Pathologic Features, Proliferative Activity, and Cyclin D1 Expression in Hurthle Cell Neoplasms of the Thyroid

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Making a histologic distinction between Hurthle cell adenomas and carcinomas sometimes may be difficult. We analyzed a series of Hurthle cell lesions to determine whether specific histologic features and expression of Ki67 and cyclin D1 could be useful in distinguishing Hurthle cell adenomas from carcinomas. Formalin-fixed, paraffin-embedded tissues from 128 Hurthle cell neoplasms, including 59 adenomas; 55 carcinomas; and 14 tumors classified as neoplasms of uncertain malignant behavior (UMB), which had equivocal capsular invasion but no vascular invasion, were analyzed for expression of Ki67 and cyclin D1 by immunostaining. The distribution of immunoreactivity for Ki67 with antibody MIB-1 was analyzed by quantifying the percentage of positive nuclei that was expressed as the labeling index.

None of the patients with adenomas or UMB tumors developed recurrent or metastatic disease after a mean follow-up of 7.8 and 7.9 years, respectively. Of the 55 patients with Hurthle cell carcinoma, 19 were associated with metastatic disease, 13 of whom died with disease. No patient with a Hurthle cell carcinoma without vascular invasion developed metastatic disease. The mean tumor size for Hurthle cell carcinomas (4.8 cm) was significantly larger than that of Hurthle cell adenomas (3.1 cm) or UMB tumors (3.7 cm). No patient with a Hurthle cell tumor smaller than 3.5 cm developed metastatic disease, even when vascular invasion was present. The Ki67 labeling index in Hurthle cell carcinomas (10.0 ± 1.2) was 3-fold higher than in Hurthle cell adenomas (3.2 ± 0.3). The Ki67 labeling index in the UMB group was 5.0 ± 0.7. Cyclin D1 showed diffuse nuclear staining in 1 of the 59 (1.7%) Hurthle cell adenomas, in 10 of the 55 (18%) Hurthle cell carcinomas, and in none of the UMB tumors.

In summary, analyses of the cell cycle proteins Ki67 and cyclin D1 in Hurthle cell thyroid neoplasms indicate that these markers may assist in distinguishing some Hurthle cell carcinomas from adenomas. Among the Hurthle cell carcinomas, large tumor size and vascular invasion are associated with clinically aggressive tumors. Our study also suggests that Hurthle cell neoplasms with only equivocal capsular invasion and no vascular invasion should behave in a benign manner.

KEY WORDS: Cyclin D1, Hurthle cell, Ki67, Thyroid.
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Hurthle cell neoplasms are uncommon thyroid tumors with Hurthle cell carcinoma composing only 2 to 3% of all cases of thyroid carcinoma (1). The majority of these tumors can be readily diagnosed by characteristic histopathologic features. However, making a distinction between Hurthle cell adenoma and Hurthle cell carcinoma sometimes can be difficult. Thus, additional diagnostic features that can assist in this determination would be clinically useful.

Recent studies suggested that Ki67, a proliferation marker, and cyclin D1, a cell cycle regulatory protein, may be important in predicting behavior of various thyroid tumors (2–6). The proliferation marker Ki67 is expressed in all stages of the cell cycle, except G0 (7). Carr *et al.* (2) showed that high rates of proliferation, as measured by the MIB-1 antibody to Ki67, may be helpful in recognizing some thyroid tumors with more aggressive behavior. Another study demonstrated that follicular carcinomas expressed significantly higher levels of Ki67 than follicular adenomas, indicating a higher proliferative rate in the malignant tumors (4).

