

REVIEW

Fibrolamellar Hepatocellular Carcinoma: Mechanistic Distinction From Adult Hepatocellular Carcinoma

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Fibrolamellar hepatocellular carcinoma (FL-HCC) has historically been classified as a rare subtype of HCC. However, unlike “classic” HCC, it occurs in children and young adults without underlying liver disease. The recent discovery of a deletion mutation in all FL-HCCs represented a major advancement in understanding the pathogenesis of this disease. This deletion results in the fusion of the genes encoding a heat shock protein (*DNAJB1*) and the catalytic subunit of pro-

tein kinase A (PKA, *PRKACA*), and overexpression of *PRKACA* and enhanced cAMP-dependent PKA activity. This review summarizes recent advancements in FL-HCC pathogenesis and characteristics of the HSP40-PKA C protein. *Pediatr Blood Cancer* 2016;63:1163–1167. © 2016 The Authors. *Pediatric Blood & Cancer*, published by Wiley Periodicals, Inc.

Key words: carcinoma; fibrolamellar; hepatocellular; protein kinase A

INTRODUCTION

Fibrolamellar hepatocellular carcinoma (FL-HCC) is a primary liver cancer that occurs in young people without underlying liver disease. It was first described as a unique subtype of HCC by Edmondson in 1956 and was named FL-HCC by Craig in 1980.[1,2] It accounts for less than 1% of all primary liver cancers, but represents the majority of HCCs in patients younger than 30 years of age.[3–6] Due to the rarity of FL-HCC and a lack of representative experimental systems, the molecular basis for carcinogenesis in these tumors has been elusive and difficult to study. Recently, however, there have been significant breakthroughs in our understanding of the pathogenesis of FL-HCC. Honeyman and colleagues discovered a novel, chimeric transcript that is present in all studied samples of FL-HCC.[7] Our group and others have confirmed that this mutation is unique to FL-HCC, and expanded our knowledge of how this mutation functions in the liver.[8,9, manuscript in press] In this review, we present data supporting the notion that the *DNAJB1-PRKACA* mutation causes FL-HCC. Further, we review a body of new information reaffirming that FL-HCC is truly a distinct entity from classic, adult HCC.

COMPARISON BETWEEN FL-HCC AND “CLASSIC” HCC

Clinical Characteristics

FL-HCC occurs in younger individuals with no underlying liver disease. The average age at presentation is 25 years, but there is a second smaller peak in incidence between 70 and 79 years.[4,10] HCC in general is typically diagnosed in adults between 40 and 70 years of age, and is more common in males, while FL-HCC does not have a gender bias. Importantly, most HCCs develop in the setting of liver cirrhosis, while FL-HCCs do not.

Macroscopically, FL-HCCs are large and pale. They frequently have a central scar surrounded by hypervascular tumor cells with interspersed hypovascular fibrotic bands. Many groups have reported various immunohistochemical markers

in FL-HCC, including HepPar1, CD68, FGFR1, cytokeratin 7, epithelial membrane antigen, and various neuroendocrine markers, none of which are specific for FL-HCC.[11–13] Serum markers found to be elevated in FL-HCC include vitamin B12 binding capacity and haptocorrin, whereas alpha-fetoprotein is rarely elevated in these patients, unlike in classical HCC.[14,15] Collier et al. first described elevated neurotensin levels in the sera of patients with FL-HCC; this finding has been confirmed by several other investigators.[16–18] In fact, Evers and colleagues demonstrated that serum neurotensin levels may distinguish FL-HCC from other liver tumors.[17]

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Abbreviations: FL-HCC, fibrolamellar hepatocellular carcinoma; PKA, protein kinase A

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Treatment

Surgery is the mainstay of treatment in FL-HCC. There is no clear benefit of any chemotherapeutic agent in these patients, including sorafenib, which has been shown to have some efficacy in typical HCC.[19] There are a number of case reports and series describing different chemotherapeutic regimens for metastatic, recurrent, or unresectable disease, though no clinical trials have documented reproducible efficacy of any drug combination.[20–22] This lack of success may be secondary to both a poor understanding of the pathogenesis of FL-HCC and the grouping of these patients with those with traditional HCC. Radiotherapy for HCC is gaining popularity, and there are sporadic reports of FL-HCC showing response to radiation.[23]

Curative treatment of FL-HCC currently relies on early diagnosis and complete surgical resection. Unfortunately, since patients with FL-HCC tend to be young, healthy, and to have no underlying liver disease, they often present with lymph node involvement and/or metastatic disease. Multiple reports describe lymph node involvement in 50–70% of FL-HCCs at the time of diagnosis,[19,24] thus lymph node dissection is recommended at the time of initial resection. In contrast to typical HCC, this aggressive surgical approach has been shown to improve outcomes in FL-HCC;[25] despite these efforts, more than 50% of FL-HCCs recur within 10–33 months.[24,26]

Outcomes

There has been some controversy about true outcomes in patients with FL-HCC, with some investigators reporting improved survival compared to other HCCs, and others finding no difference.[3,5,26,27] Eggert et al. reviewed the SEER database from 2000 to 2010, which included 95 patients with FL-HCC, and reported 34% 5-year survival in FL-HCC versus 16% in HCC.[5] However, when patients were stratified by treatment group, that is, potentially curative treatments versus chemotherapy/radiation alone, there was no difference in survival.[5] Thus the reported increased survival in FL-HCC may be due to the fact that these patients do not have cirrhosis and can therefore tolerate extensive liver resections. Similarly, Allan and colleagues reviewed all pediatric cases of FL-HCC in the SEER database and found that overall survival was increased in FL-HCC compared to HCC, but when patients were stratified by treatment groups there was no difference.[3] Weeda et al. reviewed the SIOPEL data and discovered no difference in overall survival between pediatric patients with FL-HCC versus HCC.[27] Mayo's group reviewed 91 cases of surgically treated tumors and found median survival of 75 months for patients with FL-HCC compared to 43 months for patients with HCC. When perioperative 30-day mortality was excluded, however, there was no significant difference in overall survival, suggesting that any detected survival advantage was due to the fact that patients with FL-HCC are healthier at baseline. These investigators also found that patients with FL-HCC tend to have more advanced disease at diagnosis, and overall 5-year survival for patients who undergo resection for FL-HCC with curative intent was 50%.[28] Based on these findings, it is reasonable to conclude that the improved outcomes in patients with resectable FL-HCC compared to typical HCC are due to the absence of

chronic liver disease and the overall younger, healthier FL-HCC patient population.

