

Hepatic adenomas with synchronous or metachronous fibrolamellar carcinomas: both are characterized by LFABP loss

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Rare hepatic adenomas are associated with synchronous or metachronous fibrolamellar carcinomas. The morphology of these adenomas has not been well described and they have not been subclassified using the current molecular classification schema. We examined four hepatic adenomas co-occurring with or preceding a diagnosis of fibrolamellar carcinoma in three patients. On histological examination, three of the adenomas showed the typical morphology of *HNF1-α* inactivated adenomas, whereas one showed a myxoid adenoma morphology. All of the adenomas were negative for *PRKACA* rearrangements by Fluorescence *in situ* Hybridization (FISH) analysis. All four of the adenomas showed complete loss or significant reduction of liver fatty acid binding protein (LFABP) expression by immunohistochemistry. Interestingly, the fibrolamellar carcinomas in each case also showed loss of LFABP by immunohistochemistry. One of the fibrolamellar carcinomas was negative for *PRKACA* rearrangements by FISH, whereas the others were positive. To investigate if LFABP loss is typical of fibrolamellar carcinomas in general, an additional cohort of tumors was studied (*n* = 19). All 19 fibrolamellar carcinomas showed the expected *PRKACA* rearrangements and immunostains showed loss of LFABP in each case, consistent with *HNF1-α* inactivation. To validate this observation, mass spectrometry-based proteomics was performed on tumor-normal pairs of six fibrolamellar carcinomas and showed an average 10-fold reduction in LFABP protein levels, compared with matched normal liver tissue. In conclusion, hepatic adenomas co-occurring with fibrolamellar carcinomas show LFABP loss and are negative for *PRKACA* rearrangements, indicating they are genetically distinct lesions. These data also demonstrate that LFABP loss, which characterizes *HNF1-α* inactivation, is a consistent feature of fibrolamellar carcinoma, indicating *HNF1-α* inactivation is an important event in fibrolamellar carcinoma pathogenesis.

Modern Pathology (2016) 29, 607–615; doi:10.1038/modpathol.2016.59; published online 25 March 2016

Fibrolamellar carcinoma is a rare subtype of primary liver carcinoma that affects younger patients without underlying chronic liver disease. The carcinoma is histologically distinct and shows a consistent somatic deletion leading to *DNAJB1-PRKACA* fusion transcripts.^{1,2} Although the tumorigenic mechanism for this fusion protein is not yet proven, the fusion

protein appears to drive overexpression of *PRKACA*. *PRKACA* is one of the catalytic subunits of protein kinase A, a key regulatory kinase with numerous downstream signaling targets.

Rarely, hepatic adenomas can co-occur with fibrolamellar carcinomas, either as synchronous or metachronous tumors. Although documented in a case report³ and a review article,⁴ these adenomas have not been fully described at the histological level and have not been subclassified using the current immunohistochemical panel of liver fatty acid binding protein (LFABP), C-reactive protein (CRP), serum amyloid A (SAA), beta catenin, and glutamine synthesis. In addition, questions persist as

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Received 12 November 2015; revised 11 February 2016; accepted 11 February 2016; published online 25 March 2016

to whether these adenomas are simply misdiagnosed fibrolamellar carcinomas, misdiagnosed because of solid growth patterns with limited intratumoral fibrosis, an important diagnostic pitfall in the pathology of fibrolamellar carcinomas.⁴ The initial goals of this study were to describe the morphology of these adenomas, subtype them using the current hepatic adenoma classification system, and use molecular methods to determine if these adenomas share the distinctive *DNAJB1-PRKACA* fusion transcripts of fibrolamellar carcinoma. Based on our findings, we extended the study to determine if LFABP loss was characteristic of fibrolamellar carcinomas.

Materials and methods

We identified hepatic adenomas co-occurring with fibrolamellar carcinomas by searching the consultation or institutional files of several of the authors (LMT, MST, and MWC). Representative slides were centrally reviewed (RPG and MST). Formalin-fixed paraffin-embedded tissue blocks were chosen for immunohistochemistry, mass spectrometry-based proteomics, and molecular cytogenetic studies. Based on the initial study findings, additional material from 19 fibrolamellar carcinomas was selected for LFABP testing, including a group of 11 fibrolamellar carcinomas previously shown to harbor *DNAJB1-PRKACA*¹ and an additional cohort of 8 fibrolamellar carcinomas.

Immunohistochemistry

Five micron-thick sections were cut from the tissue block and immunohistochemistry performed using antibodies to Arginase-1 (predilute, clone SP156; Cell Marque), HepPar1 (predilute, clone OCH1E5; Ventana), cytokeratin 7 (1:100, Clone OV-TL 12/30; Dako), CD68 (clone KP1, 1:50-1:100; Dako), PRKAR1A (1:2000, clone OTI6C7; Origene Technologies), LFABP (1:50, rabbit polyclonal; Abcam), CRP (1:2000, clone Y284; Abcam), SAA (1:2000, clone mc1; Dako), Glutamine synthetase (1:2000, clone GS-6; Millipore), and beta catenin (predilute, clone 14; Ventana). Steam antigen retrieval and standard clinical methods were used for each antibody.

