCC was established only when WHO criteria were met. We also studied the ECRCC which had the same gross appearance as MCRCC, but on microscopy showed expansile or solid tumor foci which did not exceed x 4 field (i.e. 5 mm). We compared the clinical and the histopathological features and the follow-up in both tumor groups.
Results: We identified 24 tumors (5% of all clear cell RCC): 13 MCRCC (2.7%) and 11 ECRCC (2.3%). All tumors had similar gross features. On histology, both MCRCC and ECRCC demonstrated mutilocular cysts that were at least partially lined by clear cells containing low nuclear grade (grade 1 or focally grade 2). ECRCC in addition showed foci of clear cells forming expansile sheets or nodules. Worrisome histologic features, such as mitoses, necrosis, perinephric/hilar fat or lymphovascular invasion and sarcomatoid change were not seen in any tumor of both groups. Patient follow-up was available in 10 (77%) patients with MCRCC and 9 (82%) patients with ECRCC. All patients were alive with no evidence of disease after a mean follow-up of 29 months (range 6 to 88). The features are summarized in the table.

Variables	MCRCC (n=13)	ECRCC (n=11)
Male/Female	6/7	6/5
Age (years, mean)	57	52
Procedure:		
Partial nephrectomy	7 (54%)	3 (27%)
Radical nephrectomy	6 (46%)	8 (73%)
Tumor size (cm, mean)	3	3.9
Nuclear grade:		
Grade 1	19 (69%)	6 (55%)
Grade 2 (focal)	4 (31%)	5 (45%)
Calcification	5 (38%)	2 (18%)
Mean follow-up in months (range)	37 (21-88)	21 (6-40)
Disease progression	None	None

Conclusions: Strictly defined ECRCC represent a small subgroup of clear cell RCC. ECRCC and MCRCC belong to the spectrum of RCC with low malignant potential. ECRCC had an excellent prognosis in this series with relatively short follow-up. However, larger series with longer follow-up are required to establish their prognosis.

879 Grade and Stage Migration for Upper Tract Urothelial Carcinoma

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Background: In the era of the advent of minimally invasive technology to manage urothelial tumors, increasing numbers of patients are managed conservatively with surveillance and serial biopsies, coupled with fulguration or resection of neoplasms. We examined our series of patients with upper urinary tract cancers who underwent endoscopic biopsy and/or radical excision to identify histopathological findings and clinical outcomes associated with these tumors.
Design: The clinicopathological records of patients treated at our institution from 1992 to 2008 for urothelial cancers were reviewed. Forty patients with upper urinary tract urothelial carcinoma identified on 2 or more consecutive biopsies and/or biopsy followed by nephroureterectomy or ureterectomy were included in the study.
Results: The median interval between biopsies was 1.5 months (range 0.5 to 59.5 months). One patient was rendered cancer-free by endoscopic resection of the tumor, as the nephroureterectomy specimen obtained 1.5 months after the initial treatment contained no malignancy. Grade migration was observed in 8 patients (20%), with 2 progressing from dysplasia to high grade papillary carcinoma and 4 patients from low to high grade. One patient had a high grade tumor initially and low grade on subsequent biopsy, and another patient regressed from low grade to atypia. Stage migration was observed in 12 patients (30%), progressing from non-invasive to invasive in 11 and vice versa in 1. The interval during which the stage migration was observed averaged 7.2 months, as compared to a 4.4 month average for the rest of the group.
Conclusions: Grade and stage migration of urothelial carcinoma may occur in patients with upper tract disease managed conservatively. This may represent true morphological change of the tumor as well as a biopsy sampling error. Therefore close surveillance with serial biopsies should be emphasized in the conservative management of the upper tract urothelial neoplasms.

880 The Value of Intraoperative Assessment of Pelvic Lymph Nodes during Radical Prostatectomy (RP)

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Background: Intraoperative frozen section (FS) diagnosis of metastatic prostatic carcinoma (MC) in pelvic lymph nodes (PLN) often determines the completion of RP. There is no agreed sampling standard by which PLN are examined. Previous studies, most done in the 1980's, reported widely variable sensitivities, in some series as low as 33%. In this study, we analyzed the accuracy of FS in PLN in a contemporary cohort.
Design: All RP with PLN dissection from 2005 to 2008 were analyzed. FS on PLN at the time of RP as well as permanent sections were reviewed.
Results: A total of 302 bilateral PLN were examined by FS. The number of nodes sampled and frozen varied: 64%, 25% and 11% of cases respectively had 1, 2 and ≥3 nodes sampled on one side. All PLN, including FS remnants, were subsequently examined after permanent embedding. MC in PLN was detected in 26 cases (8.6%), 10 of which were correctly identified on FS (sensitivity 38%) while 16 were not (62% false negative). There was no false positive. In 10 of 16 false negative cases, the positive nodes were not sampled for FS. In the other 6 cases, the positive nodes were sampled, but tumor was not present on FS but appeared on the permanent sections (except for 1 case). The size of MC was smaller in these false negative cases (Table 1). In 2 cases, small MC foci were present on FS, but disappeared on permanent sections. Eight planned RP were canceled after positive FS. In addition, 208 RP with PLN dissection were performed without FS, 16 of which had MC (7.7%).

Table 1. Characteristics of PLN with metastasis.

	Size of Metastasis (mean)	
Positive PLN	<5 mm	≥ 5mm
FN, not sampled for FS (10 cases, 11 nodes)	11 (1.5mm)	0
FN, sampled for FS (6 cases, 6 nodes)	5 (1mm)	1 (5mm)
True positive (10 cases, 11 nodes)	3 (2mm)	8 (11mm)

FN, false negative.

Conclusions: Intraoperative assessment of PLN has an impact on the course of surgery, since a radical prostatectomy may be aborted if a metastasis is discovered. Microscopic metastasis is less likely to be detected on FS, and has a higher false negative rate than grossly appreciable metastasis. Up to 62% of patients with metastasis could be missed on FS. This low sensitivity could potentially lead to prostatectomy despite positive PLN, but may be remedied by more exhaustive sampling. Small foci of metastasis could be lost during suboptimal orientation and trimming on FS remnants when they are processed for permanent sectioning.

881 Warty/Basaloid (Undifferentiated) Penile Intraepithelial Neoplasia (PeIN) Is More Prevalent Than Differentiated PeIN in a Non-Endemic Region for Penile Cancer When Compared with an Endemic Area. A Comparative Study between France and Paraguay

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Background: PeINs are classified in undifferentiated (warty/basaloid) and differentiated in concordance with their HPV related and HPV unrelated pathogenesis. High incidence of HPV- related PeIN have been reported in European countries but we found no comparative study with other regions.
Design: To test the hypothesis of a higher incidence of HPV related PeIN in geographical regions non endemic for penile cancer we standardized diagnostic criteria and compared 1- penile precancerous lesions from 78 and 25 patients from France (non-endemic) and Paraguay (endemic for penile cancer). 2- PeIN associated with invasive carcinoma from 37 and 73 patients from Paris and Paraguay. Eighteen cases of PeIN not fitting into these 2 categories were excluded from the study.
Results:

PeIN	PeIn alone		PeIn with invasive cancer	
	France (%)	Paraguay (%)	France (%)	Paraguay (%)
Diff	8 (10)	16 (64)	18 (49)	57 (78)
Undiff	70 (90)	9 (36)	19 (51)	16 (22)
Total	78 (100)	25 (100)	37 (100)	73 (100)

Conclusions: HPV- related undifferentiated PeIN was more prevalent in European patients (90 vs. 36%, $p < 0.000001$; and 51 vs. 22 %, $p < 0.004$). This is in contrast to the higher frequency of HPV unrelated invasive tumors reported in European, North American and South American patients. Possible explanations may be the higher prevalence of HPV types pathogenetically related to PeIN in the European study population or the clinical under recognition of differentiated lesions.

882 Total Lymph Node Metastasis Diameter and Post-Cystectomy Survival: A Novel Marker of Bladder Cancer Prognosis

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Background: Current nodal staging systems are based on the size of the largest positive lymph node (LN). Recently, lymph node density (LND) has been proposed as a better parameter to discriminate among patients with node-positive bladder cancer for post-cystectomy survival (PCS). The total LN metastasis diameter (TLNMD) may better reflect the burden of metastatic disease and improve the ability to predict PCS.

Design: Patients and Methods: The clinicopathologic characteristics and follow-up information of 134 patients with node-positive bladder cancer following radical cystectomy without neoadjuvant chemotherapy was modeled using Cox proportional hazards regression analysis to predict PCS. Pathological specimens were retrospectively reviewed by a single genitourinary pathologist blinded to clinical outcome to determine the diameter of LN metastatic deposits. The median follow-up of survivors was 23 months.

Results: The overall median survival was 17 months, the median LND was 17%, and the median number of lymph nodes removed was 14. TLNMD was a significant predictor of PCS after adjusting for pathological T stage, lymphovascular invasion, LND, comorbidity, and extranodal extension (adjusted HR 1.1; P = 0.02), even when restricting the analysis to patients with > 10 LN removed. The predictive accuracy of a model containing the TLNMD was superior to those without this parameter and the current TNM staging system (c-index 0.71 vs. 0.67 vs. 0.62).

Conclusions: The total diameter of LN metastatic deposits is a significant and independent predictor of PCS after adjusting for all known prognostic clinicopathologic parameters among patients with node-positive bladder cancer. Revision of the current nodal staging classification using this parameter should be considered.

883 Lipoid-Cell Variant of Urothelial Carcinoma: A Neoplasm with True Lipid and Its Association with Micropapillary Carcinoma

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Background: Urothelial carcinoma (UC) is the most frequent type of bladder carcinoma and has numerous morphologic variants. The rare lipoid-cell variant of urothelial carcinoma (LCVUC) exhibits cells which mimic signet-ring lipoblasts. The exact content of these cells has not yet been reported in humans. The frequently aggressive micropapillary subtype of UC has been found to have an association with LCVUC. We report on the immunohistochemistry (IHC) and electron microscopy (EM) of 8 LCVUC cases and its association with micropapillary carcinoma (MPC).

Design: Departmental files were searched from 1/1/2000 to 9/9/2008 for high grade UC and MPC yielding 531 cases of high grade UC and twenty cases of MPC. Eight cases with lipoblastic features were identified and the percentage found in high grade UC and/or MPC were assessed. PASD stains were performed. The IHC panel included Ker 903, S100, p63, CEA, CK 20 and CK 7. The staining was designated as: 0= <5% positivity; +1= 5-25% positivity; +2= 25-50% positivity; and +3 = > 50% positivity. Five of the eight cases of LCVUC had EM performed on the lipoid cells.

Results: Histologically, seven cases of LCVUC made up between 5-50% of MPC. One case of LCVUC was found only in high grade UC and composed 5% of the tumor. The IHC results are in Table-1. The PASD stains were uniformly negative. The EM of all five cases confirmed true lipid within the lipoid cells.

Table-1

Case	Ker 903	S100	p63	CEA	CK 20	CK 7
1	H+2 M+2 L+2	H0 M0 L0	H+3 M0 L0	H+2 M+3 L+3	H0 M+1 L0	H+1 M+3 L+3
2	H+2 M+1 L+1	H0 M0 L0	H+3 M+3 L0/+1	H+2 M+3 L+3	H+2 M+2 L+2	H+2 M+2 L+2
3	H+3 M+3 L+3	H0 M0 L0	H+3 M0 L0	H+1 M+2 L+3	H+1 M+1 L0	H+3 M+3 L+3
4	M+3 L+3	M0 L0	M0 L0	M+2 L+3	M+1 L0	M+3 L+3
5	H+3 L+3	H+1 L+1	H+3 L+1	H+2 L0	HO L0	H+3 L+3
6	H+3 M+3 L+3	H+1 M0 L0	H+3 M0 L0	H+1 M+1 L+1	H0 M0 L0	H+3 M+3 L+3
7	H+3 M+3 L+3	H0 M0 L0	H+3 M0 L+2/+3	H+2 M+1/+2 L+1/+2	H+3 M+3 L+3	H+3 M+3 L+3
8	M+2 L+2	M0 L0	M0 L0	M0 L0	M+3 L+1	M+3 L+3

H= high grade UC; M= MPC; L=LCVUC

Conclusions: LCVUC is a rare entity that seems to be associated with MPC and has the same IHC profile, particularly, its nonreactivity to p63. For the first time, lipid has been confirmed in this variant and may warrant a change from "lipoid" to lipid cell variant of urothelial carcinoma.

884 Urothelial Carcinoma of the Urinary Bladder Associated with Intestinal Augmentation Cystoplasty

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Background: Patients who have undergone intestinal augmentation cystoplasty are at risk for developing latent vesical malignancy.The present study was conducted to evaluate the histological and immunohistochemical characteristics and molecular genetic alterations in these neoplasms.

Design: Four patients developing urothelial neoplams after augmentation cystoplasty were included in the current study. Tumor specimens were assessed for morphological features and immunohistochemical expression of uroplakin III, CDX2, β-catenin, and cytokeratin 7 and 20. Gene mutations in fibroblast growth factor receptor 3 (FGFR3) gene and p53 gene were analyzed and the UroVysion fluorescence in situ hybridization (FISH) tests were performed.

Results: The mean age of the patients, including two men and two women, was 37. The latency from bladder augmentation to developing malignancy ranged from 17 to 21 years (mean 19 years). All patients died of widespread metastasis months after cancer diagnosis (mean, 5 months). All tumors were high-grade (grade 3) invasive urothelial carcinoma comprising various architectural patterns with brisk mitoses and tumor necrosis. Three harbored glandular differentiation (75%) and the remaining one showed squamous differentiation (25%). All cases revealed abnormal decreasing β-catenin expression with moderate to strong membranous staining in 30~60% of tumor cells. Two tumors (50%) showed nuclear expression of CDX2, with variable staining intensity and percentages. Moderate uroplakin III staining was focally identified in one case. All but one tumors (75%) were intensely stained by cytokeratin 7. One case (25%) displayed focal cytokeratin 20 expression. In FISH analysis, all tumors displayed characteristic chromosomal abnormalities (100%). Point mutations of both FGFR3 and p53 genes were identified in one case.

Conclusions: Neoplasms developed after augmentation cystoplasty were extremely aggressive urothelial carcinoma and exhibited distinct morphologic, immunohistochemical and genetic characteristics. These neoplasms represent a rare and specific variant of urothelial carcinoma which is uniformly lethal. The UroVysion FISH analysis may offer a surveillance strategy in patients who underwent augmentation cystoplasty.

