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mTORC1 and FGFR1 signaling in fibrolamellar hepatocellular carcinoma

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Fibrolamellar hepatocellular carcinoma, or fibrolamellar carcinoma, is a rare form of primary liver cancer that afflicts healthy young men and women without underlying liver disease. There are currently no effective treatments for fibrolamellar carcinoma other than resection or transplantation. In this study, we sought evidence of mechanistic target of rapamycin complex 1 (mTORC1) activation in fibrolamellar carcinoma, based on anecdotal reports of tumor response to rapamycin analogs. Using a tissue microarray of 89 primary liver tumors, including a subset of 10 fibrolamellar carcinomas, we assessed the expression of phosphorylated S6 ribosomal protein (P-S6), a downstream target of mTORC1, along with fibroblast growth factor receptor 1 (FGFR1). These results were extended and confirmed using an additional 13 fibrolamellar carcinomas, whose medical records were reviewed. In contrast to weak staining in normal livers, all fibrolamellar carcinomas on the tissue microarray showed strong immunostaining for FGFR1 and P-S6, whereas only 13% of non-fibrolamellar hepatocellular carcinomas had concurrent activation of FGFR1 and mTORC1 signaling ($P<0.05$). When individual samples were stratified according to staining intensity (scale 0–4), the average score in fibrolamellar carcinomas was 2.46 for FGFR1 and 3.77 for P-S6, compared with 0 and 0, respectively, in non-tumor liver. Immunoblot analyses of fibrolamellar carcinomas revealed high mTORC1 activities relative to AKT activities accompanied by reduced TSC2 expression, which was not observed in non-fibrolamellar hepatocellular carcinomas. Our findings provide evidence for mTORC1 activation and FGFR1 overexpression in human fibrolamellar carcinoma, and support the use of FGFR1 inhibitors and rapamycin analogs in the treatment of patients with unresectable fibrolamellar carcinoma.
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Fibrolamellar carcinoma is a rare variant of hepatocellular carcinoma, accounting for ~5% of all hepatocellular carcinoma cases.[1,2] The histologic hallmark of fibrolamellar carcinoma is large, eosinophilic cells with prominent nucleoli surrounded by laminated fibrous stroma, from which it derives its name. To date, no unique biomarker for fibrolamellar carcinoma has been identified; the immunophenotype is most consistent with a hepatocellular origin, with immunoreactivity for HepPar1, cytokeratin 7, epithelial membrane antigen, and CD68. Recent transcriptomic analyses of fibrolamellar carcinomas identified the overexpression of neuroendocrine genes in these tumors, but classic markers such as synaptophysin and chromogranin are typically absent in fibrolamellar carcinoma.[3] In fact, little is known about the molecular basis of fibrolamellar carcinoma to date. Pathways commonly mutated in typical hepatocellular carcinoma, such as β-catenin and p53, are not differentially regulated in fibrolamellar carcinomas, while others including RAS, MAPK, EGFR, and PI3K have been reported to be upregulated in a small number of patients.[4] In this study, we focused on the potential involvement of mTORC1 and FGFR1 signaling in fibrolamellar carcinomas.

Mechanistic target of rapamycin (mTOR) is a serine/threonine kinase that associates with other proteins to form two kinase complexes: mTOR complex 1 (mTORC1) and complex 2 (mTORC2). Among them, mTORC1 regulates protein synthesis