The cell cycle is regulated by a host of cell cyclins, cyclin-dependent kinases, and cyclin-dependent

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kinase inhibitors. Cyclin D1 is a regulator of cell cycle progression from G1 to S phase (8–11). Encoded by a gene on chromosome 11q13 (12, 13), cyclin D1 is overexpressed in many tumors (14–26). It is interesting that overexpression of cyclin D1 is seen in a variety of tumors without amplification or genetic alteration of the cyclin D1 gene (14, 21). Recent studies suggested that cyclin D1 may have a role in thyroid tumorigenesis (5, 6). Zou *et al.* (5) found a 4- to 5-fold increase in cyclin D1 expression in a subset of thyroid papillary carcinomas, as compared with benign nodular goiters. No evidence of cyclin D1 gene amplification or rearrangement was found to account for the increase in cyclin D1 expression (5). Lazzereschi *et al.* (6) also found cyclin D1 to be overexpressed in a subset of papillary carcinomas without genetic alteration or amplification. The goals of this study were to analyze the histologic features and the expression of Ki67 and cyclin D1 in Hurthle cell neoplasms of the thyroid and the usefulness of histopathologic features and immunohistochemical markers in distinguishing Hurthle cell adenomas from carcinomas.

MATERIALS AND METHODS

Cases and Tissues

Formalin-fixed, paraffin-embedded tissue blocks from 128 randomly selected patients who underwent surgery at the Mayo Clinic, Rochester, MN, between 1962 and 1995 were used. These included 59 Hurthle cell adenomas, 55 Hurthle cell carcinomas, and 14 Hurthle cell neoplasms of uncertain malignant behavior (UMB). Hematoxylin- and eosin-stained sections were reviewed independently by three of the authors (LAE, JRG, RVL) for verification of diagnoses (Fig. 1). By definition, the neoplasms were composed exclusively or predominantly (more than 75%) of follicular cells exhibiting Hurthle cell features (27). Focal architectural papillary features were identified in five cases; however, these tumors did not have the nuclear features of papillary thyroid carcinoma. Neoplasms that were composed of fewer than 75% of follicular cells exhibiting Hurthle cell features (two cases) were excluded. All tumors with oncocytic cytoplasmic features and nuclear features of papillary carcinomas as recently described (28) were also excluded.

The mean number of sections of the tumor-capsular interface evaluated in the Hurthle cell carcinoma cases was 7.2 (range, 1 to 23). Two cases of Hurthle cell carcinoma with only capsular invasion each had only one section evaluated. Both were small tumors, 1.1 cm and 2 cm, and additional deeper sections were cut from the blocks to evaluate for vascular invasion. The mean number of sections evaluated for Hurthle cell carcinomas with vascular invasion was 7.0. The mean number of sections evaluated for Hurthle cell carcinomas with only capsular invasion was 8.8. When available, the original wet tissue was reexamined and additional sections were taken to evaluate for vascular invasion.