885 Frequency of *ERG* Rearrangement in a Swedish Hospital Based Biopsy Cohort

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Background: Recurrent gene fusions between *TMPRSS2* and ETS transcription factor family members, most often *ERG*, are seen in 40-80% of prostate cancers (PCa). The frequency of TMPRSS2-ERG fusion in two recent Watchful Waiting cohorts, where tumor samples were obtained from incidental transurethral resection of the prostate (TURP) samples demonstrated *TMPRSS2-ERG* fusion frequency of 15% and 17.5%. The aim of this study was to investigate if this significant difference in ETS rearrangement frequency was particular to the Swedish population or incidental PCa.

Design: We assessed the *ERG* rearrangement by fluorescence *in-situ* hybridization (FISH) using an ERG break-apart assay on a needle biopsy based PCa cohort from Sweden. This Swedish population based biopsy cohort consists of 96 men consecutively diagnosed with localized or advanced (T1-T3, Nx, M0-M1) PCa between 1989 and 1990 at the Department of Urology, Orebro University Hospital in Sweden.

Results: We observed that 74% (71/96) of the cases could be assessed for *ERG* rearrangement. Of these cases 45% (32/71) demonstrated *ERG* rearrangement, with 62.5% (20/32) being rearranged with deletion and 37.5% (12/32) being rearranged with insertion. We detected an increase in percentage of *ERG* rearranged cases with increasing tumor stage (T1 23.1%, T2 45.5% and T3 52.8%). Gleason score and nuclear grade were not associated with *ERG* rearrangement.

Conclusions: The 45% ERG fusion PCa observed in this cohort is comparable to the frequency of other hospital-based cohorts in other countries including a recent prospective study conducted by the Early Research Detection Network (EDRN). This indicates that the fusion frequency of 15% and 17.5% in the incidental Swedish Watchful Waiting cohorts may be due to lower tumor stage or tumor location and not due to ethnic differences.

886 Chromosomal Deletions at 6q, 8p, and 13q in the Early Development of Prostate Cancer

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Background: Certain genetic alterations and accumulation are strongly associated with prostate cancer progression including metastasis. Previously, chromosomal deletions/alterations at 6q, 8p, and 13q have been reported to be important at cancer progression stage and metastasis. On the other hand, little is known for the early cancer development from pre-clinical/microscopic stage to the clinically-evident cancer.

Design: A total of 184 microscopic cancers (MC) defined as limited within a 3 mm circle and corresponding 82 clinically-evident cancer (CC) nodules were selected from radical prostatectomy specimens. Tumor volumes (TV) of MC and CC were calculated and grades were evaluated by Gleason scoring system. Thirteen microsatellite loci at 6q16-22, 8p22–23, 13q14, 21 and 33 were evaluated for loss of heterozygosity (LOH). Immunohistochemistry was simultaneously performed to detect expression of alpha-methylacyl co-A racemase (AMACR).

Results: Average TV of MC was 1.26 mm^3, and range was 0.032–11.77 mm^3. Average TV of CC was 3024.7 mm^3, and range was 42–13 816 mm^3. The results are summarized in Table 1. Frequencies of LOH at 6q16–21, 6q22, 8p23.1, 8p23.2, 13q14 were significantly higher in CC (30.9%, 40.5%, 12%, 8.7% and 20.6%) than in MC (1.0%, 2.7%, 2.9%, 1.1% and 5.4%). The results were summarized in Table 2. No significant differences of AMACR expression were detected between 2 groups (positive rate: 93.4% in MC, 100% in CC).

Table 1 TV and GS

	Average TV (mm^3)	Range (mm^3)	GS≤7	GS≥8
MC	1.26	0.032-11.77	184	0
CC	3,024.7	42-13,186	68	14

GS, Gleason score; MC, microscopic cancer; CC, clinically-evident cancer

Table 2 LOH frequency

	MC (%)	CC (%)	p
6q16-21	1/100 (1)	17/55 (30.9)	⟨0.001
6q22	2/74 (2.7)	17/42 (40.5)	⟨0.001
8p22	1/34 (2.9)	5/24 (20.8)	0.072
8p23.1	2/104 (1.9)	6/50 (12)	0.015
8p23.2	1/90 (1.1)	5/57 (8.7)	0.033
13q14	6/111 (5.4)	13/63 (20.6)	0.004
13q21	0/16 (0)	1/10 (10)	0.385
13q33	1/37 (2.7)	2/22 (9)	0.549

LOH, loss of heterozygosity; MC, microscopic cancer; CC, clinically-evident cancer

Conclusions: Chromosomal deletions at 6q16–21, 6q22, 8p23.1, 8p23.2, 13q14 are important events not only for cancer progression/metastasis but also for cancer development from pre-clinical stage to be clinically evedent. Deletions at 13q21 and 33 seems to be less important for this transition.

887 SSTR2a as a Prognostic and Therapeutic Biomarker for Prostate Cancer: An Immunohistochemical and RT-PCR Analysis

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Background: In human prostate cancers, although hormonal therapy has been useful and sensitive for androgen receptor (AR) positive cases, therapy is very limited for AR-negative, advanced or recurrent cases. Somatostatin analogues have been used as therapeutic agents in somatostatin receptor type 2a (SSTR2a) positive cancers. It has been confirmed that they can inhibit the growth and metastasis in androgen-independent prostate cancers in vitro. The purpose of this study is to detect SSTR2a in human prostatic cancers by immunohistochemistry in order to predict the response to a new therapeutic method in advanced prostate cancer.

Design: Formalin-fixed and paraffin-embedded sections from 108 prostate cancer cases (97 radical prostatectomy cases and 7 of their metastatic lymph nodes, and 11 needle biopsy cases), were analyzed. The immunohistochemical study was performed by the Envision method using SSTR2a antibody (Gramsch Laboratories). Cases were considered "positive" if they were positive on the cell membrane or luminal surface. RT-PCR analysis was performed on 11 needle biopsy and 3 metastatic cases, after selection of appropriate carcinoma cells by laser microdissection.

Results: The study showed expression of SSTR2a in 13 of the 108 cases (12%); The histological grade (Gleason) and tumor stage of the prostate cancer were directly related to SSTR2a expression (u-test, p=0.013 & p=0.0014, respectively); Among the seven cases with lymph node metastasis, SSTR2 expression was markedly higher in frequency than in the 101 non-metastatic cases (u-test, p=0.0096); SSTR2 mRNA was detected in only 4 of 11 cases by needle biopsy and 1 of 3 cases of LN metastasis by RT-PCR. Although not statistically significant, the metastatic carcinoma cells revealed higher mRNA levels than primary prostate carcinoma cells. Interestingly, in SSTR2a positive cases, SSTR2a-expression was only identified in some of the carcinoma cells. The results indicate that the SSTR2a positive carcinoma cells probably have a stronger tendency for metastasis, and this phenomenon may be applicable to androgen-independent metastatic or recurrent cases.

Conclusions: SSTR2a is a prognostic factor of metastatic potential in prostatic carcinoma. Somatostatin analogues may be beneficial for patients with advanced prostate cancer if they are positive for SSTR2a.

888 Characterization of p53 and p16 in Development of Balanitis Xerotica Obliterans

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Background: Balanitis xerotica obliterans (BXO), or penile lichen sclerosus et atrophicus, is a genital skin disease which may cause voiding complications and has a possible association with squamous cell carcinoma. The etiology of BXO is unknown. It is difficult to diagnose the early phase of BXO. In this study, we attempt to determine the immunoprofile of early and late BXO using p16 and p53. P16 is a tumor suppressor gene product, associated with high-risk human papilloma virus (HPV) infection. HPV is associated with anal and genital squamous dysplasias and carcinomas. P53 mutations and increased immunostaining have been found in squamous cell carcinoma.

Design: We studied the clinicopathological features as well as p16 and p53 immunohistochemical expression in BXO aiming to find useful diagnostic features. We morphologically classified BXO into early chronic inflammatory lesions (10 cases) and sclerotic lesions (10 cases). The p16 and p53 immunostains were performed in all cases. The normal squamous mucosa and squamous cell carcinoma were used for comparison.

Results: Early BXO demonstrated band-like infiltrates in superficial dermis with obscured basal layers. Late BXO demonstrated an atrophic epidermis, loss of basal cells and a sclerotic dermis without obvious chronic infiltrates. No p16 or p53 was seen in normal squamous epithelium, while full thickness epidermal expression of both was found in squamous cell carcinoma. In early BXO, both p16 and p53 immunostains showed strong, diffuse nuclear staining in basal cells. Loss of basal cell staining was seen in some cases. In late BXO, p16 and p53 expression levels were reduced, ranging from negative to mild staining probably related to loss of basal cell.

Conclusions: We studied the p16 and p53 immunoexpression in balanitis xerotica obliterans. Based on the p16 and p53 expression pattern, we can divide BXO into two groups. These two groups may represent early and late phases of the disease or different risk groups. The p16 and p53 staining may be useful in diagnosis of early balanitis xerotica obliterans, or in study of the development of BXO associated genital squamous cell carcinoma.

889 Urothelial Carcinoma with Mucinous Features

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Background: Variants of urothelial carcinoma include a plethora of lesions with distinct morphology and in several cases specific biological behavior. Among those, mucin expression has been reported in adenocarcinomas (micropapillary variant, intestinal type adenocarcinoma and microcystic variant). Urothelial carcinomas with abundant extracellular mucin extravasation have thus far not been reported.

Design: This study describes the clinicopathological characteristics of 13 cases and term this variant "urothelial carcinoma with mucinous features". Thirteen cases of urothelial carcinoma with mucinous features were prospectively collected over 9 years. Cases with any areas of distinct adenocarcinoma component or micropapillary features were excluded from this study.

Results: The mean age of the patients was 64 years (range 45-84). Nine of the 13 were male. All cases involved the bladder with one of the cases involving a bladder diverticulum. Treatment included TURB only in 6 patients, TURB with subsequent intravesical BCG treatment in 4 patients, radical cystectomy only in 2 patients, and neo-adjuvant chemotherapy followed by cystectomy in 1 patient. All cases with extracellular mucin were invasive urothelial carcinomas. The two most common patterns of cancer with extracellular mucin were small or medium sized nests seen in 10 cases followed by filiform cancer seen in 4 cases. Other patterns of invasive cancer associated with extracellular mucin were cords, cribriform, pseudoglandular, and individual cells. The amount of invasive urothelial carcinoma with mucinous features compared to conventional invasive urothelial carcinoma in the samples ranged from 5 to 95% (mean 50%). An unusual finding was the presence of invasive low grade urothelial carcinoma associated with extracellular mucin in 5 cases, 4 of which had an overlying low grade papillary urothelial carcinoma component. All cases evaluated were uniformly positive for CK7 and negative for CDX2. Five cases were also positive for CK20, three were positive for MUC5 in the mucinous areas and 2 were positive for MUC2 in the mucinous areas. Two of the MUC5 positive cases were also focally positive for polyclonal CEA. Histochemical stains for alcian blue with and without hyaluronidase, PAS and mucicarmine showed positivity in both intra and extracellular compartments in all cases in the areas of mucinous differentiation.

Conclusions: The current study describes a novel variant of urothelial carcinoma which may be confused with either adenocarcinoma and micropapillary urothelial carcinoma, which can have therapeutic and prognostic implications.

890 Leydig Cell Tumors of the Testis, a Clinicopathological Series with Malignant Histological Features

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Background: Malignant Leydig cell tumors of the testis are uncommon. To date, only small series have been reported.

Design: From a database of 724 Leydig cell tumors, 84 Leydig cell tumors from the Testicular Tumor Registry of the AFIP from 1979 to 1998 were selected based on the presence of one or more of the following features: cellular pleomorphism, tumor necrosis, vascular invasion, increased number of mitotic figures or atypical mitotic figures. Unclassifiable gonadal stromal tumors and cases with Sertoliform differentiation were excluded. Mitotic figures, necrosis, degree of atypia, and extension to paratesticular tissue were correlated with aggressive behavior (presence of vascular invasion or metastatic disease at the time of diagnosis). Unusual histologic features were recorded.

Results: The mean age of the patients was 55 years (range 19-94). Tumor sizes ranged from 1.1 to 14cm (mean 4.7). The tumors were characterized most often by sheets of polygonal cells with abundant cytoplasm and central nuclei. Degree of pleomorphism was coded as mild (23.8%), moderate (32.1%) and severe (44%). Unusual features included signet-ring cell changes (2%), foamy cells (%), adipose metaplasia (10%), calcification (3%), vacuolated cells (12%), myxoid changes (12%) and areas of spindling (21%). Mitotic figure counts ranged from 0 to 24/10HPF (mean 6.4). 65 cases (77%) had tumor necrosis, ranging from 5 to 80% (mean 22.9%). Tumors with metastasis at diagnosis (9, 10%) or vascular invasion (20, 23%) occurred in older patients (p=0.004), were larger (p=0.002) and had a higher mitotic count (p=0.04) compared to others. Percent necrosis, extension into paratesticular tissue, spindle cell change, presence of atypical mitoses, circumscription, fibrous bands and degree and extent of anaplasia were not significantly different between aggressive tumors at presentation and the others. Survival data from 21 patients (25%) revealed mean overall survival of 73 months (range 0-300 months).

Conclusions: Mitotic counts, tumor size and patient age correlate with presence of metastic disease at diagnosis or presence of vascular invasion and suggest aggressive behavior in a subset of Leydig cell tumors with malignant histologic features.

891 Low Grade Urothelial Carcinoma of the Upper Urinary Tract: A Clinicopathological Analysis of 63 Cases

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Background: Urothelial carcinomas of the upper urinary tract are relatively uncommon, with limited pathological data and outcomes for patients with low grade tumors. We examined our series of patients with low grade upper urinary tract cancers who underwent conservative management or radical excision to identify histopathological findings and clinical outcomes associated with these tumors.