Fluorescence *in situ* Hybridization (FISH)

FISH for *PRKACA* rearrangement was performed on 5 μ -thick formal-fixed sections. *PRKACA* FISH was performed on the hepatic adenomas and the paired fibrolamellar carcinomas. In addition, we examined the untested fibrolamellar carcinomas in the follow-up cohort ($n=8$). *PRKACA* FISH used the validated probe and methodology as described previously.¹ FISH slides were visualized and scored by two experienced technologists who each independently evaluated 50 tumor nuclei.

Mass Spectrometry-based Proteomic Analysis

On six randomly selected fibrolamellar carcinomas,¹ known to harbor the *DNAJB1-PRKACA* fusion transcript, we performed laser capture microdissection of areas of tumor and paired normal liver tissue on 10- μ tissue sections cut on to PEN membrane slides. Approximately an 500 000 μm^2 area of tissue was collected for each sample by laser capture. Collected fragments were captured into a tube containing lysis buffer, composed of 0.1 M Tris-HCl (pH=8.5) with 0.002% Zwittergent Z3-16, via laser pressure catapulting. Proteins were extracted from the tissue fragments by heating the mixture at 99 °C for 1 h. Protein concentration of each microdissection was interpolated from a concentration vs area curve, which was pre-constructed by collecting different areas on a control tissue and estimating the resulting protein concentration with a BCA assay (Thermo-Fisher Scientific).

Proteins present in the patient samples were reduced by incubating at 56 °C with 2.5 μ l of Tris in 0.1 M Tris-HCl (pH=8.5; final concentration = 10 mM) for 30 min. Reduced proteins were alkylated by adding 2.5 μ l of iodoacetamide in 0.1 M Tris-HCl (pH=8.5; final concentration of 10 mM) and incubating in the dark at room temperature for 30 min. Proteins were digested by adding 2 μ l of trypsin to an enzyme to protein ratio of 1:50 and incubating at 37 °C for 16 h. The digestion was terminated by adding 3 μ l of 0.2% trifluoroacetic acid (TFA) and the samples were diluted to a protein content of 12.5 $\mu\text{g}/\mu\text{l}$ with a solution containing 0.2% formic acid (FA), 0.1% TFA, and 0.002% Z3-16.

Peptides present in each sample were loaded onto a 0.25 μ l bed OptiPak tap (Optimize Technologies) custom-packed with 5 μm , 200A Magic C8 (Bruker-Michrom) stationary phase. The loaded trap was washed for 4 min with an aqueous loading buffer of 0.2% FA and 0.05% TFA at 10 $\mu\text{l}/\text{min}$. Following the wash, peptides were transferred onto a 35 cm \times 100 μm PicoFrit column, self-packed with Agilent Poroshell 120S 2.7 μm EC-C18 stationary phase, using a Dionex UltiMate 3000 RSLC liquid chromatography (LC) system (Thermo-Fisher Scientific). Peptides were separated using a 400 nl/min LC gradient comprised of 2–40% B in 0–120 min, 40–95%B from 120–130 min, 95%B from 130–140 min, and re-equilibrated to 2%B from 140–150 min. The mobile phase A was 2% acetonitrile (ACN) in water with 0.2% FA and the mobile phase B was ACN/isopropanol/water (80/10/10 by volume) with 0.2% FA. Eluting peptides were analyzed using a QExactive Plus mass spectrometer (Thermo-Fisher Scientific). The instrument was configured to operate in data-dependent mode by collecting MS1 data at 70 000 resolving power (measured at m/z 200) with an AGC value of 1E6 over a m/z range of 360–2000, using lock masses from background polysiloxanes at m/z 371.10123 and 446.12002. Precursors were fragmented with normalized collision

energy of 28, fragments measured at 17 500 resolving power and a fixed first mass of 140. Resulting tandem mass spectra (MS/MS) were collected on the top 20 precursor masses present in each MS1 using an AGC value of 1E5, max ion fill time of 50ms, and an isolation window of 1.5 Da.

Bioinformatics

The number of MS/MS matching to a protein in a sample is considered to be a semi-quantitative measure of its abundance.⁵ We have successfully used this method to discover biomarkers for detecting amyloid deposits present in fat aspirate specimens⁶ and use this method for subtyping amyloid deposits present in clinical specimens.⁷ We utilized the same method for detecting differentially expressed proteins between fibrolamellar carcinoma and paired normal liver.⁸

To accomplish this, the MyriMatch database search engine was used to match the MS/MS present in data files against a composite protein sequence database containing RefSeq human proteome (version 53) and sequences common contaminants (eg wool and cotton). Reversed protein sequences were appended to the database for estimating peptide and protein false discovery rates. MyriMatch was configured to use 10 ppm m/z tolerance for precursors and fragments while performing peptide-spectrum matching (PSM). The software derived semitryptic peptides from the sequence database while looking for the following variable modifications: carbamidomethylation of cysteine (+57.023 Da), oxidation of methionine (+15.994 Da), and formation of n-terminal pyroglutamic acid (−17.023 Da). IDPicker software filtered the PSMs at 2% FDR. The software was configured to use an optimal combination of MVH, mzFidelity, and XCorr scores for filtering.⁹ Protein identifications with at least two unique peptide identifications were considered to be present in the sample. Resulting proteins were clustered into groups of proteins that match the same set of peptides. QuasiTel software performed differential expression of protein groups found in two different experimental groups using a quasi-likelihood-based generalized linear mixture model. Protein groups with a *P*-value ≤ 0.05 and an absolute fold change ≥ 1 (where 0.0 signifies no change) were considered as significantly differentially expressed between fibrolamellar carcinoma and matched normal liver.