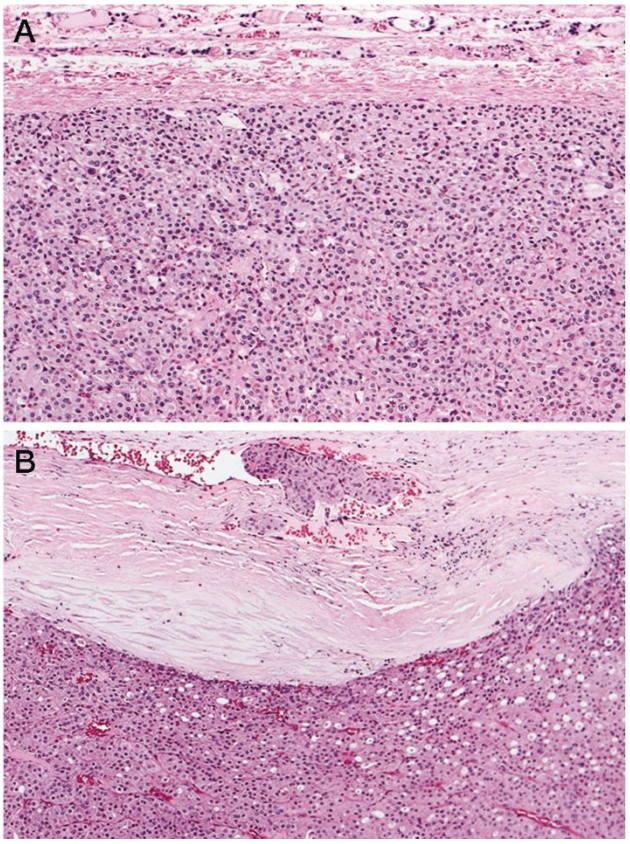

FIGURE 1. Histologic examples of Hurthle cell neoplasms analyzed. **A,** Hurthle cell adenoma composed exclusively of follicular cells exhibiting oncocytic features in a follicular growth pattern. **B,** Hurthle cell carcinoma with vascular invasion.

Neoplasms classified as adenomas were single lesions, encapsulated, and microscopically uniform and usually compressed the non-neoplastic gland. Hurthle cell adenomas did not show capsular or vascular invasion. Neoplasms classified as carcinomas showed unequivocal capsular and/or vascular invasion. Tumors with unequivocal capsular invasion had tumor cells penetrating through the entire thickness of the capsule. Vascular invasion was defined as tumor cells partly or completely obliterating a vessel located within or outside the capsule with tumor cells attached to the vessel wall. Lesions with no vascular invasion and only questionable capsular invasion (*i.e.*, nests of tumor cells present in the capsule but not penetrating completely through the capsule) were classified as Hurthle cell UMB neoplasms, analogous to the designation that Rosai *et al.* (27) used for follicular neoplasms with similar characteristics. All of the UMB tumors were originally diagnosed as Hurthle cell carcinomas,

but in reevaluation of all of the material available for this study, no unequivocal invasion, either vascular or capsular, was identified.

Patient Information

Patient history and follow-up information were obtained by chart review. Clinical information, including age, gender, mean follow-up, tumor size, and metastatic disease, are summarized in Table 1.

Immunohistochemical Analysis

Formalin-fixed, paraffin-embedded tissue sections were cut at 5 μm and treated with 0.1 mol/L citrate, pH 6.0, in an 800-W microwave oven for 15 min for antigen retrieval before immunostaining. A monoclonal antibody to Ki67 (AMAC, Westbrook, ME) was used at a 1:50 dilution. The rabbit polyclonal anti–cyclin D1 antibody was used at a 1:500 dilution as previously reported (22). Antigen retrieval by microwaving was done for 15 min as previously reported (4). Immunostaining was done with the Elite avidin-biotin-peroxidase kit (Vector Laboratories, Burlingame, CA), according to the manufacturer's specifications. Slides were counterstained with hematoxylin for 1 second. Tonsil tissues, positive for both Ki67 and cyclin D1, were used as positive controls. Normal mouse or rabbit sera were substituted for the primary antibodies as negative controls.

Quantitation

The distribution of immunoreactivity was analyzed by quantifying nuclear staining in each case without knowledge of the diagnosis or outcome. The percentages of cells expressing Ki67 were determined by counting 1000 cells per slide with the aid of an ocular 10 $\times$ 10-mm grid (LJ). Ki67-immunoreactive cells were counted in areas of highest density of staining over a minimum of 10 high power fields, because of the low percentage of cells staining positive for Ki67 in benign thyroid tissue. This method of estimating Ki67 labeling has been used by other investigators in studies of thyroid and parathyroid tumors and to evaluate mitotic activity in adrenal cortical neoplasms (4, 29, 30). The percentage of positive nuclei was expressed as the labeling index (LI). When 10% of the cases were blindly recounted for Ki67, the LIs did not vary more than $\pm$10% from the original count. LIs were expressed as the mean $\pm$ standard error of the mean for the Ki67 labeling. The percentages of cyclin D1 positive nuclei were estimated as no nuclear staining, less than 5% positive nuclei, 5 to 25% positive nuclei, and more than 25% positive nuclei for the entire section.