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in response to nutrients (eg, amino acids) and growth factors (eg, insulin).[5] Upon activation, mTORC1 phosphorylates several substrates, including eIF4E-binding proteins and ribosomal protein S6 kinases (S6Ks), to mediate TOP-mRNA translation.[6] In turn, S6K1 activates ribosomal protein S6, whose phosphorylation (P-S6) can be reliably detected in tissue using immunohistochemistry and serve as a surrogate indicator of mTORC1 activity. mTORC1 has been implicated in promoting tumor growth and proliferation in many human cancers, including hepatocellular carcinomas,[7] but its functional role in fibrolamellar carcinoma has not been established. Understanding whether mTORC1 activation acts as an oncogenic 'driver' or merely a biomarker will have significant implications in the therapeutic response to mTORC1 inhibitors, such as rapamycin and its analogs. One of the molecular signatures accompanying mTORC1 activation is feedback inhibition of PI3K/AKT; thus, the relative activities of mTORC1 and AKT in a tumor provide evidence for primary *vs* secondary functions of mTORC1. For example, tumors with underlying mutations in the *TSC1* or *TSC2* genes possess high mTORC1 activity relative to that of AKT.[8] Conversely, when mTORC1 is stimulated downstream of PI3K or by AKT-activating mutations, the relative mTORC1:AKT activity is low (ie, high AKT activity compared with mTORC1). We have confirmed these findings in our experimental models of liver cancer, in which the *Tsc1* or *Pten* gene was deleted in murine hepatocytes.[9]

In our studies of *Tsc1*-null hepatocellular carcinomas in mice, we found that mTORC1-driven cancers consistently express several receptor tyrosine kinases, including fibroblast growth factor receptor 1 (FGFR1),[9] which belongs to a family of four FGFRs that control cell growth, proliferation, and differentiation during embryonic development.[10,11] The activation or overexpression of FGF/FGFR complexes has been increasingly associated with various cancers, including those of the breast, prostate, and lung.[12,13] Although FGFR1 is normally expressed in only non-parenchymal cells in the liver, it is expressed in transformed hepatocytes in a subset of hepatocellular carcinomas, perhaps promoting hepatocarcinogenesis.[14,15] Upon ligand binding, FGFRs transduce their mitogenic signaling through phosphorylation and recruitment of FRS2, which promotes docking of adapters necessary for downstream activation of the MAPK and PI3K/AKT pathways. Indeed, we have shown that FGF2 can stimulate human hepatocellular carcinoma cells in a FGFR1-dependent manner,[9] but the relevance of the FGFR1 pathway in fibrolamellar carcinoma has not been examined.

In this study, we performed immunohistochemistry and immunoblot analyses on human hepatocellular carcinomas, focusing on mTORC1 and FGFR1 signaling in fibrolamellar carcinomas. Our findings highlight the consistent expression of P-S6, P-S6K1, and FGFR1 in fibrolamellar carcinoma, in contrast to typical hepatocellular carcinoma. Our results further suggest a primary role of mTORC1 in fibrolamellar carcinoma based on the high relative mTORC1:AKT activity in these cancers. This novel molecular characterization of fibrolamellar carcinoma provides putative therapeutic targets for these patients.

Materials and methods

Tissue Microarray

A tissue microarray was constructed using 152 formalin-fixed archived samples, including normal liver (61 samples), hepatocellular carcinomas (30 samples), fibrolamellar carcinomas (10 samples), cholangiocarcinomas (27 samples), focal nodular hyperplasia (8 samples), hepatic cell adenomas (8 samples), and metastatic lesions (6 samples) in accordance with a previously described method.[16] Briefly, formalin-fixed, paraffin-embedded tissue blocks and the corresponding H&E-stained slides were overlaid for tissue microarray sampling. A board-certified pathologist (MMY) reviewed the slides to identify and mark representative tumor areas. Triplicate cylinders (0.6 mm in diameter) were punched from the representative tumor areas and from adjacent non-tumorous liver tissue from the individual donor's tissue blocks. These cylinders were then re-embedded into a recipient paraffin block at defined positions using a tissue arraying instrument (Beecher Instruments, Silver Spring, MD, USA). Immunohistochemistry for FGFR1 and P-S6 was performed on the tissue microarrays using antibodies listed in Table 1 as described.[9] Numbers of a given specimen type varied slightly between the FGFR1 and P-S6-stained arrays because of depth of sections for each sample (eg, 29 hepatocellular carcinomas in FGFR1-stained array *vs* 30 hepatocellular carcinomas in P-S6-stained array).