Design: The clinicopathological records of patients treated at our institution from 1992 to 2008 for urothelial cancers were reviewed. Sixty-three patients with low grade upper urinary tract urothelial carcinoma were identified.
Results: Of the patients reviewed, the median patient age was 71 years (range 42 to 92) with a male to female ratio of 1:5. Twenty-six patients (41%) had a prior history of bladder carcinoma. Patients underwent nephroureterectomy (55), distal ureterectomy (4), and percutaneous resection (3) or fulguration (1) of their upper urothelial tract tumors. All tumors from biopsy of final pathological analysis were low grade with papillary urothelial carcinoma (98%), ureteritis (6%), urothelial hyperplasia (4%), squamous metaplasia (2%), and squamous differentiation in 2% of cases. Lymphovascular invasion was present in one case. Pathological staging confimed all cases were lower stage (pTis, pTa, or pT1), with one pTx. At a median follow-up of 42 months, 4 (6%) patients were dead from their disease, 8 (13%) died from unknown/other causes, and 51 (81%) were alive.
Conclusions: From our series low grade upper tract urothelial carcinoma was associated with favorable survival outcomes. Close monitoring and follow up of patients managed with conservative therapy is warranted to prevent disease progression.

892 Prostate Cancer Topography and Patterns of Lymph Node Metastasis

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Background: Pelvic lymph node dissection is the standard means of detecting lymph node metastases (LN+) in men with prostate cancer (CA). We examined the correlation of primary tumor topography in radical prostatectomies (RP) and patterns of LN metastasis.
Design: In 125 LN+ cases, entirely-submitted/whole-mounted RP specimens and corresponding lymph node dissections were reviewed. For primary CA, tumor maps were constructed with annotation of low (Gleason pattern ≤ 3) and high grade (Gleason patterns 4-5) CA. Laterality, anterior-posterior and apex/mid/base localization, extraprostatic extension [EPE], seminal vesicle invasion [SVI], lymphovascular invasion [LVI], and tumor volume [TV] were noted. Total # of LN excised and # and laterality of LN+ were also recorded.
Results: LN Quantity and Distribution - Total # LN resected: range: 4 to 48 (mean=14.5; median=13) - 76 of 125 cases had 1 LN+, 33 had 2 LN+, and 16 had >2 LN+ (range: 3 to 18) - 58 cases had LN+ on the right [R], 44 on the left [L], and 20 bilateral (unknown in 3 cases) Pathologic Variables for LN+ Cases - 74 of 115 (64%) evaluable cases showed LVI - 108 of 125 (86%) cases showed EPE and 45 (37%) demonstrated SVI (17 R, 12 L, and 17 bilateral) - Among cases with SVI, 27/46 (59%) had > 1 LN+, compared with 22/78 (28%) without SVI - Volumetric studies revealed mean and median total TV of 6.38 and 3.92 cc, respectively (range: 0.03 to 45.7) - Predominantly high grade Gleason patterns (4-5) accounted for $\geq$ 50% of total TV in 105 (84%) cases and $\geq$ 90% of total TV in 73 (58%) cases Correlation of Dominant Lesion Location and LN+ - Dominant lesions on RP: 50 R lobe, 44 L lobe, 31 bilateral lobes - R lobe dominant tumors: 14/50 (28%) showed LN+ on the L side (9 exclusively left LN+, 5 bilateral LN+). L lobe dominant tumors: 17/44 (39%) showed LN+ on the R side (8 exclusively right LN+, 9 bilateral LN+) - 102/125 were posterior/posterolateral, 18 were both anterior/posterior, and 5 were anterior only - 79 primary tumors involved the base, while 46 were located primarily in the apex-mid gland - 13 of 16 CA with LN+, but without EPE or SVI, were predominantly in the apex-mid gland.
Conclusions: LN+ cases are overwhelmingly associated with large volume, high grade, high stage ($\geq$ pT3a) disease and LVI. Interestingly, one-third of LN+ occur contralateral to the dominant RP cancer. In this series, LN+ were infrequently associated with anterior-dominant tumors. Organ-confined LN+ cases were predominantly situated in the apex-mid gland.

893 Kidney Injury Molecule-1 (KIM-1) Expression in Renal Clear Cell Carcinoma

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Background: Kidney Injury Molecule (KIM-1) is a transmembrane glycoprotein type 1 overexpressed in the epithelium of proximal tubules of human kidney under ischemia or nephrotoxic injury as well as in tubulointestitial diseases and several policystic renal diseases. Previous studies suggest that this molecule could be an specific and sensitive biomarker of renal clear cell carcinoma (RCCC) and their metastases.
Design: A serie of 100 patients with diagnosis of RCCC in our Hospital collected between 2000 and 2004 were studied. Tissue Microarrays (TMAs) were designed . For each case tumor tissue and normal tissue three representative areas were selected on the slides and then removed from the corresponding paraffin block, with a punch of 1,2 mm. KIM-1 polyclonal antibody was used for the inmunohistochemical study (1: 50 dilution). The expression was evaluated using a semiquantitative method ranging 0-300 (HScore). The results were analysed by means the SPSS Data Analysis Program 13.0.
Results: The expression of KIM-1 in RCCC was diffuse in cytoplasms with mild to strong intensity. The univariated and multivariated analysis of KIM-1 expression with the variables Fürhman grade, necrosis and microvascular permeation , no observed significative correlation. Clinical stratification in low, middle and high groups of risk (Leivobitch 2003 classification) and ranked of Hscore inmunostain (<100, 100-200 and >200) demonstrated a significant correlation in the low risk group and Hscore <100 in order the cancer specific survival and disease-free survival (p=0.039).
Conclusions: Therefore KIM-1 coul be considered as a tumor supressor gene in some renal cell carcinomas . A low KIM-1 expression could be a marker to identify a subset of low risk renal clear cell carcinomas with worse prognosis. Patients with clinical low risk RCCC (pT1a, grades 1-2, without necrosis and low levels of calcium in serum) and KIM-1 expression under 100, show an increase number of recurrences.

894 Expression of Extracellular Matrix Associated Protein Cyr61 in Prostate Cancer: An Immunohistochemical and Quantitative RT-PCR Analysis

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Background: Cysteine-rich angiogenic inducer 61 (Cyr61) is an extracellular matrix protein that plays a regulating role in several cellular pathways including cell adhesion, migration and proliferation. Recently, using gene expression arrays, we demonstrated elevated Cyr61 mRNA expression in a subset of prostate cancer (PrCa) lesions. Our current study evaluated the expression pattern of Cyr61 in PrCa, HG-PIN, paired benign prostatic tissue (BPT) and donor prostatic tissue (CTL).
Design: Frozen tissue was used for mRNA quantitative real time reverse transcriptase PCR (Q RT-PCR) analysis using Cyr61 primers (5'-GCAGCCTGAAAAAGGGCAA -3' and 5'-AACATCCAGCGTAAGTAAACCTGAC -3') and probe (5'-AGCAAGACCAAGAAATCCCCCGAACC -3'). Cyr61 mRNA was normalized against Glucuronidase ß. Amplification was performed using ABI Prism 7700 (Applied Biosystems, CA). TMA sections were constructed from 200 consecutive radical prostatectomy specimens performed at our hospital (2000-2001). Immunohistochemistry was performed using polyclonal antibody for Cyr61 (H-78; Santa Cruz). Semiquantitative visual intensity score for Cyr61 cytoplasmic expression was assigned on a scale of 1-3 in every TMA core. Visual intensity scores were validated by image analysis using ScanScope XT (Aperio Technologies, CA) and open source software packages (TMAJ and FrIDA, Johns Hopkins), in one of the TMAs.
Results: We found a strong correlation between image analysis and visually obtained Cyr61 intensity scores (pairwise CC:0.71; p0.0001). Using Q RT-PCR, we observed significant elevation in Cyr61 mRNA levels in PrCa in comparison to CTL donor tissues ($p<0.05$). Significantly higher Cyr61 immunoexpression intensity was present in PrCa compared to BPT ($p<0.0001$). Cyr61 expression intensity was higher in Gleason score ≥ 8 PrCA ($p<0.01$) compared to those with lower gardes. Cyr61 expression in HG-PIN was moderately up-regulated compared to BPT but less intense than associated PrCa ($p<0.0001$).
Conclusions: Cyr61 expression in PrCa is increased compared to associated BPT. The higher expression of Cyr61 in higher grade tumors suggests a potential prognostic role. A study correlating Cyr61 with PrCa progression is ongoing. Further studies are needed to elucidate the mechanisms by which Cyr61 may contribute to the development and progression of PrCa.

895 Chromosome 21 Copy Number but Not *TMPRSS2-ERG* Fusion Predicts Outcome in Prostatic Adenocacrcinoma: A Large Case-Control Radical Prostatectomy Cohort Analysis

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Background: *TMPRSS2-ERG* gene fusion has been reported to be present in up to 70% of all PCa with hereto conflicting findings in relation to its clinical significance. In the current study, we aimed to evaluate the role of *TMPRSS2-ERG* fusion in predicting disease progression using FISH analysis applied to a well characterized set of PCa case-control cohort.
Design: 9 TMAs containing paired tumor and normal tissues of 158 cases and 158 control radical prostatectomies matched for grade, stage, ethnicity and ptage. All RRPs were performed in our institution between1993-2000. Progression was defined as development of biochemical recurrence, clinical evidence of metastasis or death of PCa. FISH analysis was performed using break-apart probes for the 5' and 3' regions of *ERG*. Each spot was scored for presence of *TMPRSS2-ERG* gene fusion through deletion or split as well as for polyploidy (≥3 copies) at the *ERG* locus. At least 50 cells were scored per spot.
Results: Findings are summarized in table 1.

	Fusion	Deletion	Split	Duplicated Deletion	Polyploidy + Deletion	Polyploidy + Split	ch21 Polyploidy without fusion
Cases (progressed)	51.27%	36.1%	26%	9.5%	10.1%	11.4%	29.8%
Controls	57.6%	46.2%	30.4%	8.9%	8.9%	9.5%	17.1%
p value	NS	NS	NS	NS	NS	NS	0.01

Presence of fusion due to either a deletion or split event was not associated with progression. Likewise, neither the presence of duplicated *ERG* deletion, duplictated split, presence of ch21 polyploidy with single allele *ERG* deletion nor the presence of ch21 polyploidy with single allele *ERG* split were associated with progression. *ERG* gene polyploidy without fusion was significantly associated with progression in our cohort (Hazard ratio 2.05; 95% CI: 1.2-3.5, p=0.01).
Conclusions: We found no correlation between presence of *TMPRSS2-ERG* fusion (or type of fusion) and progression in our large well characterized case-control cohort. Progression was significantly associated with presence of ch21 polyploidy without fusion.

896 Phosphorylation of Histone H2A.X Is a Specific Marker To Distinguish Oncocytoma from Chromophobe Renal Cell Carcinoma

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Background: Significant morphologic overlap exists between chromophobe renal cell carcinoma (ChRCC) and oncocytoma (ONC), and the distinction between these 2 entities remains one of the most difficult differential diagnoses in renal cortical tumors, especially when dealing with limited material such as needle biopsy. Genetically, ChRCC is characterized by more extensive chromosomal losses than ONC. Accumulation of DNA repair factors is a known cellular response to DNA damage. One such factor is the phosphorylated form of histone H2A.X (pH2A.X), which is found in cells exposed to radiation, heavy metals, heat stress, growth senescence, and oncogenic transformation.

We hypothesized that DNA damage of malignant transformation can be identified by pH2A.X in ChRCC but not in ONC.
Design: Tissue microarray sections containing 32 cases of ChRCC and 12 ONC were stained by immunohistochemistry for pH2A.X and total H2A.X, using radiation-treated tumor sections as positive controls. Each case was represented by three 1-mm tissue cores, for which any reactivity was considered positive. The intensity in positive cases was scored as weak (1), moderate (2) or strong (3). The mean value was recorded for each individual case as the average intensity of all 3 cores.
Results: pH2A.X was negative in all 12 ONC cases while 25 ChRCC cases (78%) were positive (mean value 1.9) ($p<0.001$, t-test). In ONC, all tumor cells were completely negative and in positive ChRCC, almost all tumor cells exhibited exclusive nuclear labeling. All the 7 negative cases of ChRCC had classical architectural and nuclear features to distinguish them from ONC, even though 3 had oncocytic cytoplasm. H2A.X expression was found in the majority of ONC and ChRCC, but its mean values were significantly lower in ONC (mean=2.11) than in ChRCC (mean=2.55, $p=0.015$). Evaluation of pH2A.X as a diagnostic test for ChRCC vs. ONC showed 78% sensitivity, 100% specificity, 100% positive predictive value, 63% negative predictive value and 84% accuracy.
Conclusions: The majority of ChRCC express pH2A.X compared to none of ONC, making it a specific marker to distinguish between the 2 entities. Although total H2A.X was stronger in ChRCC compared to ONC, only the phosphorylated H2A.X has discriminating value in this differential diagnosis. The application of pH2A.X as a diagnostic marker in the workup of oncocytic renal cortical tumors may help establish the correct diagnosis on limited material such as needle biopsy specimens, impacting on subsequent management decision.

897 Differential Expression of Heterochromatin Protein 1 in Renal Cortical Tumors

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Background: Heterochromatin is gene poor, transposon rich, tightly packed form of DNA characterized by limited transcription, and can be best detected by heterochromatin protein (HP1) marker. HP1 is an essential, nonhistone chromosomal protein mediating gene silencing via binding and methylating histone H3. HP1 is detected in many normal tissues, including kidney, but frequently lost during tumorigenesis. We aimed to evaluate HP1 expression in benign and malignant kidney tumors, and to elucidate its potential role as a differential diagnostic marker.
Design: Tissue microarrays (TMA) were built to include 11 oncocytomas (ONC), 32 chromophobe renal cell carcinomas (ChRCC), 65 papillary renal cell carcinomas (PRCC), and 95 clear cell renal cell carcinomas (CCRCC). Three tissue cores were acquired from each case. TMA sections were subjected to immunohistochemistry with HP1 antibody (Abcam). Normal kidney tissue with strong HP1 nuclear positivity served as a positive control. HP1 nuclear staining was scored semi-quantitatively as negative (0), weak (1+), or strong (2+), and averaged for each case.
Results: HP1 expression was present in all ONC, but lost in the majority of malignant renal cell carcinomas (Table 1). Average expression levels of HP1 in ONC vs all subtypes of RCC were significantly higher (ANOVA, $p<0.001$). By comparing HP1 staining results in ONC vs all RCC subtypes we found 100% sensitivity and negative predictive value, 94% specificity, 52% positive predictive value, and 95% accuracy.