Statistics

Differences among the three tumor groups for our parameters of interest were assessed using Kruskal-Wallis and Fisher's exact tests. Pairwise differences were evaluated using paired t tests, Wilcoxon rank sum test, and Fisher's exact test. We assessed the association between metastases and our parameters of interest using log linear models for discrete parameters and logistic regression for the continuous ones.

RESULTS

The clinicopathologic features of the cases studied are summarized in Table 1. The mean follow-up period ranged from 7.8 to 10.1 years for the groups. No patient with a Hurthle cell adenoma or a UMB tumor developed metastatic disease. Metastatic disease was identified in 19 of the 55 patients with Hurthle cell carcinomas, with 11 patients showing metastatic disease at the time of thyroid surgery. Females composed approximately 68% of the patients in the adenoma group, 56% in the carcinoma group, and 50% in the UMB group. Among the Hurthle cell carcinomas, a significantly higher percentage of males (13 of 24; 54%) than females (6 of 31; 19%) developed metastatic disease ($P = .007$). Follow-up of the 55 patients with Hurthle cell carcinomas showed 27 alive without disease, 3 alive with disease, 12 dead without disease, and 13 dead of disease.

All of the Hurthle cell lesions were composed of more than 75% follicular cells with oncocytic or oxyphil features (Fig. 1). Hurthle cell adenomas were single, encapsulated, uniform tumors without capsular or vascular invasion. Hurthle cell carcinomas showed unequivocal capsular and/or vascular invasion. Forty-six of the 55 Hurthle cell carcinomas showed vascular invasion, and the other 9

TABLE 1. Clinical and Pathologic Features of Hurthle Cell Neoplasms

Diagnosis	*N*	Mean age (yr)	Female/ Male	Mean Follow-Up (yr)	Mean Tumor Size (cm)	Metastatic Disease	Dead of Disease
Hurthle cell adenoma	59	60	40/19	7.8	3.1	0	0
UMB	14	52	7/7	7.9	3.7	0	0
Hurthle cell carcinoma	55	60	31/24	10.1	4.8[a]	19	13

UMB, uncertain malignant behavior.

[a] Hurthle cell carcinomas were significantly larger than Hurthle cell adenomas ($p = .0003$).

cases showed only capsular invasion. Hurthle cell neoplasms of uncertain malignant behavior showed no vascular invasion and only indefinite capsular invasion. In predicting the development of metastatic disease on the basis of histopathologic features, the type of invasion (vascular *versus* capsular) was significant ($P = .017$). Nineteen of the 46 Hurthle cell carcinomas with vascular invasion were associated with metastatic disease, whereas none of the patients with Hurthle cell carcinoma with only capsular invasion (no vascular invasion) developed metastatic disease.

The mean tumor size of Hurthle cell carcinomas (4.8 cm; range, 1.1 to 12 cm) was significantly larger than that of Hurthle cell adenomas (3.1 cm; range, 0.5 to 8 cm; $P = .0003$; Table 1). Hurthle cell carcinomas with vascular invasion (5.3 cm; range, 1.5 to 12 cm) were significantly larger than those with only capsular invasion (2.5 cm; range, 1.1 to 4 cm; $P = .0017$; Table 2). The mean tumor size of Hurthle cell carcinomas with metastatic disease (7.1 cm; range, 3.5 to 12 cm) was significantly greater than that of those without metastatic disease (3.5 cm; range, 1.1 to 8 cm; $P = .0000$; Table 3). No patient with a Hurthle cell carcinoma smaller than 3.5 cm developed metastatic disease, even in the presence of vascular invasion.