Table 1 Antibodies used for immunohistochemistry or immunoblotting

Antibody	*Company*	*Clonality*	*Dilution*	*Pretreatment*
FGFR1	Novus Biologicals	Polyclonal	1:200	Sodium citrate (pH 6)
Phospho-S6 Ribosomal protein (Ser235/236)	Cell Signaling	Polyclonal	1:100	Sodium citrate (pH 6)
CD68	Cell Marque	Monoclonal	1:4000	EDTA buffer

Selection of Cases for Further Analyses

To perform more detailed analyses, archived formalin-fixed paraffin-embedded primary tumor specimens from 13 patients with histologically proven fibrolamellar carcinoma were obtained from Seattle Children's Hospital and the University of Washington Medical Center. The internal review boards at both institutions approved these studies (Seattle Children's Hospital IRB #14393), and the medical records of these patients were reviewed.

Immunohistochemistry

Immunohistochemical analyses were conducted on formalin-fixed, paraffin-embedded tissue sections from patients with fibrolamellar carcinoma. After deparaffinization, rehydration, and quenching, antigen unmasking was conducted in 0.01 M citrate buffer of pH 6.0 in a microwave for 10 min. After incubation with the primary antibodies listed in Table 1, sections were developed with an avidin–biotin technique using the VECTASTAIN Elite ABC kit (Vector Laboratories) and counterstained with Gill's hematoxylin.

Histological Storing

A liver pathologist (MMY) semi-quantitatively scored immunohistochemical staining intensities in the tissue microarrays and large resection specimens. The following scoring system was used: 0, no stain or $<10\%$ of cells positively stained; 1, weak staining or 10–24% positively stained; 2, weak to moderate or 25–49% stained; 3, moderate to intense or 50–74% stained; and 4, intense or $>74\%$ stained.

Immunoblotting

Protein lysates were prepared from frozen fibrolamellar carcinoma samples, normal liver specimens, and archived typical hepatocellular carcinoma samples, and quantified using the BCA Protein Assay (Pierce, Rockford, IL, USA). Lysates were subjected to SDS-PAGE, transferred to PVDF membranes, and membranes were immunoblotted per standard protocols using antibodies listed in Table 1. Resulting radiographs were scanned and densitometric analysis performed using Image J software. Relative levels of phosphorylation for each target were calculated by normalizing each sample to total protein intensity and transforming using a base 10 logarithm.

Statistical Analyses.

Densitometry values from immunoblots were compared using Student's *t*-test, with statistical significance reached for $P<0.05$.

Results

To investigate mTORC1 and FGFR1 signaling in fibrolamellar carcinoma, we performed immunohistochemical analyses using a set of human liver tumor tissue microarrays containing a variety of liver tumors and normal liver samples as described in Materials and methods. Interestingly, all 10 fibrolamellar carcinomas in this data set were strongly positive for FGFR1 (Table 2), and 9 of 10 fibrolamellar carcinomas were also positive for P-S6 (Table 3). Near-uniform expression of these proteins in fibrolamellar carcinomas was significantly higher than in other hepatocellular carcinomas, of which 9 of 29 expressed FGFR1 and 4 of 30 expressed P-S6. Given these data, we were compelled to further investigate these two markers, both to confirm their activation status and to determine the cell type in which they are expressed.

We acquired archived primary fibrolamellar carcinoma samples and reviewed the medical records of 13 patients with fibrolamellar carcinomas who underwent resection at Seattle Children's Hospital or the University of Washington Medical Center. Patients included in this cohort ranged from 13 to 32 years of age at the time of diagnosis; clinical characteristics of the patients in our data set are depicted in Table 4. Five patients underwent resection alone for localized disease, had surgical margins that were negative for tumor and an absence of nodal metastases, and are currently disease-free, 3 months–9 years after diagnosis. One additional patient has no evidence of disease 5 years after a liver transplant, which was performed 1 year after diagnosis following neo-adjuvant chemoembolization and systemic sorafenib. Two patients presented with advanced disease are alive but are undergoing