Table 1. HP1 expression in kidney tumors

Histotype (N cases)	Negative	1+	2+
Normal kidney (N=9)	0	0	9 (100%)
ONC (N=11)	0	2 (18%)	9 (82%)
ChRCC (N=32)	28 (88%)	2 (6%)	2 (6%)
PRCC (N=65)	62 (95%)	3 (5%)	0
CCRCC (N=95)	91 (96%)	3 (3%)	1 (1%)

Conclusions: ONC maintains high nuclear HP1 expression similar to renal tubular epithelium. In contrast, the vast majority of renal cell carcinomas exhibit loss of HP1 (88% of chromophobe, 95% of papillary and 96% of clear cell carcinomas). The striking loss of HP1 in the majority of RCC subtypes, but not in benign ONC, indicates that it is associated with malignancy. Differential expression of HP1 across benign and malignant tumors can be used as a diagnostic aid in challenging overlapping cases, when loss of HP1 can be interpreted in favor of carcinoma.

898 Biochemical Recurrence Related to Tertiary Gleason Patterns at Radical Prostatectomy (RP)

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Background: Only limited data from heterogeneous patient cohorts are available to evaluate the long-term clinical significance of a higher tertiary Gleason pattern at RP.
Design: We evaluated data on 7,219 men who underwent RP at our institution from 1984-2005 and had available pathology and clinical outcome data. Tertiary Gleason patterns were defined as Gleason pattern $\geq$4 for Gleason score 6 and Gleason pattern 5 for Gleason score 7 or 8; Gleason scores 9-10 were not considered to have tertiary Gleason patterns.
Results: There were 406 men (6%) with tertiary Gleason patterns. Men with tertiary Gleason patterns had significantly higher preoperative serum PSA levels ($p=0.017$) and biopsy Gleason score ($p<0.0001$) than men without tertiary Gleason patterns. For men with tertiary Gleason patterns, recurrence-free survival curves were intermediate between those of men without tertiary Gleason patterns in the same Gleason score category and men in the next higher Gleason score category. For example, tumors with Gleason score 3+4=7 with tertiary pattern 5 had a prognosis in between those of 3+4=7 without tertiary pattern 5 and those of 4+3=7 without tertiary pattern 5. For men with Gleason 4+3 in particular, the presence of tertiary Gleason pattern 5 decreased recurrence-free survival to a level very close to that of Gleason score 8 without tertiary Gleason pattern 5. The presence of a tertiary Gleason pattern was an independent prognostic factor in a proportional hazards model that included RP Gleason score, pathology stage, PSA and year of surgery, hazard ratio=1.3 (95% CI: 1.02-1.62), $p=0.033$.
Conclusions: In this the largest study to date of tertiary Gleason patterns at RP, the presence of tertiary Gleason patterns was associated with lower recurrence-free survival for all Gleason score categories. These data suggest that tertiary Gleason patterns should be evaluated and included in pathology reports of RP specimens. In most cases the presence of tertiary Gleason patterns produce survival intermediate between two adjacent Gleason score categories.

899 Pathologic Predictors of Tertiary Gleason Pattern 5 in Radical Prostatectomies Provide a Rationale for Total Embedding of Selected Prostates with Gleason Score 7 Cancer

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Background: Prostate cancer patients with Gleason score 7 cancer and a tertiary Gleason pattern 5 component (TP5) have biochemical recurrence rates similar to those of patients with Gleason score 8 to10 cancer (Pan, '00). Given concerns about unnecessary cost and time, strategies have been developed to sample only sufficient tissue from radical prostatectomies (RP) as to not miss margin positivity, extra-prostatic extension and to obtain an accurate Gleason score (Sehdev, '01; Cohen, '94). We questioned whether we could identify pathologic parameters of Gleason score 7 cancers that would identify those cancers that had a tertiary pattern 5 component and that would thus warrant total embedding of the prostate to find that component.
Design: 723 men who underwent RP with Gleason score 7 cancer and no neoadjuvant therapy were identified. Pathologic correlates of TP5 were determined using multivariate logistic regression analysis adjusting for pathologic stage, surgical margin status, preoperative serum PSA, tumor volume, and prostate gland volume.
Results: Of 723 men who underwent RP with Gleason score 7 cancer, 92 (13%) had a tertiary pattern 5 component (TP5). Compared with patients without TP5, patients with TP5 had higher tumor volume (median 4.0 cc vs 2.3 cc, $p < 0.0001$), more commonly had primary Gleason pattern 4 (61% vs. 20%, $p < 0.001$) and exhibited extracapsular extension (52% vs. 25%, $p < 0.001$). In multivariate analysis, patients with primary Gleason pattern 4 (OR 6.16, 95% CI 3.68-10.3) and extracapsular extension (OR 2.63, 95% CI 1.52-4.53) were more likely to have TP5. Additionally, increased tumor volume was associated with an increased probability of having TP5 ($p < 0.001$).
Conclusions: A tertiary Gleason pattern 5 component in a Gleason score 7 cancer is more frequent in patients with more voluminous, higher stage tumors in which the primary Gleason pattern is pattern 4. Since the pattern 5 component represented < 10% of each tumor and could only be found by total embedding of each prostatectomy specimen (being present in only one block in > 90% of our cases), we propose that RP specimens from patients whose prostate carcinoma is Gleason score 7 with a primary pattern that is 4, a tumor volume > 4 cc, and extracapsular extension be totally embedded to identify this subgroup of high risk patients for possible adjuvant therapy.

900 Comparative Analysis of Gene Expression Signatures of 3-D Culture Specific Genes and Epithelial Specific Genes from Clinicaly Localized Human Prostate Normal and Cancer Specimens

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Background: Development of prostate cancer (CaP) is associated with the dysregulation of the normal homeostatic process responsible for maintaining glandular structure. Recently, a method to recapitulate acinar morphogenesis using a benign prostatic epithelial cell line in three-dimensional culture (3-DC) was established. We found that 3-DC specific genes are cooperatively regulated when making acinar morphology. We hypothesized that these genes would be important in maintaining prostatic acini in vivo and dysregulation of these genes may play a role in CaP initiation and development. Therefore, we assessed the expression of these 3-DC specific genes in 80 microarray data sets of matching tumor and benign specimens isolated by laser capture microdissection (LCM).
Design: Human benign prostatic epithelial cell line, RWPE-1 and TA2 were cultured in 2-D with 2% matrigel or 3-DC. Total mRNA was extracted from cells in each culture condition and from LCM specimens. All the samples were amplified by the T7-based linear amplification method. Gene expression analyses were performed by Genesprings and Ingenuity Pathway Analyses using Affymetrix microarrays.
Results: In 3-DC, consistent down regulation of 126 genes and up regulation of 175 genes were observed when compared to 2D matrigel cultures. In silico pathway analyses revealed the suppression of the transforming growth factor beta (TGFbeta) signaling pathway and upregulation of epidermal growth factor receptor (EGFR) and integrin signaling pathways. Analysis of human benign and CaP samples confirmed the importance of these pathways and further identified altered expression of nephronectin, ribonucleotide reductase M2 polypeptide, osteoblast specific factor 2, and E74 like factor 3 specifically in CaP.
Conclusions: Both EGFR and TGFbeta signaling pathways are suggested to be essential pathway for cancer initiation and development. The up-regulated genes, NPNT, RRM2 and OSF2 appear to be associated with the cellular growth through EGFR and integrin pathways by analyses in silico. The ELF3 gene was reduced and associated with dedifferentiation through TGFbeta receptor downregulation.

901 Prognostic Factors of Clear Renal Cell Carcinoma in pT1a Cases

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Background: The proportion of clear renal cell carcinoma (CRCC) cases diagnosed at pT1a is known to be increasing significantly. Although their prognosis is excellent in general, some cases show distant metastasis. Most of proposed prognostic factors are based on relatively large sized CRCC data. The prognostic factors for small sized CRCC, pT1a cases aren't well described.

Design: One hundred ninety-eight pT1a CRCC cases were retrieved from authors' institution files. For each case, the following pathological parameters were recorded: patient age, tumor location (upper, middle, low), Furhman grade, presence of capsule, presence of lympho-vascular invasion, growth pattern (expansive or infiltrating), presence of scar, presence of hemorrhage, and presence of necrosis.

Results: Male to female ratio was 4. Patient's age ranged from 21 to 85 years (median 59 years, mean 58.1 years). Follow up duration ranged from one to 265 months (median 62 months). Seventeen cases showed distant metastasis. Furhman grade (grade 1+2+3 vs. 4), presence of lympho-vascular invasion, infiltrating growth pattern, and presence of necrosis were statistical significant ($p<0.0001$).

Conclusions: Furhman grade (less than 3 vs. 4), presence of lympho-vascular invasion, growth pattern, and presence of necrosis can be prognostic factors in CRCC in pTa cases. Growth pattern may be a new prognostic factor in CRCC.

902 Different Immunohistochemical Expression of CD44v6 and ALDH1 in Prostate Cancer before and after Androgen Deprivation Therapy

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Background: According to the hierarchical or stem cell model of cancer, only a relatively small subpopulation of stem cells is capable of initiating tumor growth. If this subpopulation of stem cells survives nonsurgical therapies such as anti-androgen receptor therapy, radiation- or chemo-therapy, then there is opportunity for tumor to regrow. Aldehyde dehydrogense 1 (ALDH1), a stem cell marker, is present in the secretory cells of both normal and cancer cells. CD44v6, a splice variant of a protein involved in cell adhesion and signaling, is present on the luminal surfaces of prostatic basal cells. The aim of this study was to determine the pattern of ALDH1 and CD44v6 prevalence after neoadjuvant hormone blockade / androgen ablation therapy in prostate adenocarcinoma as putative markers of cancer stem cells (CSC).

Design: 65 cases of prostate core biopsies containing prostatic adenocarcinoma (38 cases with Gleason grade 3+3=6, 15 cases with Gleason grade 3+4=7, 7 cases of Gleason grade 4+3=7, and 5 cases with Gleason grade 8) were identified. Each patient was treated by radical prostatectomy following neoadjuvant hormone blockade for 3 to 6 months. Diagnostic biopsies as well as the corresponding prostatectomy specimens were stained with CSC markers ALDH1 and CD44v6.

Results: In the prostate core biopsies before therapy, ALDH1 was positive in 68% of cases (44 of 65) while CD44v6 was positive in 12% (8 of 65). Four cases disclosed 8% of cells with co-localization. Following hormonal treatment, ALDH1 decreased and/or was unchanged in 89% (58 of 65), and enriched in 11% (7 of 65) of cases. In contrast, CD44v6 was enriched in 54% (35 of 65), and decreased in 5% (3 of 65) of cases. Thirteen cases disclosed 2% of cells with co-localization. The decrease in ALDH1 and enrichment in CD44v6 following hormone ablation therapy were both significant ($p<0.001$).

Conclusions: The putative CSC markers CD44vs and ALDH1 behave discordantly following neoadjuvant hormone blockade / androgen ablation therapy. The enrichment in CD44v6 suggests that CSC probably reside in the basal cell compartment. Additional stem cell markers are needed to more accurately define this population of cells.

903 Worrisome and Atypical Features in Renal Oncocytoma: Clinicopathological Analysis of 76 Cases

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Background: Although renal oncocytoma is a benign neoplasm, it may show worrisome and atypical morphologic features that overlap with malignant renal tumors. These unusual features have been systematically documented only in few larger studies.

Design: We retrieved 76 oncocytomas from our institutional database, resected between 01/00 and 08/08, of which 58 (76%) were found on radical nephrectomy, 16 (21%) on partial or wedge resection and 2 (3%) on biopsy. We reviewed the clinical and the gross findings from the reports. Three genitourinary pathologists reviewed all slides. On microscopic review, we particularly focused on identifying the worrisome and the atypical features.

Results: Patients had a mean age of 65 years (range 36-89), with male to female ratio 1.4:1 and right to left side ratio 1.1:1. Multiple tumors were found in 9 (12%) patients and one patient had bilateral solitary oncocytomas and a separate papillary carcinoma in one kidney. Mean tumor size was 4.6cm (range 1.5-11cm). Foci of hemorrhage were noted on gross examination in 22 (30%) tumors and central scar was seen in 25 (33%) tumors. On microscopy, 36 (47%) tumors contained at least focal cysts. Dominant cystic architecture was present in 8 (10.5%) tumors and dominant tubular and trabecular patterns were seen in 4 (5.5%) tumors each. However, the most common dominant patterns in oncocytoma were nested in 34 (44.5%) and solid in 26 (34%) cases. In 9 (12%) tumors, small papillae were noted, typically within the cysts. Invasion into the perinephric fat was present in 14 (18.5%) tumors and lymphovascular invasion was found in 4 (5%) oncocytomas. Hypocellular stroma involving >50% of the tissue was present in 6 (8%) tumors. Mitoses and coagulative necrosis were documented in only one tumor (1.3%) each. Focal clear cell change was found in 9 (12%) tumors. Focal chromophobe carcinoma-like areas with perinuclear halos and crumpled nuclei were found in 12 (16%) tumors. Psammoma bodies or calcifications were noted in 3 (4%) cases. Nuclear pleomorphism, oncoblasts and entrapped tubules were commonly seen and were documented in 21 (28%), 33 (43%) and 33 (43%) tumors, respectively.

Conclusions: In this third largest series of oncocytoma collected so far, we demonstrated that renal oncocytomas often show unusual morphology. Presence of worrisome or atypical features should not dissuade the pathologist from establishing the oncocytoma diagnosis with confidence, if otherwise typical morphology is present.

904 Variation among Urogenital Pathologists in Diagnostic Opinion of Single Small Atypical Foci in Prostate Biopsies

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Background: We investigated the level of diagnostic agreement (kappa) for small foci of atypical glands on prostate biopsy among experts specialized in urogenital pathology and all-round pathologists with extensive experience in reading prostate biopsies.

Design: We retrieved 20 prostate biopsies with small atypical foci, originally reported by urogenital pathologists of University Health Network, Toronto as benign (n=4), suspicious for adenocarcinoma (n=8) and adenocarcinoma (n=8). HE stained slides and –if available- matched immunostained slides (n=10) were digitalized. Five experts in urogenital pathology (UGE) and eight members of the pathology committee of the European Randomized study of Screening for Prostate Cancer ERSPC were asked to examine the virtual slides, available through internet. Their diagnoses were transformed into three categories, benign, suspicious and adenocarcinoma. Multirater kappa statistics were applied to determine agreement and significant differences between experts and ERSPC members.