Results

Gross and Microscopic Findings

Four cases of hepatic adenomas with fibrolamellar carcinomas were identified in three patients (Table 1). In each case, the hepatic adenomas were separate lesions from the fibrolamellar carcinomas on both gross examination and microscopic examination. No transition zones were seen.

The first set of three adenomas occurring in two individuals are discussed first, because the adenomas had the same morphology. One individual had both a primary resection for fibrolamellar carcinoma and a subsequent resection for recurrent disease: each resection specimen showed a single adenoma in addition to the fibrolamellar carcinoma, with the adenomas measuring 2.7 cm in the primary resection and 0.5 cm in the resection for recurrent fibrolamellar carcinoma. A second individual had hepatic adenomatosis, with >10 adenomas. In all of these resection specimens, the hepatic adenomas were distinctly separate nodules from the fibrolamellar carcinoma, though they were adjacent to the fibrolamellar carcinoma in two specimens. Both individuals were women, with an average age of 27 years. All three hepatic adenomas were morphologically similar, with histological findings typical of *HNF1-α* inactivated adenomas (Figure 1a). They all showed macrovesicular steatosis, from 10 to 70%, with no balloon cells, no significant intratumoral inflammation, and no intratumoral fibrosis. In the hepatic adenomatosis case, all of the adenomas showed similar features. None of the adenomas showed cytological atypia. None had the cytological features that typify fibrolamellar carcinoma: big pink cells, prominent nucleoli, and intratumoral fibrosis. In contrast, the co-occurring fibrolamellar carcinomas all showed the classic features of large eosinophilic cells with granular cytoplasm, prominent nucleoli, and abundant intratumoral fibrosis (Figure 1b). The fibrolamellar carcinomas had no other unusual histological features. They ranged in size from 7.4 to 8.5 cm.

The fourth adenoma case was resected in a 14-year-old female with a maternal history of the Carney complex and was previously reported as part of an array comparative genomic hybridization study.³ In this case, the adenoma measured 10 cm and was characterized by the recently described myxoid morphology (Figure 1c).¹⁰ The tumor

Table 1 Summary of index cases

Index patient	Gender	Temporal relationship of tumors	Hepatic adenoma size (cm)	Adenomatosis	FLC size (cm)	HCA type
First	F	Synchronous	Primary resection, 2.7 Recurrence resection, 0.6	N	Primary, 8 Recurrence, 7.5	1 1
Second	F	Synchronous	Adenomatosis	Y	8.5	1
Third	F	Metachronous	10	N	7.4	4