Table 2 Immunohistochemistry of FGFR1 expression on tissue microarray

	Fibrolamellar carcinoma	*Hepatocellular carcinoma*	*Cholangio-carcinoma*	*Focal nodular hyperplasia*	*Hepatic cell adenoma*	*Metastatic lesions*	*Normal liver*
Negative	0	20	16	8	8	5	61
Positive	10	9	11	0	0	1	0
Total *N*	10	29	27	8	8	6	61
% Positive	100	31	40	0	0	16	0

Table 3 Immunohistochemistry of pS6 expression on tissue microarray

	Fibrolamellar carcinoma	*Hepatocellular carcinoma*	*Cholangio-carcinoma*	*Focal nodular hyperplasia*	*Hepatic cell adenoma*	*Metastatic lesions*	*Normal liver*
Negative	1	26	27	8	8	5	56
Positive	9	4	0	0	0	0	2
Total *N*	10	30	27	8	8	5	58
% Positive	90	13	0	0	0	0	3

Table 4 Patient data

No.	*Sex*	*Age (years)*	*AJCC*	*BCLC*	*Treatment regimen (chronologically)*	*Outcome*
1	M	13	I	A	Partial hepatectomy	NED at 1.5 years
2	F	15	III	B	Chemoembolization, sorafenib, OLT, adjuvant systemic chemotherapy	NED at 5 years after OLT (6 years after diagnosis)
3	F	19	I	A	Partial hepatectomy	NED at 6.5 yrs
4	M	14	IV	C	Partial hepatectomy, lung metastasectomy, sorafenib, traditional chemotherapy, abdominal lymphadenectomy, lung metastasectomy	Died 2 years after diagnosis
5	M	22	IVB	C	Neoadjuvant chemotherapy, partial hepatectomy, adjuvant chemotherapy, mediastinal lymphadenectomy, multiple courses of additional chemotherapy, RFA of liver recurrence, mediastinal and abdominal lymphadenectomy, repeat partial hepatectomy, chemotherapy, repeat abdominal lymphadenectomy, lapatinib, XRT, RFA of liver recurrence, sorafenib	Died 6 years, 8 months after diagnosis
6	F	15	IVA	C	Chemoembolization, partial hepatectomy with diaphragm resection, sorafenib, chemotherapy, RFA of liver recurrence, mediastinal lymphadenectomy, LSO for pelvic recurrence, cyberknife for liver recurrence, chemotherapy, chemoembolization, resection of intraabdominal recurrence	Alive 6 years, 8 months after diagnosis but increasing lung nodules
7	M	27	IVB	C	Chemotherapy, PARP inhibitor, sorafenib, lenalidomide	Died 28 months after diagnosis
8	F	21	I	A	Partial hepatectomy	NED at 9 years
9	M	17	IVA	C	Chemotherapy, sorafenib, retroperitoneal lymphadenectomy, chemotherapy	Died 13 months after diagnosis
10	F	31	I	A	Laparoscopic partial hepatectomy	NED at 3 years, 4 mo after dx
11	M	23	IVB	C	Partial hepatectomy and mediastinal lymphadenectomy, adjuvant chemotherapy, evirolimus	Still being treated—13 months after diagnosis
12	M	27	IVB	C	Sorafenib, rapamycin, multiple chemotherapeutic regimens, Yttrium-90 via hepatic artery, chemotherapy	Died 52 months after diagnosis
13	M	32	I	A	Partial hepatectomy	NED at 3 months after diagnosis

Abbreviations: AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; OLT, orthotopic liver transplantation; NED, no evidence of disease; RFA, radiofrequency ablation; XRT, radiotherapy; LSO, left salpingo-oophorectomy.
Note that AJCC and BCLC indicate stage at presentation, and 'chemotherapy' indicates traditional cytotoxic regimens (mostly 5-FU based), while newer, targeted regimens are specified. All lymphadenectomies were performed for enlarged nodes on imaging, all of which were confirmed to be positive for metastatic FLC histologically.

chemotherapy, and the remaining patients died from their fibrolamellar carcinomas, between 13 and 80 months from the time of diagnosis. These outcomes are consistent with reports in the literature.[2]