Results: Five UGE and 7 of 8 invited ERSPC pathologists participated in the study. The kappa of the UGE (0.39 (95%CI: 0.29-0.49)) was higher than that of the ERSPC pathologists (0.21 (95%CI: 0.14 –0.27). A 100% agreement was reached by UGE for 7 biopsies (4 adenocarcinomas, 2 benign, 1 suspicious) and by ERSPC pathologists for 1 biopsy (benign). UGE and ERSPC pathologists rendered, in 5 and 9 biopsies, respectively a diagnosis ranging from benign to carcinoma. The UGE diagnosed cancer (49%) more often ($p< 0.001$) than the ERSPC pathologists (32%), mainly at the expense of the number of benign diagnoses. If the agreement was calculated for originally diagnosed cancers and benign diagnoses the kappa's of GUE increased to 0.42 and 0.55, respectively and those of ERSPC pathologists to 0.26 and 0.27 respectively.

Conclusions: Although UGE demonstrate a better agreement in their diagnostic opinion of small atypical foci in prostate biopsies than their non-specialized ERSPC counterparts, their level of agreement remains poor. UGE rendered more often a diagnosis of carcinoma than the ERSPC pathologists.

905 Impact of Pathology Review and Substage on the Clinical Outcome of pT1 Bladder Cancer

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Background: The clinical behaviour of pT1 bladder cancers is highly variable. Therefore we wished to evaluate the impact of pathology review and substaging, using a recently described simple substaging method, on the clinical outcome of pT1 bladder cancers at first presentation.

Design: The slides of 155 first diagnosis pT1 bladder tumors from multiple institutions of two countries (the Netherlands, n=69 and Canada, n=86) were reviewed and substaged, separating pT1micro-invasive (pT1m) and pT1extensive-invasive (pT1e) carcinomas, as previously described.[1] All patients initially diagnosed as pT1 were managed conservatively. Grade review was done according to both the WHO 1973 and 2004 classifications systems. Clinical data were collected by chart review and prognostic impact was determined by Kaplan Meier analysis and multivariate analysis.

Results: Mean follow-up was 6.52 years (range 0.8-21.3 yrs), 28 patients were female and mean age was 69 years at first diagnosis. Fifty-two patients (34%) remained recurrence-free. A cystectomy was performed in 31 patients (20%) and pT2 disease was found in 25 of them. Progression to pT2 or metastasis was detected in 44 (28%) patients and 24 patients died of their disease. After review the pT1 carcinomas were sub-staged as follows: 23 pTa, 40 pT1m, 87 pT1e and 5 pT2. Grade review resulted in 71 G2 and 84 G3 lesions according to the 1973 system and 37 low-grade and 118 high-grade lesions according to the 2004 system. Kaplan Meier analysis demonstrated significant differences for pTa, pT1m, pT1e and pT2 carcinomas. Within the reviewed pT1 carcinomas only substage, but not grade or tumour size proved of independent significance in multivariate analyses with progression ($P<0.001$) and disease-specific survival ($P=0.001$) as endpoints.

Conclusions: Review pathology is indicated in pT1 bladder cancer. pT1 substage (pT1m and pT1e) is the only prognosticator for progression and disease-specific survival in pT1 bladder cancer. 1. Van der Aa MNM, van Leenders GJLH, Steyerberg EW, van Rhijn BWG, Jöbsis AC, Zwarthoff EC, van der Kwast TH. A new system for substaging pT1 papillary bladder cancer: a prognostic evaluation. Hum Pathol 2005;36:981-6.

906 The Length of Positive Surgical Margins Correlates with Biochemical Recurrence after Radical Prostatectomy

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Background: The presence of positive surgical margins (+SM) in a radical prostatectomy (RP) specimen is an important negative prognostic factor, but it is still uncertain whether the extent of +SM affects clinical outcome as an independent factor. We evaluated the prognostic role of the linear length of +SM for biochemical recurrence (BCR) after RP for prostate cancer.

Design: Data of 267 consecutive RP specimens with +SM were analyzed. All RP specimens were sectioned at 4-mm intervals and completely embedded. Evaluation was done by one uropathologist. BCR was defined as two consecutive PSA levels above 0.10 ng/mL. Data were analyzed using Kaplan-Meier survival analysis and proportional hazards models.

Results: In the total group of 267 patients, the length of +SM ranged from 0.4 to 174.5 mm (median 11.2 mm; mean 21.9 mm) and was positively correlated with preoperative PSA ($p< 0.001$), pathologic stage ($p < 0.001$), tumor volume ($p = 0.001$), number of positive sites ($p <0.001$), Gleason grade at the positive margin ($p < 0.001$) and with Gleason score ($p = 0.015$). The mean length of +SM in 93 patients with persistent increased postoperative PSA levels and/or adjuvant therapy was significantly higher than in the remaining 174 patients (32.6 mm vs. 15.4 mm; $p<0.001$). In the 174 patients with an undetectable postoperative PSA level (median follow-up of 36.3 months) the 5-years risk of BCR was 29%. The risk of BCR for patients with +SM ≤ 10 mm and > 10 mm was 21% and 39%, respectively. In multivariate analyses BCR was associated with an increasing length of +SM (HR 2.15; 95% CI 1.12-4.15; $p = 0.022$), but not with preoperative PSA, pathological stage, Gleason score, tumor volume or Gleason grade at the resection margin.

Conclusions: The length of the positive surgical margin is an independent predictive factor for PSA recurrence after radical prostatectomy.

907 Percutaneous Core Needle Biopsy; a Helpful Tool in the Diagnosis of Hereditary Kidney Tumors

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Background: Accurate preoperative diagnosis of renal masses is crucial for clinical evaluation and treatment of patients. Percutaneous imaging-guided biopsy of renal masses is becoming an important tool in the diagnosis and treatment of renal cancer. In this study we report our experience with imaging guided percutaneous core biopsies (PCB) performed in patients with known hereditary kidney tumors.

Design: We present 41 PCB specimens obtained from 40 patients presenting with renal tumors. Family clinical history included VHL, HPRCC type I, BHD syndrome, Succinate dehydrogenase deficiency and Tuberous sclerosis. Material was obtained for special studies, IHC and EM.

Results: There were 18 female and 22 male patients, ranging in age from 6-87 years, (mean 51) Definite diagnoses was performed on PCB in 40 (98%) cases. Thirty tumors were malignant, 1 had neuroendocrine features, 4 had oncocytic characteristics and 5 were benign. Only 1 sample was insufficient for diagnosis. Malignant lesions included renal cell carcinoma (RCC) clear cell type (13 cases), papillary RCC or with papillary features (9 cases), RCC with eosinophilic and/or clear cell features (2 cases), chromophobe RCC (2 cases), medullary carcinoma (1 case), RCC with features of clear cell sarcoma (1 case) and poorly differentiated carcinoma (2 cases). Subtypes of tumors with papillary features included 8 cases suggestive of papillary type I and 1 case HLRCC. Benign cases comprised 4 oncocytomas and 1 angiomyolipoma. The remaining tumors included 4 oncocytic and 1 neuroendocrine neoplasms. Tumors with eosinophilic features were challenging when differentiating between oncocytomas, chromophobe and hybrid tumors. Resected specimens confirmed the preoperative pathologic diagnosis in 11 of 13 (85%). A clear cell RCC by PCB was considered as a possible Pecoma but could not be confirmed in the specimen. One PCB diagnosed as oncocytic neoplasm was a chromophobe RCC in the resected specimen. The neuroendocrine neoplasm was a neuroendocrine carcinoma. The patient with an insufficient core biopsy was diagnosed with malignant mesothelioma.

Conclusions: Percutaneous imaging guided core biopsies are an excellent tool to diagnose Hereditary renal tumors. Definite diagnosis was performed in 98% of tumors. However caution is recommended in this group of patients when tumors with eosinophilic and papillary features are encountered, since hybrid and papillary features may lead to a wrong interpretation.

908 Elevated EZH2 Expression in Invasive Urothelial Carcinoma of the Bladder

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Background: The enhancer of Zeste homologue 2 (EZH2) gene functions as a transcriptional repressor via chromatin remodeling and histone methyltransferase activity. EZH2 has been reported to be overexpressed and associated with progression in a variety of solid tumors. We investigated expression of the EZH2 protein in bladder urothelial carcinoma (UrCa) in relation to clinical outcome.

Design: Tissue microarrays (TMA) were constructed from 122 cystectomy specimens obtained from our institutional archives. At least triplicate tissue samples of UrCa and paired benign urothelium were spotted from each cystectomy. A total of 584 TMA spots were evaluated (296 invasive UrCa, 44 non-invasive UrCa, 45 CIS and 199 benign urothelium). IHC analysis was performed using affinity-purified rabbit polyclonal antibodies against human EZH2 (Zymed, CA). Positive nuclear reactivity of EZH2 was scored as the product of percentage and intensity of staining in each spot.

Results: A significantly higher EZH2 expression score was associated with invasive UrCa compared to benign urothelium (mean 1.91 vs 0.33, $p< 0.0001$). Significantly increased levels of EZH2 expression were observed in invasive vs non-invasive UrCa (mean 1.91 vs 0.96, $p = 0.0004$). Significantly elevated EZH2 expression was also observed in non-invasive UrCa vs benign urothelium (mean 0.96 vs 0.33, $p =0.002$). Among 88 matched pairs of invasive UrCa and benign urothelium, expression of EZH2 was again significantly higher in the invasive UrCa ($p< 0.00001$). Among 20 matched pairs of invasive UrCa and CIS, expression of EZH2 was significantly higher in invasive UrCa ($p<0.0001$). Although an increased EZH2 expression was observed in UrCa from patients with subsequent recurrence compared to those without recurrence, the difference was not statistically significant (mean score 2.33 vs 1.85, $p = 0.27$). EZH2 expression did not correlate with pathologic stage ($p = 0.65$) or presence of lymph node metastasis ($p =0.53$).

Conclusions: We report significant elevation in EZH2 protein expression in invasive UrCa compared to non-invasive UrCa and benign urothelium. Our findings suggest a potential role of EZH2 in the progression of urothelial carcinoma of the bladder.

909 Renal Cell Carcinoma Shows Ethnic-Related Histological Patterns

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Background: Renal cell carcinomas (RCC) account for the most common renal cancers in adults. Cytogenetic findings and new molecular markers have been included in the WHO 2004 renal tumor classification for the guidance of diagnosis, prognosis, and therapy of this disease. Tobacco, obesity, hypertension, unopposed estrogen therapy, chemical agents, and end-stage renal disease have been shown to be risk factors associated with RCC. But no epidemiologic data regarding incidences of RCC of various histological types in different racial groups have been reported. Our aim was to review clinico-pathologic data in a cohort of RCC cases and to determine if there are any differences among racial/ethnic populations.

Design: Ninety-nine RCC cases have been reviewed between 2002 and 2008 from two hospitals affiliated with State University of New York in Brooklyn, where a mixed ethnic population exists. New WHO 2004 Classification is applied to the review of the pathological findings.

Results: Clear cell carcinoma remains the most common RCC accounting for 52.53% (52/99) of all the cases, followed by papillary RCC 34.34% (34/99), chromophobe RCC 7.07% (7/99), acquired cystic disease-associated RCC 2.02% (2/99), collecting duct carcinoma 1.01% (1/99), multilocular cystic RCC 1.01% (1/99), medullary RCC 1.01% (1/99), and one pediatric RCC with unconventional features. Clear cell RCC, as has been reported, is the most common RCC in white population (79.49%, 31/39) and other non-black ethnic groups; while papillary RCC is significantly prevalent in African Americans accounting for 59.26% (32/54) of all RCC cases in this population. Papillary RCC in African American patients accounts for the majority of this type of RCC among all cases (32/34). There are nearly two folds more type 1 than type 2 papillary RCC. Type 2 papillary RCC showed more unfavorable clinical courses than type 1 cases. More chromophobe RCC cases are seen in the whites than in the African Americans.

Conclusions: We reported for the first time that African Americans seem to have an significantly increased risk of developing papillary RCC than general population. This ethnic-related difference of histological presentations may reflect the complex underlying genetic and molecular basis of the pathogenesis of RCC.

910 Immunohistochemical Analysis of Oncocytic Renal Tumors

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Background: Renal oncocytic neoplasms include oncocytoma (Onc), chromophobe renal cell carcinoma (ChRCC) and oncocytic type of conventional RCC (OnRCC). Due to overlapping morphologic features, classification of these tumors can sometimes be challenging, even in the hands of experienced pathologists. There are scattered reports that using various immunohistochemical markers to help with the differential diagnosis. We have selected a number of these markers and evaluated their usefulness in the differential diagnosis of these tumors.

Design: Forty-five renal oncocytic tumors including 16 classic Onc, 15 ChRCC, 11 oncocytic neoplasms with atypical features (Onc-Aty), and 3 OnRCC were selected. Onc-Aty included a group of tumors with histological features suggestive of oncocytoma but ChRCC or OnRCC could not be excluded based on morphology. Standard immunohistochemical staining using manufacturer's protocols were performed with antibodies to c-kit (CD-117), cytokeratin (CK) 7, epithelial cell adhesion molecule (EpCAM), survivin and Carbonic Anhydrase IX (CA IX). The percentage of positive tumor cells as well as the intensity of staining were evaluated and scored.

Results: C-kit was expressed in 14/15 of ChRCC, 16/16 classic Onc, 8/11 of Onc-Aty and was negative in OnRCC (0/3). CK7 was strongly and diffusely positive in 14/15 of ChRCC; All oncocytomas showed scattered, starry sky pattern of CK7 staining, which was completely different from the diffuse membrane staining seen in ChRCC. One of 11 Onc-Aty showed diffuse CK7 staining. EpCAM was expressed in all but one ChRCC (14/15) with a diffuse membrane staining pattern; while only presented in 4/16 oncocytoma, 3/11 Onc-Aty and none of the three OnRCC. CA IX was only positive in 1/3 OnRCC with membrane staining and negative in all other oncocytic tumors. Survivin was positive in 5/14 ChRCC, 14/16 oncocytoma, 5/11 of Onc-Aty and 1/3 OnRCC with largely nuclear staining and occasional cytoplasmic staining.