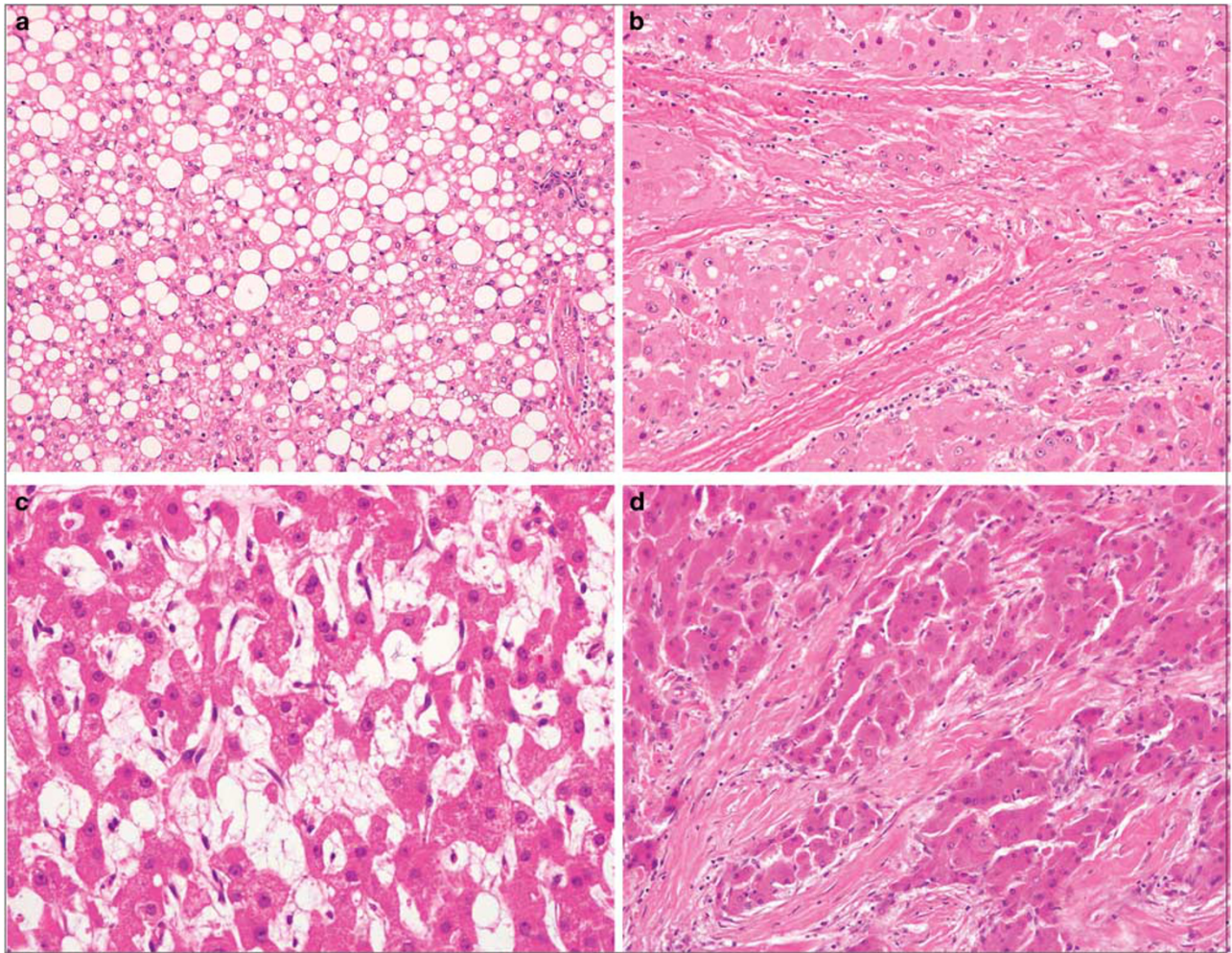


Figure 1 Morphological findings in livers with hepatic adenomas and fibrolamellar carcinomas. (a) The hepatic adenomas in the first three cases showed bland tumor cells with mild fatty change, findings typical of HNF1 alpha inactivated adenomas. (b) The co-existing fibrolamellar carcinomas showed the classical histological findings, with no unusual features. (c) In the fourth case, the adenoma showed a myxoid morphology. (d) The fibrolamellar carcinoma in the fourth case was also histologically typical.

showed cords and trabeculae of cytologically bland tumor cells widely separated by abundant extracellular myxoid material. Five years after the adenoma was resected, a fibrolamellar carcinoma developed in the same lobe of the liver. The resected fibrolamellar carcinoma showed classic histological features, with no unusual findings (Figure 1d).

Immunohistochemical and Molecular Findings

All four hepatic adenomas were negative for *PRKACA* rearrangements by FISH. The three synchronous adenomas were all classified as type 1 hepatic adenomas, or *HNF1-α* inactivated adenomas, by immunohistochemistry. They all showed complete loss of LFABP staining (Figure 2a), with negative or patchy weak staining for CRP and SAA, and membranous beta catenin labeling without nuclear accumulation. Glutamine synthetase did not show strong diffuse staining in any of the cases.

In contrast to these findings, the adenoma in the patient whose mother had the Carney complex demonstrated partial loss of LFABP (Figure 2b) as well as partial positivity for CRP (Figure 2c), and normal results for the beta catenin, SAA, and glutamine synthetase. This adenoma was thus classified as type 4 in the current classification system (unclassifiable).

Immunostains were also performed on the fibrolamellar carcinomas. All were positive for either Arginase-1 ($N=3$) or HepPar 1 ($N=1$) and were diffusely and strongly cytokeratin 7 and CD68 positive ($N=4$). For additional studies, the three primary fibrolamellar carcinomas were tested, but not the fibrolamellar carcinoma recurrence of the first case. In each of these three cases, the expression of LFABP was markedly diminished or absent (Figure 2d). All three were negative for nuclear accumulation of beta catenin and all showed patchy weak expression of SAA and were positive for CRP. By FISH, two of the three fibrolamellar carcinomas