A liver pathologist (MMY) histologically confirmed the diagnosis of fibrolamellar carcinomas in all specimens, including confirming positive immunoreactivity for CD68 by immunohistochemistry (data not shown). Immunohistochemistry for FGFR1 and P-S6 was then performed on these archived tumor samples. All 13 fibrolamellar carcinomas were positive for FGFR1 (example shown in Figure 1). The staining was within tumor cells, predominantly cytoplasmic, and could be clearly contrasted from the surrounding stroma. When stratified according to staining intensity (scale 0–4), the average score in the fibrolamellar carcinomas was 2.46 for FGFR1, compared with 0 in non-tumor liver parenchyma. We similarly found that all 13 fibrolamellar carcinomas expressed P-S6 (example shown in Figure 2), indicating mTORC1 activation in these tumors. Immunoreactivity for P-S6 in fibrolamellar caricnomas was cytoplasmic, as expected, in strong contrast to unstained stroma and normal liver surrounding

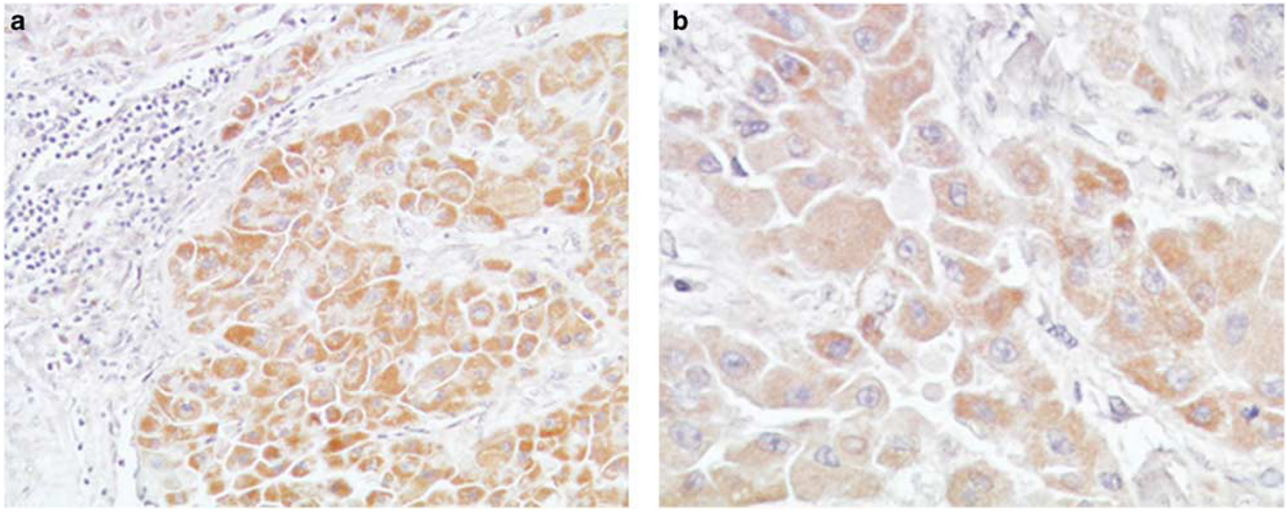

Figure 1 FGFR1 immunohistochemistry in fibrolamellar carcinoma. Immunoreactivity for FGFR1 in fibrolamellar carcinoma cells is seen at ×20 (**a**) and ×40 (**b**).