Immunohistochemical staining revealed nuclear localization of both Ki67 and cyclin D1 proteins in all groups of Hurthle cell tumors (Fig. 2). Analysis of the three groups of Hurthle cell neoplasms showed a lower percentage of adenomas and UMB cells immunostaining for Ki67 than carcinoma cells (Fig. 3 and Table 4). Hurthle cell adenomas had a slightly lower Ki67 LI than UMB tumors. Hurthle cell carcinomas (10.0 ± 1.2) had a significantly higher Ki67 LI than Hurthle cell adenomas (3.2 ± 0.3; $P < .0001$) and UMB tumors (5.0 ± 0.7; $P = .0439$; Table 4). Hurthle cell carcinomas with vascular invasion had a significantly higher Ki67 LI (11.0) than the carcinomas without vascular invasion (LI, 4.5; $P = .0174$; Table 5). Similarly, Hurthle cell carcinomas with metastatic disease had a significantly higher Ki67 LI (14.1) than carcinomas without metastatic disease (LI, 7.8; $P = .0033$; Table 2).

TABLE 2. Size, Ki67, and Metastatic Disease in Hurthle Cell Carcinomas with and without Vascular Invasion

Vascular Invasion	*N*	Tumor Size (cm) (mean ± SEM)	Ki67 Labeling Index (mean ± SEM)	Metastatic Disease
Absent	9	2.5 ± 0.3	4.5 ± 1.0	0
Present	46	5.3 ± 0.4^a	$11.0^b \pm 1.4$	19^c

SEM, standard error of the mean.

[a] Hurthle cell carcinomas with vascular invasion were significantly larger than those without vascular invasion ($p = .0017$).

[b] Hurthle cell carcinomas with vascular invasion had a significantly higher K167 labeling index than those without vascular invasion ($p = .0174$).

[c] Metastatic disease was seen in 19 Hurthle cell carcinomas with vascular invasion but in no tumors with only capsular invasion ($p = .011$).

TABLE 3. Size and Ki67 in Hurthle Cell Carcinomas with and without Metastases

Metastatic Disease	*N*	Tumor Size (cm) (mean ± SEM)	Ki67 Labeling Index (mean ± SEM)
Absent	36	3.5 ± 0.3	7.8 ± 1.3
Present	19	7.1 ± 0.5^a	14.1 ± 2.2^b

SEM, standard error of the mean.

[a] Hurthle cell carcinomas with metastatic disease were significantly larger than those without metastatic disease ($p < .0001$).

[b] Hurthle cell carcinomas with metastatic disease had a significantly higher Ki67 labeling index than those without metastatic disease ($p = .0033$).

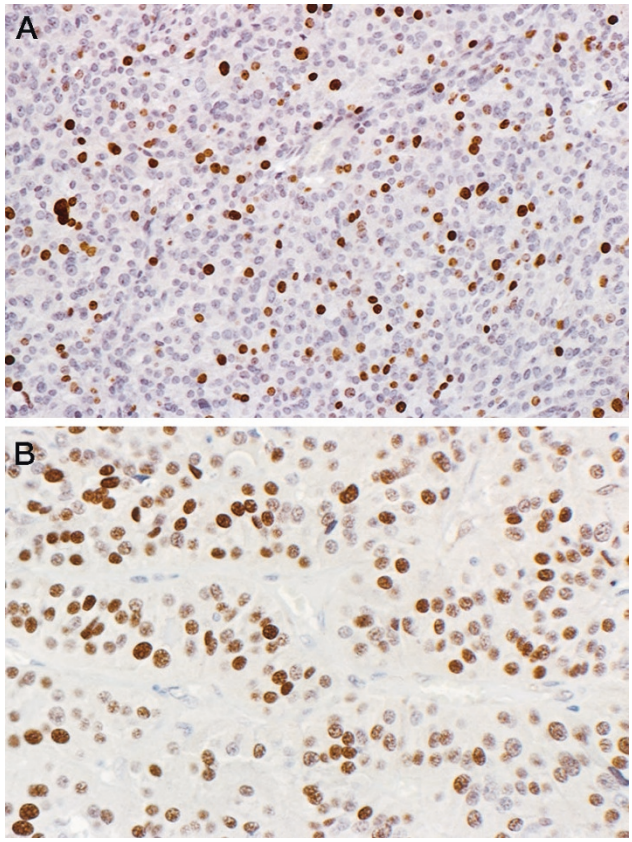

FIGURE 2. Ki67 and cyclin D1 expression in Hurthle cell neoplasms. **A,** Hurthle cell carcinoma with many nuclei staining strongly for Ki67 and a labeling index of 37%. **B**, diffuse staining for cyclin D1 in a Hurthle cell carcinoma (more than 25%).