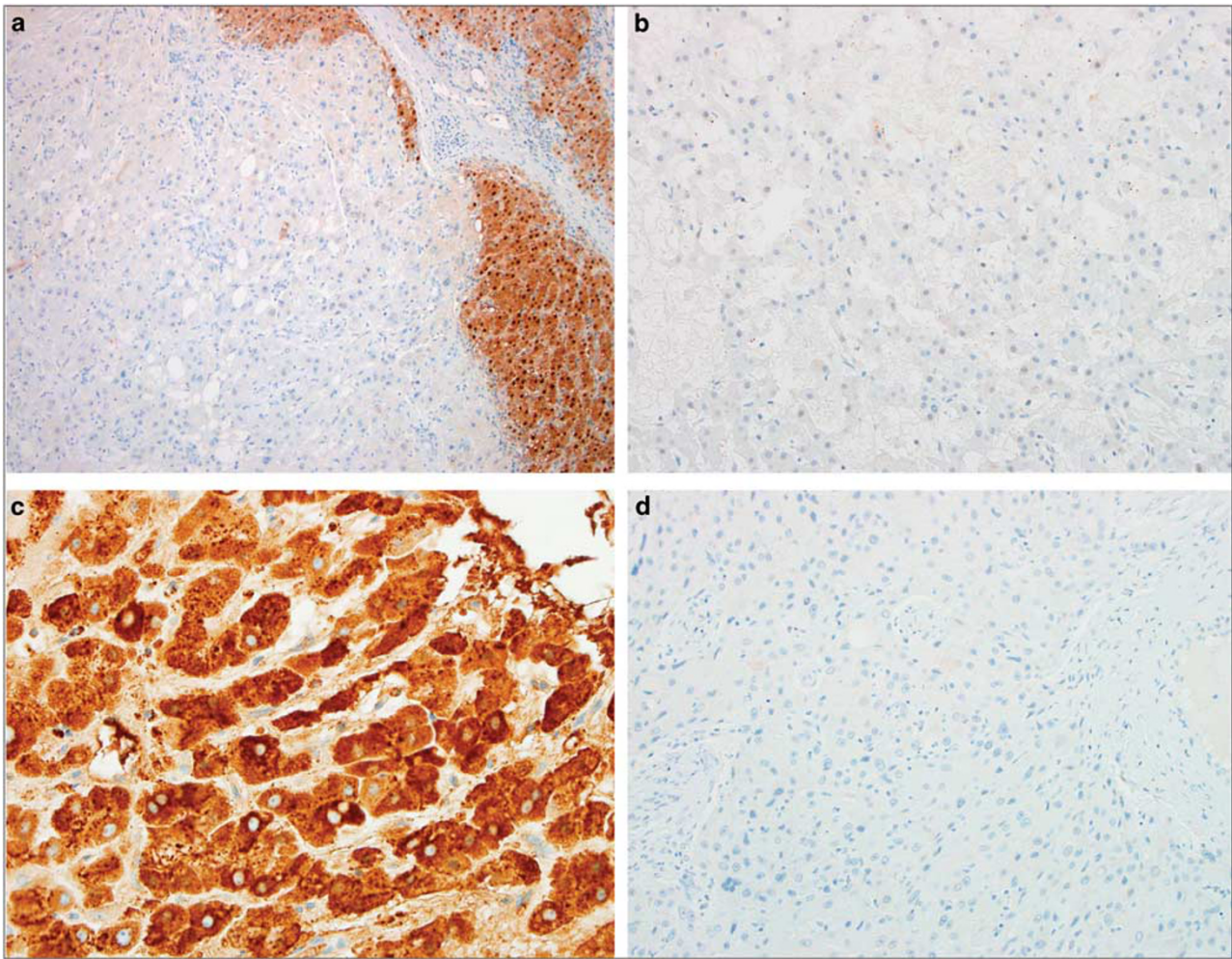


Figure 2 Immunostains in adenomas and fibrolamellar carcinomas. (a) The hepatic adenomas in case one although all three showed complete loss of LFABP staining. (b) In the fourth case, LFABP staining showed patchy loss. (c) The fourth case also showed patchy C-reactive protein (CRP) staining. (d) The fibrolamellar carcinomas in each of the four cases showed loss of LFABP staining.

harbored *PRKACA* rearrangements within all of the neoplastic cells of the (Figure 3a). However, the fibrolamellar carcinoma arising in the individual with a family history of the Carney Complex did not harbor *PRKACA* rearrangements by FISH (Figure 3b). RT-PCR was then performed and again showed no *PRKACA* rearrangements. However, re-review of the H&E stains confirmed that the morphological findings were typical of fibrolamellar carcinoma and the diagnosis was further confirmed by co-expression of CK7 and CD68.¹¹

Of note, the history of the Carney complex strongly suggests a germline mutation in *PRKAR1A*, which could represent an alternative mechanism of abnormal activation of protein kinase A. To follow-up on this possibility, we studied the expression of *PRKAR1A* in the fibrolamellar carcinoma negative for *PRKACA* rearrangements and in five control fibrolamellar carcinomas that had the classic *PRKACA* rearrangements by FISH. By immunohistochemistry, *PRKAR1A* expression was negative

to weakly positive in the background liver. In the fibrolamellar carcinomas with the classic *PRKACA* rearrangements by FISH, *PRKAR1A* was strongly overexpressed, most likely in response to activation of the protein kinase A pathway. In contrast, the fibrolamellar carcinoma that lacked the *PRKACA* rearrangements by FISH was negative for *PRKAR1A* (Figures 4a and b).

The consistent loss of LFABP was not anticipated in the fibrolamellar carcinomas that co-occurred with or followed resection for hepatic adenomas, so we studied LFABP expression in a second cohort of 19 fibrolamellar carcinomas, including 11 previously published tumors¹ and an additional new set of 8 tumors. The diagnosis in the additional set of eight fibrolamellar carcinomas was first confirmed at the genomic level by detection of the *PRKACA* rearrangement by interphase FISH: all were positive. By immunohistochemistry, all 19 cases displayed reduced or complete loss of LFABP expression, similar to the three index fibrolamellar carcinomas