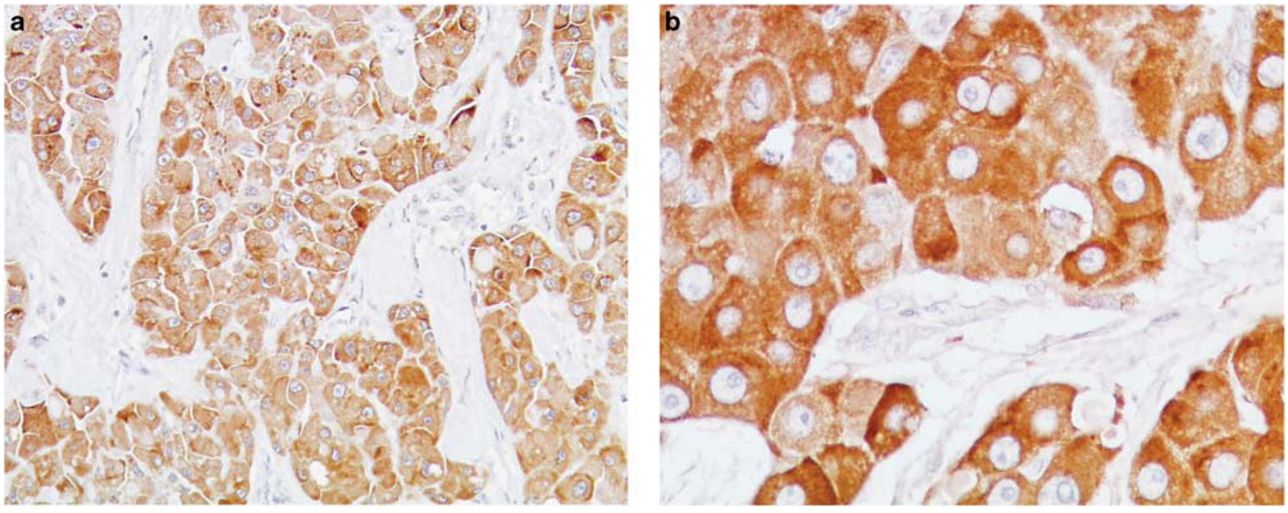

Figure 2 P-S6 immunohistochemistry in fibrolamellar carcinoma. Immunoreactivity for P-S6 in fibrolamellar carcinoma cells is seen at ×20 (**a**) and ×40 (**b**).

tumor nodules. When stratified according to staining intensity (scale 0–4), the average score in the fibrolamellar carcinomas was 3.77 for P-S6, compared with 0 in non-tumor liver parenchyma.

Next, we set out to determine the relative activities of mTORC1 and AKT in fibrolamellar carcinomas. As immunohistochemical results are difficult to quantify, and commercially available antibodies to detect AKT phosphorylation at either Ser473 or Thr308 (as markers of AKT activation) do not give reliable results in immunohistochemical analyses, we turned to immunoblot analysis of frozen tissues procured from a limited number of primary fibrolamellar carcinomas. Figure 3a shows the expression of the phosphorylated, activated forms of $S6K1^{Thr389}$, $S6^{Ser235/236}$, and AKT^{Ser473} in fibrolamellar carcinomas compared with normal liver samples. When normalized to their respective total proteins, we found that the relative mTORC1 activity (as evidenced by P-S6K1 and P-S6) in fibrolamellar carcinomas was significantly greater than that of normal livers, whereas the levels of AKT phosphorylation were similar between fibrolamellar carcinomas and normal livers. Further, when compared with hepatocellular carcinomas found in non-cirrhotic livers from patients with NASH or the hepatitis B virus, the relative mTORC1:AKT activity was greater in fibrolamellar carcinoma (Figure 3b). Finally, we hypothesized that loss of TSC2 in fibrolamellar carcinoma might lead to the demonstrated increase in mTORC1 activity in these tumors. Consistent with this hypothesis, we detected a significant reduction in TSC2 expression in fibrolamellar carcinoma compared with other