The results of immunostaining with the antibody to cyclin D1 are summarized in Table 5. Diffuse nuclear cyclin D1 expression (more than 25% of nuclei positive) occurred more frequently in Hurthle cell carcinomas (18.2%) compared with adenomas (1.7%) or UMB tumors (0.0%; $P = .006$; Table 5). Diffuse nuclear staining was seen in 10 of the 55 (18.2%) Hurthle cell carcinomas, 1 of the 59 (1.7%) Hurthle cell adenomas, and none of the UMB tumors. Eight of the 10 Hurthle cell carcinomas with diffuse cyclin D1 immunostaining showed vascular invasion. Diffuse staining for cyclin D1 was seen in a greater percentage of Hurthle cell carcinomas with metastatic disease (5 of 19; 26%) than

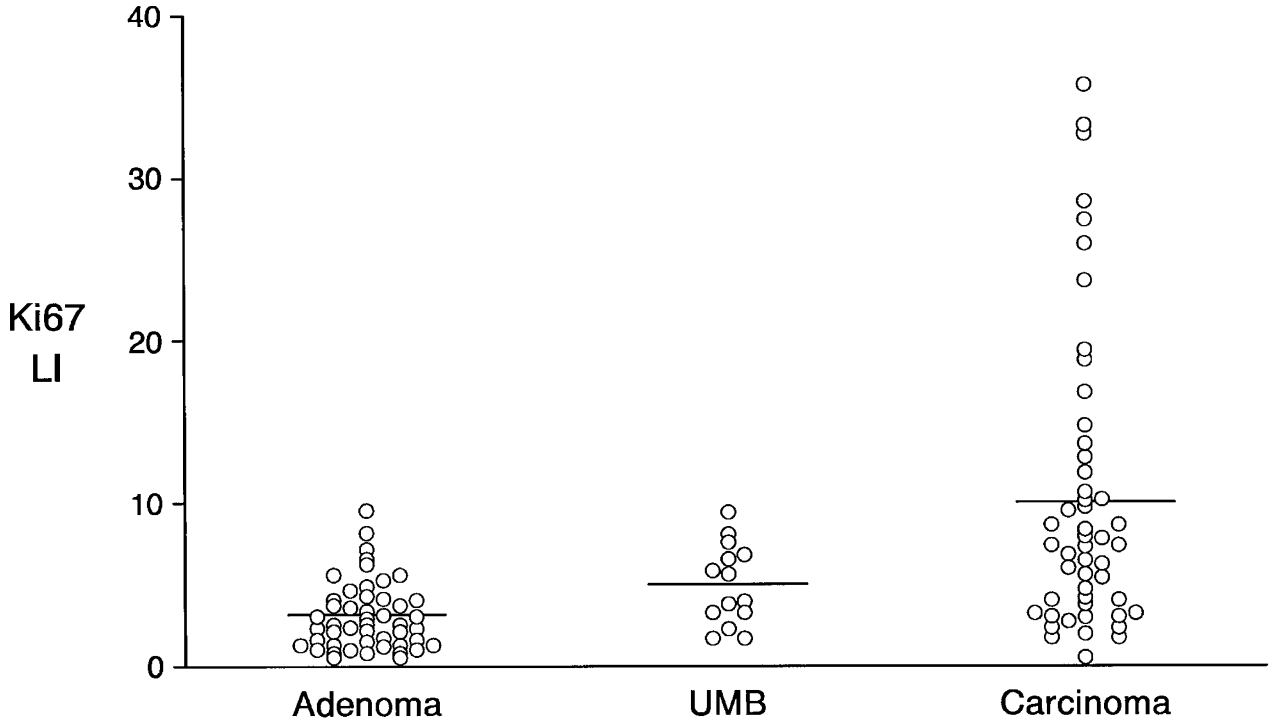

FIGURE 3. Analysis of Ki67 in Hurthle cell neoplasms. The distribution of Ki67 labeling indices and the mean labeling index for each group of neoplasms is shown. Hurthle cell carcinomas had the highest Ki67 labeling index, 3-fold higher than that of Hurthle cell adenomas.

TABLE 4. Ki67 Immunoreactivities in Hurthle Cell Tumors

Diagnosis	*N*	Ki67 Labeling Index (mean ± SEM)
Hurthle cell adenoma	59	3.2 ± 0.3[a]
UMB	14	5.0 ± 0.7
Hurthle cell carcinoma	55	10.0 ± 1.2[b]

UMB, uncertain malignant behavior.

SEM, standard error of the mean.

[a] Ki67 for Hurthle cell adenomas compared with UMB group was significantly different ($p = .0105$).

[b] Ki67 for Hurthle cell carcinomas compared with adenomas and the UMB group were significantly different ($p < .0001$ and $p = .0439$, respectively).

TABLE 5. Percentages of Cells Immunoreactive with Cyclin D1 Protein in Hurthle Cell Tumors

Diagnosis	*N*	Cyclin D1			
		0%	<5%	5–25%	>25%
Hurthle cell adenoma	59	42	14	2	1
UMB	14	8	4	2	0
Hurthle cell carcinoma	55	30	11	4	10[a]

UMB, uncertain malignant behavior.

[a] Although considerable variability was seen among the three groups, diffuse (>25% nuclei positive) staining for cyclin D1 occurred more frequently in Hurthle cell carcinomas compared with adenomas or UMB tumors ($p = .006$).