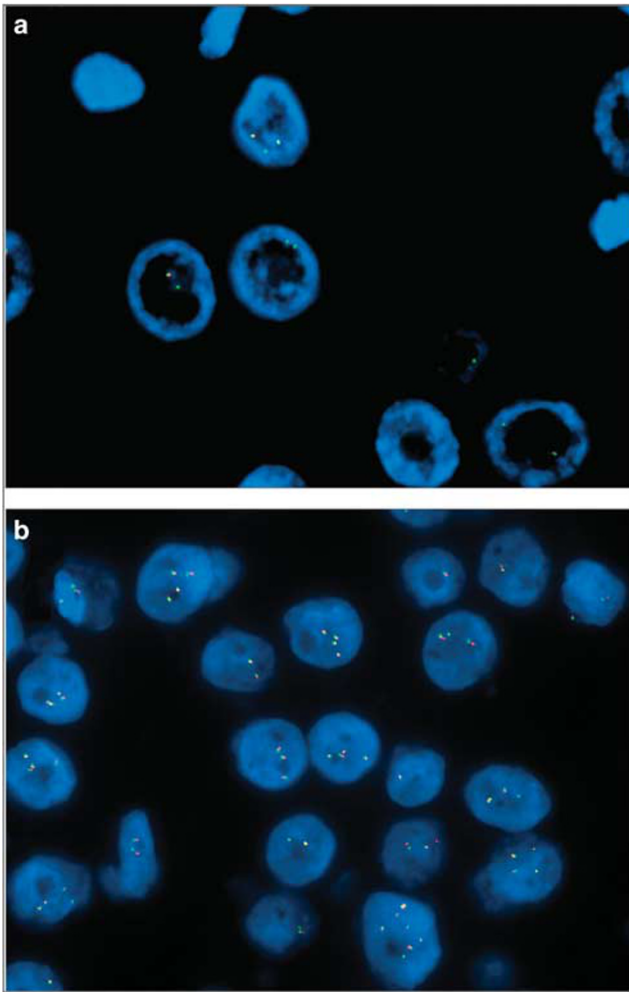


Figure 3 Fish results for *PRKACA* rearrangements. (a) The fibrolamellar carcinomas in each of the first three cases with adenomas all showed rearrangements of the *PRKACA* locus. (b) In contrast, the fibrolamellar carcinoma in the fourth case was negative for rearrangements of the *PRKACA* locus.

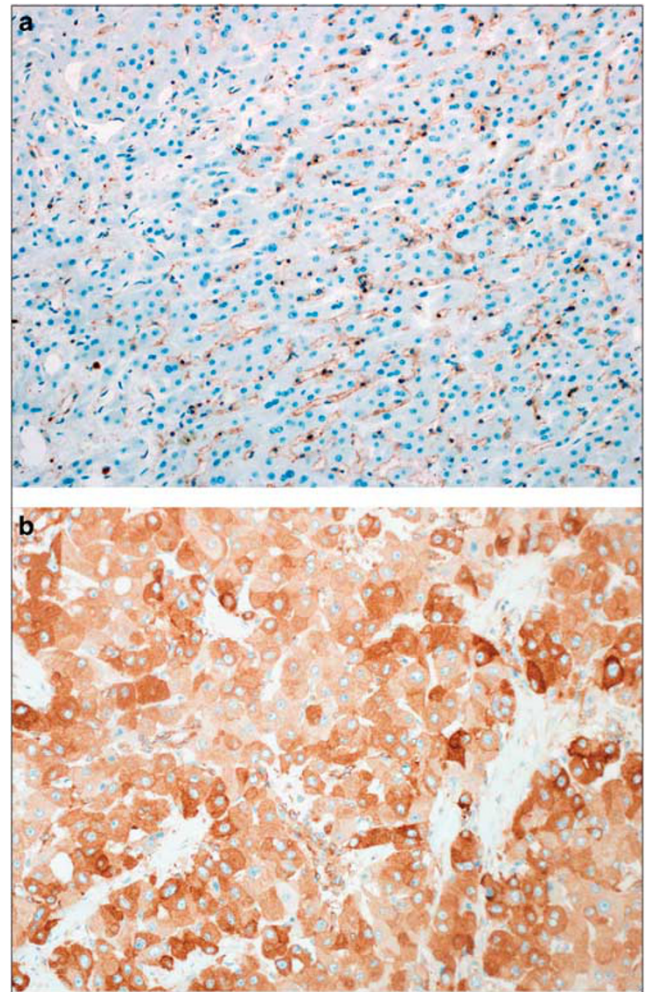


Figure 4 *PRKAR1A* expression in fibrolamellar carcinomas. (a) The fibrolamellar carcinoma negative for the *DNAJB1-PRKACA* fusion shows loss of *PRKAR1A* expression. (b) In contrast, fibrolamellar carcinomas with the *DNAJB1-PRKACA* fusion show retained expression of *PRKAR1A*.

associated with hepatocellular adenomas (Table 2; Figure 5a). In fibrolamellar carcinomas that retained some LFABP expression, the tumors typically showed a mosaic pattern, with scattered individually positive tumor cells in a background of negative to very faintly positive tumor cells (Figure 5b).

To confirm the loss LFABP protein expression in fibrolamellar carcinomas, mass spectrometry-based proteomic analysis was performed on six fibrolamellar carcinomas, comparing protein levels between matched tumor and non-tumor pairs. As expected, mass spectrometry revealed overexpression of cytokeratin 7 and anterior gradient-2 (AGR2; Table 3). In addition, *PRKACA* was clearly overexpressed at the protein level. LFABP was 10-fold reduced in tumor cells relative to non-neoplastic cells (Table 3). As an internal control, Arginase-1 levels were similar between tumor and non-neoplastic cells.