Conclusions: A phenotype of diffuse CK7, EpCAM and C-kit staining with negative CA IX strongly favors ChRCC while negative CK7, EpCAM and C-kit favor OnRCC. The scattered, starry sky staining pattern with CK7 seen in oncocytomas and Onc-Aty is quite characteristic and may prove useful in differentiating these tumors from ChRCC and OnRCC. A positive CA IX result basically excludes a diagnosis of Onc or ChRCC. Survivin appears to be of no clinical value at this point.

911 Analysis of Novel Immunohistochemical Markers with a Cluster Analysis Approach To Define an Optimal Panel for the Differential Diagnosis of Renal Epithelial Neoplasms with Eosinophilic Cytoplasm

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Background: Renal epithelial neoplasms with eosinophilic cytoplasm often share overlapping morphologic features which pose difficulties in their classification, specifically in limited samples.

Design: To analyze and compare a panel of immunohistochemical markers useful in the differential diagnosis of eosinophilic renal tumors (S100A1, CD10, AMACR, RCC, Vimentin, CAIX, CK7, MOC-31, EPCam, Claudin 7 and 8, PR, C-kit, E-cadherin, and Parvalbumin), we stained a tissue microarray of 75 renal tumors with eosinophilic cytoplasm (18 chromophobe renal cell carcinomas (ChRCCs), 20 oncocytomas (ROs), 20 Papillary RCC (PRCCs), 17 clear cell RCC (CRCCs)), and analyzed the data with cluster analysis. Expression was categorized as none, weak/focal, or strong (0-2 scale). For CK7, a score of 2 was considered positive, while for other antibodies, a score of 1 or 2 was considered positive.

Results: Cluster analysis sorted tumors in five groups, with like tumors clustering together. ChRCC clustered separately as a distinct group from other entities. RO were a distinctly different group, but clustered closer to CRCC and PRCC. Some PRCC clustered together, but others demonstrated overlap with the CRCC group. Expression pattern of EPCam, MOC-31, and Claudin-7 clustered together, as did C-kit and E-cadherin, CD10 and AMACR, and RCC and Vimentin. S100A1 expression excluded ChRCC with 89% sensitivity and 84% specificity. A panel of CK7 (negative/focal) and S100A1 (positive, score 1 or 2) distinguishes RO from ChRCC with 90% sensitivity and 94% specificity. A panel of vimentin (negative) and C-kit (positive) distinguishes RO from CRCC and PRCC with 85% sensitivity and 86% specificity. Staining results in figure 1. PR and Claudin-8 had minimal utility on initial analysis and were excluded.

	Percent of Cases Staining for Each Antibody											
	S100A1	CD10	AMACR	RCC	VIMEN	CK7 (2)	MOC31	EpCAM	Claud7	Ckit	Ecad	PARV
ChRCC	11	17	78	0	0	72	94	94	94	100	100	100
RO	90	55	25	0	5	5	45	45	40	90	95	100
CRCC	81	94	82	58	82	0	6	76	57	6	29	44
PRCC	85	75	90	81	86	45	65	67	60	10	67	90

Conclusions: Our results show that hierarchical cluster analysis is an effective approach to analyze high volume immunohistochemical data to generate an optimal useful panel. A panel of Vimentin, C-kit, CK7, and S100A1 appears to be the most effective panel for difficult cases or for small biopsies.

912 Nested Urothelial Carcinoma: A Clinicopathologic and Immunohistochemical Analysis of 33 Cases

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Background: Urothelial carcinoma (UC) with nested growth pattern is a rare and less studied variant of UC, characterized by deceptively bland morphology resembling small von Brunn's nests, yet aggressive biology.

Design: We analyzed a series of 33 patients with nested UC who underwent definitive treatment to better understand its clinicopathological spectrum, immunophenotype and clinical outcome. Tumors demonstrating either pure or mixed nested morphology with variable component of conventional UC were included.

Results: Patients age ranged from 41 to 81 years (avg 65) with a male: female ratio of 2.7:1. Pure nested UC accounted for 33% of cases. When seen along with conventional UC, the nested component ranged from 30% to 90%. Of the 22 cases associated with conventional UC, 29% had an additional component of divergent differentiation. Muscle invasion at TURBT and extravesical disease (≥T3) at cystectomy were observed in 82% and 83% of cases respectively ($p < 0.0001$ compared to non-nested UC). Angiolymphatic invasion was common (73%). In comparison to pure conventional high grade UC, nested UCs were associated with metastatic disease (72%), in the form of lymph node and/or distant metastases (compared to 16% for conventional UC, $p < 0.0001$). Behavior was similarly aggressive whether tumor was pure nested or mixed nested. Despite overall bland morphology, focal areas of high grade morphology in the deeper invasive component were noted in most tumors. Nested component uniformly demonstrated random cytological atypia, and growth patterns ranging from haphazard small isolated nests to areas with confluent nested growth with minimal desmoplasia. When associated with conventional UC, the nested component was part of the deeply invasive tumor. 94% of cases were positive for CK7, 68% CK20 positive, 93% K903 positive, and 93% p63 positive. Follow-up was available on 31 patients, and ranged from 1 to 31 months (average 12). 18% of patients died of disease, and another 36% of patients were alive with disease at last follow-up. 16/33 patients (48%) received neoadjuvant chemotherapy. Only two of these patients responded to this therapy. 24 patients were treated with cystectomy, 1 with nephroureterectomy, another 3 were treated with TURBT only, and 5 received radiation. Seven (21%) patients received adjuvant chemotherapy.

Conclusions: UC demonstrating pure or variable component of nested morphology is an aggressive tumor with uniformly bad outcome. Increased awareness of the spectrum of this tumor and its early recognition is important due to its subtle and heterogeneous morphology.

913 MMR IHC in Upper Urinary Tract Urothelial Carcinoma

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Background: Hereditary nonpolyposis colorectal cancer syndrome (HNPCC) tumors characteristically have a high level of microsatellite instability (MSI-H) due to a mutation in one of the DNA mismatch repair (MMR) genes: MLH1, MSH2, MSH6, or PMS2. MMR defects can be demonstrated by immunohistochemistry (IHC). The MSI phenotype can occur in up to 15% of sporadic colorectal cancers, which do not exhibit germline mutations in the MMR genes. The MSI phenotype has been studied in other tumors associated with HNPCC, including ovarian and endometrial cancers. We assessed MMR IHC and the MSI phenotype in upper urinary tract urothelial carcinomas, which have a lifetime risk of 1-12% in HNPCC patients.

Design: 80 patients who had a resected urothelial carcinoma of the renal pelvis or ureters entered in our hospital's electronic surgical pathology database between September 1992 to July 2008 were identified. All had archived, formalin-fixed, paraffin-embedded tissue and clinical follow-up. We performed the MMR IHC panel (MLH1, MSH2, MSH6, and PMS2) on all cases. We tested for MSI in 35 cases using PCR based techniques. Clinicopathologic features were documented.

Results: 5/80 (6%) cases showed loss of at least one MMR marker: two cases with loss of MSH6 alone, one with loss of PMS2 and MLH1, and two with loss of MSH2 and MSH6. One of the latter was a previously known HNPCC case with a germline MSH2 mutation; it was not tested for MSI due to a limited specimen. Four of the 5 cases (80%) were invasive and high grade, as compared to 47/75 cases (63%) with normal MMR IHC. Two of the four cases tested were MSI-H. One case with loss of MMR expression exhibited mucinous morphology. The mean age for patients with loss of MMR was 64 vs. 72 for those with normal MMR IHC. Four of the patients with loss of MMR expression had no family history suggestive of HNPCC, but one case had a personal history of prostate cancer, renal clear cell carcinoma, and melanoma.

Conclusions: Six percent of upper urinary tract urothelial carcinomas exhibited loss of MMR expression; 2 (of 4 tested) of these were MSI-H. The most common defect in MMR was in MSH6, differing from colorectal cancers, in which MLH1 is the most common defect. Our data demonstrates that some of upper urinary tract urothelial carcinomas do have the MMR phenotype associated with HNPCC. MLH1 methylation studies, which are ongoing, may further define this association.

914 Utility of Intraoperative Frozen Section in Assessing the Surgical Margin of Robotic Prostatectomy Specimens

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Background: Intraoperative frozen section utilized to achieve surgical margin free of prostatic glands has rarely been reported in robotic prostatectomy.

Design: From 584 robotic prostatectomies performed between '07 - '08, frozens were sent in 116 cases. Frozens were requested by the surgeon because of visual suspicion for prostatic glands at the prostatic bed. To evaluate the utility of frozen in assessing prostatic glands (benign/malignant) at the periphery of the specimens, all the frozen and corresponding permanent sections from these cases were retrospectively reviewed.

Results: A total of 165 frozens were performed. The results were summarized in Table 1.

Table 1: Summary of the margins and the diagnoses in frozen section (FS) and corresponding permanent section (PS)

115 Frozen sections showing fibromuscular/fibroconnective tissue:
--- PS showing fibromuscular/fibroconnective in 108 cases
-------41 Apex
-------44 Base
-------23 Mid-lateral
--- PS showing benign ptostatic glands in 7 cases
-------4 Base
-------2 Mid-lateral
-------1 Apex
39 FS showing benign prostatic glands
--- PS showing benign prostatic glands in 38 cases
--------8 Apex
--------22 base
------- 8 Mid-lateral
--- PS reverted into prostatic carcinoma in 1 case
9 FS showing prostatic carcinoma
--- PS showing ptostatic carcinoma in 8 cases
-------2 Apex
-------3 Base
-------3 Mid-lateral
--- PS reverted into benign prostatic tissue in 1 case

The predictive value of a positive and negative carcinoma by frozen was 89% and 97%, respectively. The positive predictive value of a negative prostatic gland by frozen was 93%. When prostatic glands were present on frozen, the predictive value of a positive carcinoma by frozen was 19%. The predictive value of a positive prostatic gland (including carcinoma) by intraoperative macroscopic assessment was 34%.

Conclusions: Frozen section has both high positive and negative predictive values in assessing prostatic glands/carcinoma. Given the adverse effect of a carcinoma margin on disease recurrence and survival, a 19% of positive predictive value for carcinoma when prostatic glands present on frozen merits an attention and obtaining of a margin free of prostatic glands should be sought. Intraoperative macroscopic assessment has low predicative value for prostatic glands, which may be related to the degree of accuracy of the examining device used. In conclusion, frozen section is a reliable method to obtain margins free of prostatic glands, including carcinoma.

915 Expression of the Glucocorticoid Receptor in Renal Cell Neoplasms: Diagnostic, Histogenetic, and Prognostic Implications

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Background: Glucocorticoids (GCs) are steroid hormones involved in a variety of physiologic and pathologic processes, such as cellular differentiation, growth, inflammation, and the immune response. GCs mediate their effect by binding to the

glucocorticoid receptors (GRs), members of the steroid hormone receptor superfamily, which are expressed in a variety of target tissues, including the kidney. Our goal was to determine the expression pattern and prognostic significance of GR in renal cell neoplasms (RCNs).

Design: Paraffin embedded microarray specimens from 187 consecutive patients with RCNs were analyzed for GR expression by IHC. A rabbit polyclonal Ab PA1-511A from Affinity Bioreagents was used as the primary Ab. Cases were stratified into 132 clear cell renal cell carcinomas (CRCC), 25 papillary RCC (PRCC), 16 chromophobe RCC (CHRCC), and 14 oncocytomas (OC). The intensity of protein expression was scored semiquantitatively on a scale of 0-3+.

Results: Strong nuclear GR expression was present in normal renal glomeruli and in the proximal convoluted tubules, whereas distal convoluted tubules and collecting ducts were negative. In the RCNs the staining pattern was similar to that in normal kidney with predominant nuclear GR localization. GR expression was found in the majority of CRCC (68%), in 24% of PRCC, and in only 6% of CHRCC and 14% of OC (P<0.005). Within the CRCC group the vast majority of positive cases (88%) demonstrated strong nuclear reactivity (2+ and 3+), whereas none of the CHRCCs and OCs showed strong expression. Univariate analysis revealed a significant direct correlation between GR expression and overall survival in the CRCC group (*P*=0.03). By the end of follow-up 88% of CRCC patients with positive GR staining were alive as opposed to 59% of the patients whose tumors were negative. Multivariate analysis indicated that GR expression was an independent predictor of survival (*P*=0.04).

Expression of GR in RCNs

GR	CRCC	PRCC	CHRCC	OC
Positive	90/132 (68%)	6/25 (24%)	1/16 (6%)	2/14 (14%)
Strong (2+-3+)	79/132 (60%)	1/25 (4%)	0/16 (0%)	0/14 (0%)

Conclusions: The majority of CRCC strongly express GR, distinguishing them from CHRCC and OC. This expression pattern reflects the histogenetic origin of CRCC from the proximal nephron. GR may be considered in the IHC panel to more accurately subtype RCNs. Moreover, GR expression proved to be a strong predictor of patient survival in CRCC.

916 How Should Hilar Paratesticular Soft Tissue Invasion Be Staged in Germ Cell Tumors (GCT)

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Background: Extratesticular extension (ETE) is defined as tumor penetration through the entire thickness of tunica albuginea. To our knowledge, only one study evaluated the ETE in GCT and found that it occurs only in the testicular hilum. Although invasion of extratesticular structures, such as tunica vaginalis, epididymis, spermatic cord and scrotum is recognized in the current TNM system, involvement of hilar paratesticular soft tissue (PST) is not addressed.

Design: We investigated the frequency and the location of ETE in GCT, and focused on the hilum of the testis. We reviewed the slides and the pathology reports of 289 consecutive testicular GCT (209 seminomas, 80 mixed GCT), which were resected in our institution between 09/1999 and 12/2006. When ETE was identified, we assessed the tumor extension into the following structures: tunica vaginalis, epididymis, hilar (PST) and spermatic cord.

Results: ETE was identified in 58 (20%) of all testicular GCT: 34 (16%) in seminomas and 24 (30%) in mixed GCT. When ETE was present, it involved the hilar (PST) in all cases and in 36 (62%), it was the only extratesticular site involved without invasion of other structures. Additional invasion of epididymis and spermatic cord was found in 16 (27%) and 10 (17%) tumors with ETE, respectively. Tunica vaginalis invasion was found only in 2 (3%) of tumors with ETE, which represented 0.7% of all GCT. Four tumors demonstrated extensive hilar involvement and it was difficult to distinguish hilar (PST) invasion from a true spermatic cord invasion. When we compared histopathological variables between tumors with and without ETE, presence of ETE was strongly associated with vascular invasion (p<0.001) and rete testis invasion (p<0.001). Tumors with ETE had larger mean size vs. tumors without ETE (4.9 vs. 3.8cm; p=0.027). Distribution of histologic tumor type (seminoma vs. mixed GCT) did not differ significantly in tumors with and without ETE.