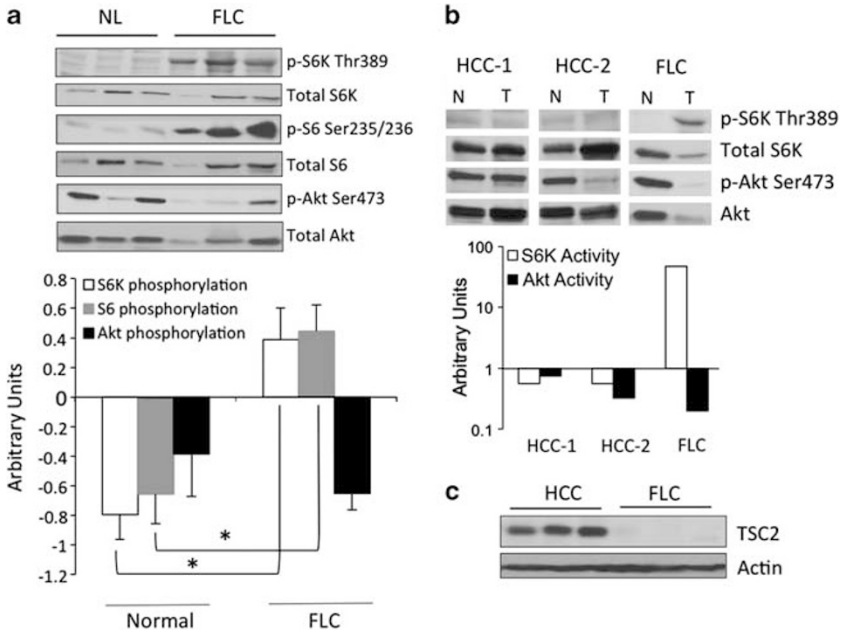

Figure 3 mTORC1 and AKT signaling in fibrolamellar carcinoma (FLC). (**a**) Immunoblot analyses of phosphorylation of S6K1, S6, and AKT in FLC compared with normal livers. The graph shows relative S6K, S6, and AKT phosphorylation of fibrolamellar carcinomas and normal liver (NL) normalized to the respective total proteins based on densitometry. Shown are logarithmic transformed data. $*P<0.01$. (**b**) Comparison of S6K1 and AKT phosphorylation of tumor (T) and normal liver (N) in NASH-related hepatocellular carcinoma (HCC-1), HBV-related hepatocellular carcinoma (HCC-2), and FLC. Relative AKT and S6K1 activities are shown in the graph below, based on densitometry. (**c**) Immunoblotting for TSC2 in FLCs and other hepatocellular carcinomas shows an absence of TSC2 expression in FLC. Actin serves as loading control.

hepatocellular carcinomas (Figure 3c). However, the mechanism of loss of TSC2 protein in fibrolamellar carcinomas is yet unknown.

Discussion

Fibrolamellar carcinoma affects young patients, with a mean age of ~25 years, who have no pre-existing liver disease or cirrhosis.[1] Typical hepatocellular carcinoma, on the other hand, frequently arises in cirrhotic livers of older individuals, with a mean age in the mid-sixties.[17] The difference in the presence of underlying liver disease may explain why patients with fibrolamellar carcinoma are more likely to undergo and survive resection or transplantation, and in some studies have been shown to have a better prognosis than those with typical hepatocellular carcinoma. However, this latter finding is controversial.[18–20] A recent study of 95 patients from the Fibrolamellar Carcinoma Consortium reported a median survival of 6.7 years,[2] and based on data from SEER 18 registries from 2000 to 2010 the overall 5-year survival was significantly better among fibrolamellar carcinoma patients (34%) compared with other patients with hepatocellular carcinoma (16%).[20] However, in patients who have received a potentially curative form of treatment (resection, transplantation, or radiofrequency ablation), there was no difference in survival between the two groups.[19,21] Weeda *et al*[19] compared hepatocellular carcinoma without cirrhosis with fibrolamellar carcinoma in the pediatric population, and found no significant difference in long-term survival. Regardless, fibrolamellar carcinoma often presents late with frequent nodal metastases, and there is no effective systemic therapy.[22]