Discussion

Fibrolamellar carcinoma is a unique type of primary liver carcinoma that is distinct from typical hepatocellular carcinomas at the histological, immunophenotypical, and molecular levels. These unique findings are used to recognize this tumor in diagnostic surgical pathology. The morphological findings are the foundation for the diagnosis, but because of histological overlap with other tumors, the diagnosis also benefits from confirmatory tests. The combination of typical morphology and CK7 and CD68 co-expression are useful to diagnose fibrolamellar carcinomas and separate them from their mimics.¹¹ An alternative method is detection of recurrent somatic *DNAJB1-PRKACA* in fibrolamellar carcinoma.¹

Although rare, cases of hepatic adenomas can develop in livers with synchronous or metachronous fibrolamellar carcinomas. This is the first series of

Table 2 Summary of the results of LFABP immunohistochemistry on fibrolamellar carcinomas

Case	Semiquantitative extent of LFABP-positive neoplastic cells (%)	Intensity of LFABP staining	Evaluated with mass spectrometry ^a
First index	0	NA	ND
Second index	20	Weak	ND
Third index	10	Weak	ND
1	10	Moderate	ND
2	10	Moderate	Yes
3	40	Moderate	Yes
4	0	NA	ND
5	0	NA	ND
6	0	NA	ND
7	20	Weak	ND
8	0	NA	ND
9	0	NA	ND
10	0	NA	Yes
11	0	NA	ND
12	70	Moderate	ND
13	5	Weak	ND
14	30	Weak	ND
15	20	Weak	ND
16	0	NA	ND
17	5	Weak	ND
18	10	Weak	ND
19	0	NA	ND

Abbreviations: ND, not done; NA, not applicable.

^aThe other three cases of fibrolamellar carcinoma randomly chosen for mass spectrometry were not evaluated with LFABP immunohistochemistry.

such cases and shows several key findings. First, the adenomas and the fibrolamellar carcinomas are morphologically distinct: they look different histologically and are physically separated. None had transition zones. Second, none of the adenomas had a *PRKACA* rearrangement. Third, the hepatic adenomas were type 1 adenomas (*HNF1-α* inactivated) in three specimens, with a steatotic histology and loss of LFABP expression. Fourth, the fibrolamellar carcinomas were histologically classic, with no unusual features.

The adenoma that arose in the setting of the Carney complex had a myxoid morphology and was unclassifiable using immunohistochemistry, but showed partial LFABP loss. Interestingly, the metachronous fibrolamellar carcinoma lacked a *PRKACA* rearrangement by FISH and RT-PCR, but had classical morphology and CK7 and CD68 co-expression. More than 70% of families with the Carney Complex have germline mutation in *PRKAR1A*, a regulatory subunit of protein kinase A. Inactivation of *PRKAR1A* could potentially lead to excess protein kinase A signaling, providing an alternative molecular lesion that, like a *PRKACA* rearrangements, would lead to excess protein kinase A signaling. Although direct molecular confirmation of a *PRKAR1A* mutation was not possible, there was loss of *PRKAR1A* expression. This finding has clinical testing implications and confirms the

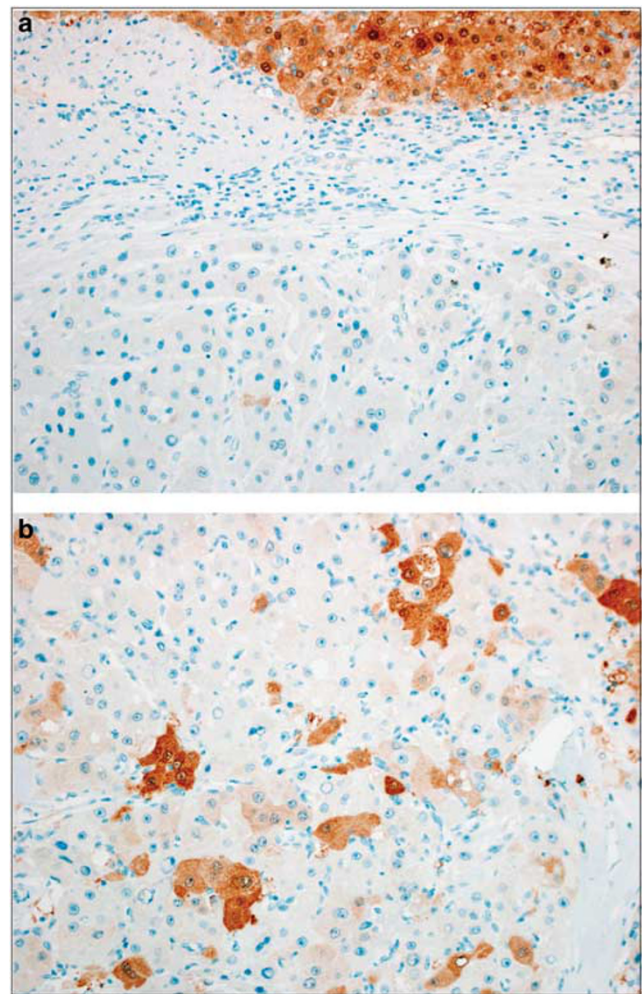


Figure 5 LFABP loss in fibrolamellar carcinomas. (a) The degree of LFABP loss varied. In most cases, the tumors showed extensive loss, with either no or residual trace staining. The non-neoplastic liver is seen at the top of the image. (b) Other cases showed a mosaic staining pattern, with scattered strongly positive tumor cells in a background of otherwise negative staining.