Conclusions: ETE occurs exclusively through the hilum of the testis. Most common location of ETE is the hilar (PST). ETE is strongly associated with presence of vascular and rete testis invasion and it is more commonly seen in larger tumors. Tunica vaginalis invasion is an exceptionally rare finding in GCT. Hilar (PST) invasion should be incorporated in the future revisions of the TNM staging for testicular GCT and further studies should validate its prognostic significance.

917 Novel Immunohistochemical Biomarkers for Renal Cell Carcinoma Derived from Expression Microarray Data

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Background: Biomarkers discovered through microarray technology have been exploited in the development of several novel immunohistochemical assays for tumor diagnosis. We used this approach to identify novel diagnostic markers for renal cell carcinoma (RCC).

Design: RCC microarray datasets were assessed for quality control with the CaCorrect algorithm, and candidate biomarkers were identified with the OmniBiomarker algorithm. Candidate RCC biomarkers were verified by quantitative RT-PCR, using total RNA from fixed tissues of 17 clear cell, 13 papillary and 7 chromophobe RCC. Selected biomarkers were further verified by immunohistochemistry, performed on KIC1501 tissue microarray (Clonagen), which includes 66 clear cell, 16 papillary and 12 chromophobe RCC. Antibodies used were: anti- Carbonic anhydrase IX (CA9); anti-Schwannomin-interacting protein 1 (SCHIP1); and anti- Cytochrome c oxidase subunit 5a (COX5A). Intensity of staining was graded on a 0/1+/2+/3+ scale.

Results: CA9 was strongly overexpressed in clear cell RCC by microarray, and this pattern was verified by quantitative RT-PCR and immunohistochemistry ($p < 0.001$). SCHIP1 was strongly overexpressed in papillary RCC by microarray and quantitative RT-PCR. By immunohistochemistry, staining was seen in papillary and clear cell RCC, but 3+ intensity was significantly more frequent in papillary RCC ($p < 0.001$). COX5A was strongly overexpressed in chromophobe RCC by microarray and quantitative RT-PCR. By immunohistochemistry, staining for COX5A was seen in all RCC subtypes; however, 3+ intensity was significantly more frequent in chromophobe RCC ($p < 0.02$).

Conclusions: Biomarker expression profiling is expected to become more important for renal tumor classification, with both diagnositic and therapeutic implications. CA9 is a known clear cell RCC biomarker and potential therapeutic target. SCHIP1 has not been described in RCC previously, and should be considered as a supplementary biomarker for papillary RCC diagnosis. COX5A is a mitochondrial gene product; many such proteins are overexpressed in chromophobe RCC. These candidate markers should be evaluated in larger prospective studies to validate their clinical diagnostic utility.

918 Differential Protein Expression in Renal Cell Carcinoma: Biomarker Discovery and Clinical Applications

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Background: Renal cell carcinoma (RCC) is the most common neoplasm in the adult kidney. Unfortunately, there are currently no biomarkers for the diagnosis of RCC. In addition to early detection, biomarkers have potential use for prognosis, monitoring recurrence after treatment, and as predictive markers for treatment efficiency.

Design: In this study, we identified proteins that are dysregulated in RCC, utilizing a quantitative mass spectrometry analysis. We compared the protein expression of kidney cancer tissues to their normal counterparts from the same patient using LC-MS/MS. iTRAQ labeling permitted simultaneous quantitative analysis of four samples (cancer, normal, and two controls) by separately tagging the peptides in these samples with four cleavable molecular weight tags (114, 115, 116, and 117). The samples were then pooled and the tagged peptides resolved first by strong cation exchange chromatography and then by nanobore reverse phase chromatography coupled online to nano-electrospray MS/MS.

Results: We identified a total of 937 proteins in two runs. There was a statistically significant positive correlation of the proteins identified in both runs ($rp = 0.695$, $p < 0.001$). Using a cutoff value of 0.67 fold for underexpression and 1.5 fold for overexpression, we identified 168 underexpressed proteins, and 156 proteins that were overexpressed in RCC compared to normal. These dysregulated proteins in RCC were statistically significantly different from those of urothelial carcinoma and end-stage glomerulonephritis. We performed an in-silico validation of our results by different approaches. We also experiemtally validated our findings by Western blotting. In silico analysis showed that many of these proteins are involved in cancer-related pathways and can serve as potential diagnostic and/or therapeutic targets.

Conclusions: Our results suggest that protein expression profile in RCC is significantly different from that of other kidney diseases. Identified proteins have the potential as tumor markers. Larger scale analyses are needed to examine whether these proteins are consistently differentially expressed in RCC.

919 Unilateral Severe Chronic Injury of the Kidney without Uremia Can Give Rise to Renal Cell Carcinoma with or without Acquired Cystic Kidney Diseases: Proof of Concept

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Background: Renal cell carcinoma (RCC) of different histologic types is a well known complication of end-stage renal disease (ESRD). This can happen with or without a background of ACKD. It is currently believed that the systemic milieu of ESRD in patients maintained by long-term dialysis constitutes the major cause of this condition. However, alternative pathogenesis has not been explored.

Design: All nephrectomy specimens with RCC, with or without associated ACKD, that developed against a background of severe renal changes characteristic for ESRD, including glomerulosclerosis, tubular atrophy, interstitial fibrosis, and vascular wall thickening, within a ten-year period(1997-2007) were reviewed.

Results: Twenty six such cases were found. In 24 cases, the patients had ESRD and were maintained on long-term dialysis. The remaining two patients **did not** have ESRD. In one of them, the nephrectomy specimen showed one RCC with typical features of the "ACKD-specific" type, multiple clear cell RCCs, multiple papillary adenomas, against a background of severe chronic renal injury identical to the type of changes seen in ESRD; but cystic changes were not seen. In the other patient, the left nephrectomy specimen showed one papillary RCC, one tubulocystic RCC, multiple papillary adenomas, multiple large cysts with or without mural tumor nodules characteristic for ACKD, against the background of severe chronic renal injury similar to that of the other case. The right partial nephrectomy showed a 3 cm papillary RCC, but was otherwise histologically normal. Virtual karyotyping with the 250K Nsp mapping array (Affymetrix, Santa Clara) on the papillary and tubulocystic RCCs showed similar chromosomal profiles characteristic for papillary RCC. In both patients, the atrophic changes were unilateral by renal imaging and probably related to the renal arterial stenosis of the same side. Both patients had normal serum creatinine pre-and post-operatively.

Conclusions: These observations provide proofs of the novel concept that unilateral severe chronic injury of the kidney can give rise to RCC with or without associated ACKD in the absence of ESRD. A common chromosomal imbalance profile of

histologically diverse tumors in this context further suggests a common derivative for these RCCs.

920 FAS-N Expression in Prostate Cancer: Can It Be Used To Distinguish Cancer from Benign in Prostate Biopsies?

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Background: Fatty acid synthase (FAS) is a cytosolic polypeptide that is required for de novo lipogenesis. FAS has been found to be over expressed in different cancers, including prostate cancer. Further studies have shown that FAS over expression occurs early in the development of prostate cancer. However, its expression in detecting prostate cancer in prostate core biopsy with the hope of using it as a new modality to diagnose prostate cancer has not been fully investigated, especially where H & E staining is limited.

Design: A total of 121 cases identified from the years 2000 to 2006 with prostate core biopsy tissue containing adenocarcinoma and confirmed at prostatectomy with a Gleason score (GS) of 6 or greater were retrieved from Division of Anatomic Pathology and immunohistochemically stained for FAS-N using a monoclonal antibody from DAKO, Carpenteria, CA. The stains were independently evaluated by three observers and a case was considered negative (0) if <1% of the cells of interest exhibited immunoreactivity. Positive stains were graded as weak (1+), intermediate (2+) or strong (3+); as well as focal if 1-50% of the cells stained or diffuse if >50% of the cells stained.

Results: We had a total of 121 patients whose ages ranged from 43 to 88yrs with a median of 68yrs. Patients cancer ranged from GS of 6 to 10 with a median of GS 8. Approximately 27% of the sample had GS 6. In each of the GS 7, 8 and 9 there was 21% of the sample. 8% had GS 10. Table 1 shows frequency for each of the 4 staining levels. Atrophic tissue stains the least with 99% of it showing no staining at all. The majority (75%) show no staining. On the reverse side, most cancers show either 3+ (44%), 2+ (27%) or 1+ (28%), only 1 case had no staining. High grade PINs mostly (55.4%) show no staining.

Table 1: Frequency table (with percentages) for each staining intensity

Intensity	0	1+	2+	3+
Atrophy	120 (99.2%)	1 (0.8%)	0 (0.0%)	0 (0.0%)
Benign	91 (75.2%)	30 (24.8%)	0 (0.0%)	0 (0.0%)
Adenocarcinoma	1 (0.8%)	34 (28.1%)	33 (27.3%)	53 (43.8%)
Hg PIN	67 (55.4%)	18 (14.9%)	24 (19.8%)	12 (9.9%)

Conclusions: FAS-N can be used to distinguish adenocarcinoma from benign including atrophic prostate tissue in biopsy with high sensitivity (99%) and specificity (100%) when the staining intensity is 2+ or 3+. When the staining intensity is weak (1+) or the differential diagnosis is an Hg PIN, a routine basal cell layer marker should be added to aid in diagnosis.

921 Lef1 Expression in Androgen-Independent Prostate Cancer

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Background: A major problem in prostate cancer treatment is the progression of cancer cells to androgen-independent cancer. Markers for this progression are critically needed. Recent evidences suggest aberrant expression of AR (androgen receptor) may play a role in cancer progression and treatment failure. AR expression is potentially regulated by LEF1 in androgen-independent prostate cancer. We examined the expression of LEF1 in androgen-dependent and -independent prostate cancer.

Design: Immunohistochemical study with LEF1 antibody was used to characterize its expression in 99 prostate samples, including 24 cases of benign, 56 cases of androgen-dependent and 19 cases of androgen-independent prostate cancer. Androgen-dependent specimens were derived from patients who were diagnosed with prostate cancer by TURP, having high grade (Gleason 8 or higher) and volume of disease. Androgen-independent samples were derived from patients who underwent TURP at least 6 months after surgical orchiectomy. The immunostaining signal is scored as a combination of intensity (0 as negative, 1+ as weak, 2+ as moderate and 3+ as strong expression) and percentage of positive cells (<10% as 1+ and ≥10% as 2+) as in any given case with highest score as 5.

Results: Of 24 benign cases, LEF1 is not expressed in luminal cells in all cases and is only expressed in basal cells in certain cases. LEF1 is expressed in 9 of 56 (16%) androgen-dependent cases ranging from 20% to 60% positive cells for a given case. We observed a statistically significant ($p<0.01$) difference of LEF1 expression between androgen-dependent and -independent cases. LEF1 is expressed in 13 of 19 (66%) androgen-independent cases. The expression of combined score is statistically significantly higher in androgen-independent than -dependent cases ($p=0.016$).

Conclusions: We demonstrated a dramatic increase of LEF1 expression in androgen-independent disease. The results of this study is consistent with our in vitro data that LEF1 increased AR expression and consequently enhanced growth and invasion ability in androgen-independent cancer cell line. Thus, we identified LEF1 as a potential marker for androgen independent disease.

922 Detection of *TMPRSS2* Gene Deletions and Translocations in Carcinoma, Intraepithelial Neoplasia and Normal Epithelium of the Prostate by Direct Fluorescence *In Situ* Hybridization

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Background: *TMPRSS2* gene fusions with *ETS*-transcription factor family members *ERG*, *ETV1* or *ETV4* have been recently discovered as a common molecular event in prostate cancer. Much attention has been focused on exploring their clinical application as a genetic tumor marker for diagnosis, prognosis, and prediction of response to therapy. However, detection of *TMPRSS2* genetic alterations using direct fluorescence *in situ* hybridization (FISH) has not been well studied. We examined cancers, intraepithelial neoplasia (PIN), and normal prostate epithelium to determine frequency, specificity, tissue heterogeneity, and prognostic value of the *TMPRSS2* genetic alterations.

Design: 71 cases (161 samples) of normal prostate, 60 cases (153 samples) of PIN, and 61 cases (142 samples) of carcinoma in formalin-fixed paraffin-embedded tissue microarray were tested using a direct *TMPRSS2* dual-color break-apart FISH probe cocktail. This FISH probe cocktail theoretically should detect all known *TMPRSS2*-associated deletions or translocations.

Results: 62% of prostate carcinomas demonstrated *TMPRSS2* gene alterations, including 39% translocation, 16% deletion and 7% mixed patterns. Tissue heterogeneity for *TMPRSS2* gene alterations was identified in 28% of prostate carcinomas. No difference in frequency of *TMPRSS2* gene alterations was found between Gleason 6 and 7. 17% of PIN had *TMPRSS2* gene alterations and presented the same FISH pattern as the corresponding carcinoma. None of 161 normal prostate samples showed *TMPRSS2* translocation or deletion.

Conclusions: Direct *TMPRSS2* dual-color break-apart FISH probe cocktail provides a simple and reliable method for detection of *TMPRSS2*-related genetic alterations in formalin-fixed paraffin-embedded tissue. *TMPRSS2* genetic alterations detectable by this method are highly specific for prostate neoplasia, and can be identified in the majority of prostate carcinomas. Tissue heterogeneity for *TMPRSS2* alterations is common, and it should be considered when sampling and evaluating biopsy specimens.

923 High Grade Foamy Gland Prostatic Adenocarcinoma on Biopsy or Transurethral Resection: A Morphological Study of 55 Cases

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Background: Foamy gland carcinoma is a variant of adenocarcinoma of the prostate that typically is assigned a Gleason score 3+3=6. The morphological features of high foamy gland carcinoma have not been previously studied.

Design: We analyzed 55 cases of high grade (Gleason score ≥7) foamy gland carcinoma of the prostate in needle biopsy (n=49) or TURP (n=6).