Hurthle cell carcinomas without metastatic disease (5 of 36; 14%), although these results did not reach statistical significance.

DISCUSSION

Analysis of the cell cycle proliferation marker Ki67 showed significant differences between benign and malignant Hurthle cell tumors of the thyroid. In this study, Ki67 was effective in distinguishing between some Hurthle cell carcinomas and adenomas, although there was overlap at the lower Ki67 LIs. As a group, the Ki67 LI was 3-fold higher in Hurthle cell carcinomas as compared with Hurthle cell adenomas. Our recent study found a 3-fold increase in the LI of Ki67 in follicular carcinomas as compared with follicular adenomas, similar to the present study of Hurthle cell neoplasms (4). The Ki67 LIs for Hurthle cell carcinomas (10.0 ± 1.2) and Hurthle cell adenomas (3.2 ± 0.3) are lower than the recently reported Ki67 LIs of follicular carcinomas (15.6 ± 3.1) and follicular adenomas (4.5 ± 0.6) (4). Although the number of cases of Hurthle cell tumors was greater than the follicular tumors in the previous study (4), these differences in Ki67 immunostaining suggest a higher proliferation rate in follicular tumors.

Hurthle cell carcinomas with vascular invasion showed a higher proliferative rate than tumors with only capsular invasion. Van Heerden *et al.* (31) reported that follicular carcinomas with vascular invasion had higher rates of distant metastases than follicular carcinomas with only capsular invasion, indicating that the type of invasive growth is important in predicting the behavior of follicular thyroid carcinomas. In our study, the nine cases of Hurthle cell carcinoma without vascular invasion

neither had metastases at diagnosis nor subsequently developed metastases during the follow-up period. These findings suggest that vascular invasion may also be important in predicting the behavior of Hurthle cell thyroid carcinomas as it is in the closely related follicular carcinomas, which agrees with the previous report (31).