Table 3 The expression of various proteins in the tumor cells of fibrolamellar carcinoma compared with the matched non-neoplastic cells for six cases is shown

Protein	Average fold change compared with matched normal tissue
Cytokeratin 7	4.4
Anterior gradient-2	> 100
PRKACA	2.2
Arginase-1	1.1
LFABP	0.1

importance of morphology as the basis for the diagnosis of fibrolamellar carcinoma, followed by confirmatory testing.

Importantly, these data also highlight LFABP loss as a consistent finding in fibrolamellar carcinomas,

one that was confirmed by two different methods: immunohistochemistry and mass spectrometry-based proteomics. The advantages of mass spectrometry-based proteomics are the improved quantitative capability and precision, as well as the ability to assess multiple analytes simultaneously, compared with conventional immunohistochemistry. Our data from this approach confirmed markedly reduced LFABP expression and simultaneously confirmed overexpression of other markers known to be overexpressed in fibrolamellar carcinoma; namely anterior gradient-2,¹² cytokeratin 7, and PRKACA.^{1,2}

Taken together, these data suggest that *HNF1-α* inactivation is a key recurrent event in the biology of fibrolamellar carcinomas. LFABP is a downstream target of *HNF1-α*¹³ and biallelic loss of *HNF1-α* is associated with loss of LFABP expression, an observation that forms the foundation for using LFABP immunohistochemistry as a diagnostic tool.¹⁴ It is unknown how the loss of the tumor suppressor *HNF1-α* contributes to neoplasia, but biallelic loss of *HNF1-α* can lead to activation of glycolysis and lipogenesis,^{15,16} as well as upregulation of ERBB2 and mTOR. Other possible targets include upregulation of cyclin D1 to promote cell division and upregulation of PDGFA and PDGFB, facilitating angiogenesis.

Loss of LFABP1 by immunohistochemistry is a key part of the diagnosis of type 1, or *HNF1-α* inactivated, hepatic adenomas. However, LFABP loss is not unique to type 1 hepatic adenomas. Loss of LFABP1 by immunohistochemistry is also seen in conventional hepatocellular carcinomas,^{17,18} in myxoid hepatic adenomas,¹⁰ and in fibrolamellar carcinomas (current study), implying that *HNF1-α* inactivation is a broadly targeted pathway in hepatocellular neoplasia. In this respect, *HNF1-α* inactivation shares parallels with the Wnt signaling pathway, both of which are broadly targeted by both benign and malignant hepatocellular tumors.

Interestingly, *HNF1-α* inactivation in hepatic adenomas typically results from *HNF1-α* mutations. In contrast, *HNF1-α* mutations have not been identified in genomic or transcriptomic study of fibrolamellar carcinomas.^{2,19,20} This suggests an alternate mechanism for the downregulation of LFABP in fibrolamellar carcinoma, perhaps through methylation or microRNAs. The mosaic pattern seen in some of the fibrolamellar carcinomas is also unusual, with scattered strongly positive tumor cells in a background of more diffuse loss of staining in tumor cells. The significance of this staining pattern is not clear, but the mosaic staining would support a possible epigenetic mechanism.

Overall, the lack of *HNF1-α* mutations in fibrolamellar carcinomas is similar to conventional hepatocellular carcinomas, where ~40% of conventional hepatocellular carcinomas show loss of LFABP, but mutations are found in only 5% of cases.^{21,22} The diminished or absent LFABP expression in all cases

of fibrolamellar carcinoma suggests *HNF1-α* inactivation is an important molecular event in tumorigenesis and should be added to the short list of other distinctive, consistent molecular events, including AGR2 overexpression and protein kinase A activation, which results from *DNAJB1-PRKACA* fusion or possibly loss of PRKAR1A protein expression.

In conclusion, hepatic adenomas rarely co-occur with fibrolamellar carcinomas. Most are type 1 adenomas, sharing with fibrolamellar carcinomas the key biological event of *HNF1-α* inactivation. Our data also suggests that protein kinase A activation can rarely occur through other molecular events in fibrolamellar carcinomas. We also report the first well documented fibrolamellar carcinoma that is negative for the *DNAJB1-PRKACA* fusion, with the diagnosis of fibrolamellar carcinoma confirmed by histological review by liver pathologists and by co-expression of CK7 and CD68.

Acknowledgments

We are grateful for the funding support of the Department of Laboratory Medicine and Pathology Research Committee through Discretionary funds. We thank the Pathology Research Core (supported by CA 15083), Cytogenetics Core and Proteomics Core of the Mayo Clinic Medical Genome Facility for excellent technical work.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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