Although the clinical and histological differences between fibrolamellar carcinoma and other forms of hepatocellular carcinoma are very well defined, the molecular differences between the two are more ambiguous. A number of immunohistochemical studies have been conducted on hepatocellular carcinoma and fibrolamellar carcinoma, and the elevated expression of some markers in fibrolamellar carcinoma has been conclusively shown. Data from the Fibrolamellar Carcinoma Consortium show that eight of nine fibrolamellar carcinoma tumors (89%) stained positively for cytokeratin 7 (CK7) and three of four (75%) for epithelial membrane antigen (EMA).[2] These results are corroborated by a similar study, which demonstrated that 100% of fibrolamellar carcinomas expressed CK7 and EMA, compared with only 28% of other hepatocellular carcinoma samples, a difference that was statistically significant.[23] Recently, CD68 immunoreactivity was detected in the vast majority of fibrolamellar carcinomas (31/32) while only 10/29 other hepatocellular carcinomas showed cytoplasmic staining for CD68.[24] Despite these contrasting features, fibrolamellar carcinomas and other

hepatocellular carcinomas also share a number of similarities: HepPar1 has shown immunoreactivity in both hepatocellular carcinoma and fibrolamellar carcinoma;[2,23] CK34 expression has been found in both cancers as well.[25] These findings highlight our lack of understanding of the molecular drivers of the fibrolamellar variant of hepatocellular carcinoma.

In this study, we showed that fibrolamellar carcinomas have upregulated mTORC1 signaling, as evidenced by P-S6K and P-S6 expression. Unlike cancers that are driven by PI3K/AKT activation, fibrolamellar carcinomas show high mTORC1 activities compared with that of AKT, a unique pattern that is most compatible with a primary event in mTORC1 dysregulation. An example in which a disease process is driven by mTORC1 activity includes the 'loss-of-function' mutations involving *TSC1* and *TSC2*, two negative regulators of mTORC1. Consistent with this notion, we detected significant reduction in TSC2 expression in fibrolamellar carcinomas, but the underlying epigenetic and genetic causes of the loss of TSC2 expression remain to be defined. In contrast to our findings of mTORC1 activation in all of our fibrolamellar carcinoma samples, most investigators report evidence of upregulation of mTORC1 pathway in up to 50% of typical hepatocellular carcinomas.[26] In our institutional cohort of 51 non-fibrolamellar HCCs, we found evidence of increased mTORC1 activity in 25% of cases (data not shown). Furthermore, mTORC1 activation in hepatocellular carcinoma has been associated with a worse prognosis regardless of the etiology of the patient's liver disease.[7]

Our investigation of mTORC1-driven hepatocellular carcinoma in a murine model (hepatocyte-specific *Tsc1* knockout mice),[9] led us to appreciate the frequent upregulation of FGFR1 in these tumor cells. We found that FGFR1 signaling can contribute to AKT and MAPK activities, but the relative balance between mTORC1 and AKT activities remain high in these murine hepatocellular carcinomas. Translating these findings to human fibrolamellar carcinomas, we found consistent FGFR1 expression in these cancers, although its role in tumorigenesis is unclear. In prostate carcinoma, where FGFR1 is frequently upregulated, functional studies suggest a mitogenic role of FGFR1 in disrupting the epithelial–stroma interaction, which ultimately leads to tumor progression.[12] Given the prominent extracellular deposition of collagen matrix in fibrolamellar carcinoma, which could be laden with growth factors such as FGF ligands, the role of FGFR1 in fibrolamellar carcinoma pathogenesis needs to be further investigated.

Together, our findings show for the first time evidence of an mTORC1-driven event in the pathogenesis of fibrolamellar carcinoma, along with overexpression of FGFR1. If these pathways prove to be functionally relevant, they will provide a novel therapeutic strategy to target mTORC1 and FGFR1 in combination, using clinically available drugs. There is currently an ongoing multi-center trial to evaluate the efficacy of everolimus and estrogen deprivation therapy in unresectable fibrolamellar carcinoma (NCT01642186). To date, attempts at FGFR inhibition with clinically available agents, such as brivanib alaninate or pazopanib in patients with fibrolamellar carcinoma have not been described. In the short term, defining the genetic mechanisms that lead to mTORC1 activation in fibrolamellar carcinoma using genome-wide analyses of clinical specimens is of paramount importance. In parallel, establishing pre-clinical models that mimic the molecular and histologic phenotypes of fibrolamellar carcinoma will greatly aid in our understanding of their pathogenesis.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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