Results: On average, 3.4 cores were involved by high grade foamy gland carcinoma. On average, 84% of the total tumor volume was foamy gland carcinoma, with high grade foamy gland cancer averaging 73% of the total foamy gland carcinoma. The most common architectural pattern was cribriform (73%), followed by fused/poorly defined glands (55%), cords/single cells (11%) and solid sheets (5%). Nuclear enlargement was observed in 82% of cases. Prominent nucleoli were either absent or infrequent in 69% of cases, and seen more frequently with Gleason score ≥8. Mitotic figures were observed in 40% of cases, more frequently in Gleason score ≥8. In 56% of cases, intraluminal dense pink secretions were identified. Perineural invasion and extraprostatic extension identified on the biopsy specimens were noted in 33% and 9% of cases, respectively. In 33% of cases, there was at least a moderate stromal reaction. In 6 cases, there was a peculiar extensive desmoplastic reaction almost obscuring the carcinoma component, 5 of which were Gleason scores 4+4=8. Concurrent ordinary acinar non-foamy adenocarcinoma was encountered in 47% of the cases: Gleason 6 (27%); Gleason 7 (27%); and Gleason 8-10 (46%). Of the 19 cases with available immunohistochemical stains for high molecular weight cytokeratin, 7 (37%) showed nonspecific labeling of cancer cells in a non-basal cell pattern. A similar finding was seen in 1 of the 7 (14%) of the cases with available stains for p63. α-Methyl-CoA racemase (AMACR) positivity was noted in all 9 cases stained.

Conclusions: High grade foamy gland cancer shares certain morphological features with more typical lower grade foamy gland cancer including relatively bland nuclei with more difficult to identify nucleoli as well as frequent intraluminal dense pink secretions. However, consistent with their higher architectural grade, high grade foamy gland cancers had more prominent nucleoli and increased mitotic figures compared to lower grade foamy grade cancer. A unique subset of high grade foamy gland carcinoma poses particularly difficult diagnostic challenges, with scattered, scant, relatively bland foamy glands imbedded in an extensive densely sclerotic desmoplastic stroma.

924 Xp11.2 Translocation Renal Cell Carcinoma(RCC) in Adults – A TMA Study of 120 RCC Cases

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Background: Xp11.2 translocation renal cell carcinomas (RCC), a distinctive entity in the 2004 WHO renal tumor classification, are rare neoplasms and are often encountered in the pediatric and young adult population. The diagnosis of Xp11.2 translocation carcinomas has also been reported in elderly patients. This incidence is probably underestimated partly due to unavailable genetic studies and overlapping histopathological morphology with clear cell and papillary RCC. TFE3 immunohistochemical assay has been proven as a sensitive and specific method for the diagnosis of the Xp11.2 translocation RCC, however, the weaker nuclear stain remains problematic for interpretation.

Design: A total of 120 consecutive adult (>18Y) RCC patient specimens (FFPE during the period 2001-2008) from our institute, were collected to construct a tissue micro array(TMA). Immunohistochemical analysis was performed on TMA using TFE3 (P-16) antibody. The staining intensity was graded according to previous report (AJSP 2003; 27:750). We have investigated few cases with strong and weaker nuclear stain using RT-PCR(1 case with weaker nuclear stain and 2 cases with strong nuclear stain) and Western blot analysis (2 cases with no staining, 4 cases with weaker nuclear stain and 2 cases with strong nuclear stain).

Results: Among the 120 RCC, 11(9.2%) cases were TFE3 positive (strong nuclear stain) with a mean age of 54 (range 31-77). There were 6 females and 5 males. The percentage of positive cases in different age groups, were 20% (3/15) in the age group 18-40; 5.9%(3/51) in the age group 41-60 and 9.3%(5/54) in the age group older than 60. No TFE3 fusion transcripts (ASPL, PRCC, CLTC, PSF and Nono) were detected by RT-PCR in the tested case with weak nuclear stain. The two tested cases with strong nuclear staining showed the ASPL-TFE3 transcript. Full length TFE3 protein was detected by western blot in all 4 tested cases with weaker nuclear staining.

Conclusions: 1) Xp11.2 translocation RCC is not an uncommon neoplasm. The incidence in this group of 120 patients is 9.2% in adults and up to 20% in patients between 18-40 years. 2) TFE3(P-16) IHC is a valuable assay for the diagnosis Xp11.2 translocation RCC. The strong nuclear TFE3 stain is most likely indicative of Xp11.2 translocation. The weaker nuclear stain appears to be due to expression of full length TFE3 protein, rather than chimeric fusion protein due to translocation.

925 *TMPRSS2-ERG* Gene Fusion in Prostate Cancer of Different Ethnic Groups

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Background: Prostate cancer (PCa) exhibits significant differences in prevalence and mortality among different ethnic groups. The genetics underlying these differences is not well understood. The *TMPRSS2-ERG* fusion is a common recurrent chromosomal aberration in this malignancy and is thought to represent an early event in the prostate carcinogenesis. In this study, we examined the frequency of *TMPRSS2-ERG* gene fusion in PCa from Caucasion, Asian and African American patients.

Design: A tissue microarray of PCa from 51 Caucasian, 22 Asian and 59 African American patients who underwent radical prostactomies was contructed. PCa Gleason score (GS) was 7 in all Caucasian patients; GS 6 in 31 and GS 7 in 28 African American patients; and GS 6 in 4, GS 7 in 8 and GS≥8 in 10 Asian patients. The *TMPRSS2-ERG* fusion status was determined using a multi-color interphase fluorescence in situ hybridization assay for *ERG* break-apart. A nucleus without ERG rearrangement has 2 pairs of juxtaposed red and green (yellow) signals. A nucleus with an *ERG* break apart (indicative of a *TMPRSS2-ERG* fusion) show split-apart of one juxtaposed red-green signal pair resulting in a single red and green signal for the transolcated *ERG* allele and a still combined (yellow) signal for the nontranslocated *ERG* allele in each cell. The telomeric green signal may be lost due to deletion and results in one yellow and one red signal in a nucleus with *ERG* rearrangement through deletion.

Results: *TMPRSS2-ERG* gene fusion was present in 45.1% (23/51) Caucasian, 13.6% (3/22) Asian, and 33.9% (20/59) African American patients (p=0.034 by chi-square test). The gene fusion through translocation, deletion or both occurred in 56.5% (13/23), 39.1% (9/23) and 4.3% (1/23) in Caucasian, 66.7 (2/3), 33.3 (1/3) and 0% (0/3) in Asian and 20%, 60% (12/20) and 20%(4/20) in African American patients, respectively (p=0.098 by chi-square test).

Conclusions: The frequency of *TMPRSS2-ERG* was determined to be significantly different in PCa of Caucasion, Asian and African American patients. This is a small study so the potential limitations to this study include why the patients were screened as they came from different populations. Further studies are needed to address whether such difference may account for the difference in the prevalence and mortality among different ethnic groups.

926 Atypical Cribiform Lesions of the Prostate: Implications for Diagnosis in Prostate Biopsies

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Background: Atypical cribiform lesions of the prostate (ACL) are cribiform structures consisting of cytologically malignant cells with partial or complete basal cell lining. They can represent "intraductal carcinoma" which is invariably associated with high grade prostate carcinoma (PCa) (cancer associated ACL, ACL-PCa), or cribiform high grade PIN which can be an isolated finding not associated with PCa (non-cancer associated ACL, ACL-nonPCa). We report the histological differences of these 2 lesions on radical prostatectomy (RP) and implications for diagnosis in prostate biopsies (NBX).

Design: RP was reviewed for ACL. The presence of basal cells was confirmed by basal cell immunostaining. ACL intermixed with, or within 2 mm from the border of PCa was categorized as ACL-PCa. The following histological features were reviewed: number of ACL/prostate gland, size, glandular contour (round, irregular, branching), architectural pattern (trabecular, cribiform, solid), comedonecrosis, nuclear feature (round and uniform, round with varying sizes, pleomorhpic, giant nuclei [≥6x adjacent nuclei]).

Results: 35 cases with ACL-PCa and 17 cases with ACL-nonPCa were studied (Table). The mean number of ACL per prostate was 25.3 for ACL-PCa and 2.4 for ACl-nonPCa (p=0.008). The size ranged from 0.2-6.5 mm for ACL-PCa, and 0.2-1mm for ACL-nonPCa. The branching contour was present in 29/35 ACL-PCa, but in only 1/17 ACL-nonPCa (p<0.001). The architectural patterns were not different between the two (p=0.21). Comedeonecrosis was present in 14/35 ACL-Pca, and none of ACL-nonPCa (p=0.002). The pleomorphic nuclei or giant nuclei that were ≥6X of the adjacent nuclei were present in 9 of ACL-PCa, but none of ACL-nonPCa (p=0.049).

	ACL-PCa	ACL-nonPCa	P value
# Cases	35	17	
Mean # of ACL/prostate gland	25.3	2.4	0.008
Size (range, mm)	0.2-6.5	0.2-1	
Branching glandular contour	29/35	1/17	<0.001
Comedonecrosis	14/35	0/17	0.002
Nuclear features: pleomorhpic or giant (x6 adjacent nuclei)	9/35	0/17	0.049

Conclusions: NBXs demonstrating several features of large foci, architectural complexity with large branching glands, pleomorphic or giant nuclei, or comedonecrosis are invariably associated with invasive cancer. A note indicating such should be included in the biopsy report. As there is overlap in histological appearance between ACL-PCa and ACL-nonPCa, the presence of atypical cribriform lesions in NBX should prompt repeat biopsy to rule out concomitant PCa.

927 Establishment of Laminin and Pankeratin Dual Immunostain for the Evaluation of Urothelial Carcinoma

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Background: Incorrect staging due to misinterpretation of lamina propria invasion is one of the most commonly described diagnostic errors in bladder specimens. Lamina propria invasion can be difficult to assess due to the presence of only a small microinvasive focus or fragmentation artifact. Immunohistochemical assessment for basement membrane or epithelial cells may help corroborate the histologic impression of lamina propria involvement. We sought to create a dual immunostain containing a basement membrane marker (laminin) and an epithelial marker (pankeratin) to facilitate the interpretation of lamina propria invasion.

Design: 504 cases of paraffin embedded urothelial carcinoma were obtained, including 58 whole tissue sections (cystectomies, transurethral resections and biopsies) and 446 cases using tissue microarray. The tissue was incubated with laminin primary monoclonal antibody (1:25) with 3,3'-diaminobenzidine as the chromogen followed by pankeratin cocktail incubation (prediluted) using Vulcan fast red as the secondary chromogen. Pankeratin was semi-quantitatively analyzed as 0, < 5% of cells stained; 1+, 5-10% of cells stained; 2+, 11-50% of cells stained; or 3+, >50% of cells stained. Invasive and noninvasive urothelial components as well as blood vessels were assessed for laminin.

Results: Pankeratin stained benign and malignant urothelial cells in the vast majority of the 504 cases (0, 2%; 1+, 1; 2+, 1%; 3+, 96%) with intense staining and little background, allowing for clear visualization. Within the 58 whole tissue sections, 40 contained only invasive carcinoma, all of which completely lacked laminin expression surrounding tumor. 18 of the 58 tissue sections contained a noninvasive component, demonstrating laminin separating the urothelium from the lamina propria. Dual staining highlighted vascular invasion in 5 of the 58 cases. One of the 5 cases containing vascular invasion was not diagnosed in the corresponding pathology report.

Conclusions: We created a dual immunostain of laminin and pankeratin. Invasive carcinoma was strongly positive for pankeratin and lacked laminin. Noninvasive components showed dual expression. The dual immunostain of laminin and pankeratin shows promise as a tool to facilitate the interpretation of invasion in bladder specimens. Additionally, this dual stain highlights vascular invasion, allowing for the assessment of both lamina propria and vascular invasion on the same slide.

928 MicroRNA Expression Profiling Distinguishes Conventional and Chromophobe Renal Cell Carcinomas from Normal Renal Parenchyma

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Background: MicroRNAs (miRNA) are small non-coding RNAs that modulate expression of protein-encoding genes by binding and inactivating messenger RNA. Renal cell carcinoma (RCC) subtypes are traditionally distinguished by morphologic, immunophenotypic, and cytogenetic characteristics. miRNA expression patterns have not been previously reported for RCC subtypes. The aims of this study are to 1) determine if unique miRNA expression profiles are present among 2 RCC subtypes (conventional (CON) and chromophobe (CHR)) and normal renal parenchyma (NRP) and 2) identify specific miRNAs that may contribute to underlying mechanisms of renal tumorigenesis.

Design: Sixteen specimens of frozen, banked human renal tissue with 4 in each group (CON, CHR, NPR) yielded high integrity RNA that was labeled without amplification and hybridized on microarrays (Exiqon, Denmark) covering all annotated miRNAs in the miRBase (1168 probes with 4 replicates/array). Statistically significant differential expression was determined by the Significance Analysis of Microarray (SAM) multiclass test (Q=0.03) and post-hoc t-test of individual transcripts.

Results: Differential expression was observed in 32 miRNAs among the 3 specimen classes. CON specimens exhibited 14 miRNAs increased from 1.6 to 8.4 fold compared to NRP. These miRNAs were not differentially expressed between CHR and NPR. However, CHR samples revealed an additional 14 distinct miRNAs elevated (2.3 to 9.4 fold) versus NPR specimens that were not differentially expressed between CON and NPR specimens. These miRNAs were all expressed at significantly different levels between CON and CHR samples. *miR-21* was among the identified miRNAs increased in CON, consistent with upregulation in other malignancies, including breast, colorectal and cervical carcinoma.

Conclusions: The expression pattern obtained from the RCC tumor samples provided an exclusionary signature for each CON and CHR tumor class when compared to NRP, and from each other. Our data suggest that post-translational regulation may contribute to RCC tumorigenesis as well as to the molecular differentiation pathways resulting in histologic subtypes of RCC. We are currently assessing miRNA profiles in papillary RCC and oncocytoma and evaluating messenger RNA profiles in tumors from the corresponding cases to extend the molecular characterization of miRNA expression in renal neoplasia.

Gynecologic

929 Metastatic Pancreatic Adenocarcinoma to the Ovary: A Clinicopathologic Review of Twenty Nine Cases

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Background: Metastatic pancreatic adenocarcinoma to the ovary (MPAO) can represent a diagnostic dilemma. The number of studies on this topic is limited. In this study, we present the clinicopathologic features of twenty nine such cases seen at our institution.