Several studies have suggested that tumor size is predictive of malignancy in Hurthle cell neoplasms (32–34). Recently, Chen *et al.* (32) reported that patients with Hurthle cell carcinomas had significantly larger tumors (4.0 cm) than those with Hurthle cell adenomas (2.4 cm) and concluded that size was predictive of malignancy in Hurthle cell neoplasms. Our observation that Hurthle cell carcinomas had a significantly larger mean tumor size than adenomas (4.8 cm *versus* 3.1 cm) agrees with previous studies, although considerable overlap was present. Carcangiu *et al.* (35) evaluated 153 Hurthle cell neoplasms and found that all tumors smaller than 1 cm were benign and all tumors larger than 10 cm were malignant. In the present series, we found that all tumors smaller than 1.1 cm were benign and all tumors larger than 8 cm were malignant. Perhaps even more important than the diagnosis of malignancy is the actual behavior of the neoplasm. Hurthle cell carcinomas with metastases had a significantly larger mean tumor size than Hurthle cell carcinomas without metastases. No patient in our study with a Hurthle cell neoplasm smaller than 3.5 cm in size had metastatic disease, even in the presence of vascular or capsular invasion. The larger mean tumor size of Hurthle cell carcinomas with metastases compared with those without metastases confirms the prognostic importance of tumor size in Hurthle cell neoplasms.

The Hurthle cell neoplasms of uncertain malignant behavior in our study behaved like Hurthle cell adenomas. No patient in the Hurthle cell adenoma group or UMB group developed metastatic disease. In their study of 153 Hurthle cell neoplasms Carcangiu *et al.* (35) included 35 cases classified as "indeterminate." They also found that the indeterminate lesions behaved in a clinically benign fashion. Tallini *et al.* (36) suggested that indeterminate categories not be used for Hurthle cell neoplasms "because in the absence of invasion, these neoplasms almost invariably behave in a benign fashion." We suggest that if extensive histologic evaluation reveals only equivocal capsular invasion and no vascular invasion, then the indeterminate lesions such as those in our UMB group should behave in a benign manner.

Recent studies have suggested that cyclin D1 may have a role in thyroid tumorigenesis (5, 6, 37). In our study, diffuse staining for cyclin D1 was seen in a greater percentage of Hurthle cell carcinomas than adenomas or UMB tumors. These findings indicate that cyclin D1, which is a regulator of cell cycle progression, may be overexpressed in some Hurthle cell carcinomas. Zou *et al.* (5) found a 4- to 5-fold increase in cyclin D1 expression in a subset of thyroid papillary carcinomas, compared with benign nodular goiters. Lazzereschi *et al.* (6) also found cyclin D1 overexpressed in a subset of papillary carcinomas without genetic alterations or amplification. In a recent study of 116 cases of thyroid tumors, Wang *et al.* (37) found cyclin D1 expression in 37 (63%) cases of follicular variant of papillary carcinomas and in 34 (60%) cases of follicular adenomas. There was no difference in cyclin D1 expression between these two groups. In the present study, we found that diffuse cyclin D1 protein expression was more common in Hurthle cell carcinomas compared with Hurthle cell adenomas. Because cyclin D1 staining was present in only a relatively small number of tumors, technical problems such as antigen preservation in formalin-fixed, paraffin embedded tissues probably contributed to suboptimal staining for cyclin D1 protein.

Historically, some authors had suggested that all Hurthle cell neoplasms should be regarded as malignant or potentially malignant (33). Our study indicates that Hurthle cell lesions without histologic evidence of vascular invasion after extensive sampling behave in a benign manner.

In summary, analyses of the cell cycle proteins Ki67 and cyclin D1 in Hurthle cell thyroid neoplasms indicate that these markers may assist in distinguishing between Hurthle cell carcinomas and adenomas in diagnostically difficult cases. Among the Hurthle cell carcinomas, large tumor size and vascular invasion are associated with clinically aggressive tumors. Our study also suggests that Hurthle cell neoplasms with only equivocal capsular invasion and no vascular invasion should behave in a benign manner.

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