Molecular Characteristics

Understanding the molecular pathogenesis of FL-HCC has been a challenge due to the rarity of this disease. Using transcriptome analysis, Kannangai et al. discovered overexpression of MAPK, PI3K, RAS, and xenobiotic pathways in FL-HCCs.[29] Kakar and Wilkens compared CGH profiles of FL-HCCs and other HCCs; they found that none of the pathways that are frequently mutated in typical HCC, such as TP53, Wnt/ β -catenin, or survivin, are mutated in FL-HCC.[30, 31] Other genes that are overexpressed in FL-HCC include aromatase, EGFR, anterior gradient 2, and certain neuroendocrine markers such as PCSK1, neurotensin, calcitonin, and DNER.[32] Classic markers of neuroendocrine tumors, such as chromogranin-A and synaptophysin, are not expressed in FL-HCC, however. Our group has demonstrated overexpression of FGFR1 and activation of mammalian target of rapamycin complex 1 (mTORC1) in FL-HCC; these results were supported by recent whole genome analysis by Cornella et al. who found mammalian target of rapamycin (mTOR) pathway activation in 51% of their FL-HCC cohort.[8,33] Unfortunately, a randomized, phase II clinical trial evaluating the effectiveness of treating unresectable FL-HCC with everolimus, letrozole + leuprolide, or all three drugs in combination (NCT 01642186) was closed early due to lack of efficacy.

Recently, next-generation whole transcriptome sequencing and whole genome sequencing (WGS) lead to major breakthroughs in our understanding of the pathogenesis of FL-HCC. First, Honeyman and colleagues reported that 15 of 15 FL-HCC samples had a single copy 400 kB deletion on chromosome 19, resulting in an in-frame fusion of exon 1 of the gene for heat shock protein 40 (*DNAJBI*) fused to the majority of the gene encoding the catalytic subunit of protein kinase A (PKA, *PRKACA*).[7] This mutation has been confirmed in several additional FL-HCC cohorts.[8,9,34,35] Of note, these molecular studies have primarily focused on FL-HCC in children and young adults; the presence of *DNAJBI-PRKACA* in elderly patients with FL-HCC has not been established. Cornella et al. were further able to stratify FL-HCCs into three subtypes based on unique molecular characteristics; they identified an eight-gene signature that was linked to poor outcomes.[8] Importantly, all three groups that performed WGS on FL-HCC samples did not uncover any additional consistent mutations, suggesting that *DNAJBI-PRKACA* plays a prominent role in FL-HCC pathogenesis.[8,9,34] With over 200 cases analyzed, each mutation leads to the same chimeric transcript, though the actual breakpoints on chromosome 19 are unique among individuals. The predilection for the *DNAJBI-PRKACA* gene rearrangement in FL-HCC strongly argues against the role of individual genes that are lost in the ~400 kB deletion. Additionally, there are no reported cases of other tumors harboring this unique rearrangement. Another key finding suggesting that FL-HCC is a distinct entity from other HCCs is the fact that FL-HCCs have a stable genome with little copy number variation and no microsatellite instability, unlike classic HCCs.[8,9,34] The strikingly high prevalence of the *DNAJBI-PRKACA* fusion, along with an absence of other consistent mutations in FL-HCC, leads to the

hypothesis that the *DNAJBI-PRKACA* fusion product is a driver of FL-HCC tumorigenesis and thus warrants further study.

PKA SIGNALING AND CANCER

To date, the role of cyclic AMP-dependent PKA in liver cancer is unknown. PKA is composed of two regulatory (R) subunits and two catalytic (C) subunits. In the presence of cAMP, the C subunits disassociate from the R subunits in the holoenzyme and are thus able to phosphorylate downstream effectors. There are two classes of R subunits, RI and RII, of which there are two different splice variants for each (RI α , RI β , RII α , and RII β). [36,37] RI and RII are similar in structure, but differ in their localization and cAMP sensitivity. The subcellular targeting of the R subunits is governed by their interactions with A-kinase anchoring proteins, which thus regulate the localization of the PKA holoenzyme. [38]

One intriguing aspect of the *DNAJBI-PRKACA* fusion in FL-HCC is that elevated PKA activity has been previously implicated in benign and malignant tumors of endocrine differentiation. [39] Activation of PKA is initiated by ligand-mediated release of G $_{\alpha s}$ to stimulate adenylyl cyclase to synthesize cAMP. Consequently, activating mutations in the G-protein alpha stimulatory subunit (*GNAS*) gene, which encodes G $_{\alpha s}$ proteins, usually result in permanent association with GTP, leading to constitutive adenylyl cyclase activation and production of cAMP. Arg²⁰¹ or Gin²²⁷ activating mutations in *GNAS* lead to McCune-Albright syndrome [40], which involves excess secretion of various hormones; similar mutations have been identified in benign solitary secreting pituitary adenomas and hepatocellular adenomas. [41,42]

Somatic mutations in the PKA C subunit that result in an inability to bind to the R subunits have been reported in cortisol-secreting adrenal adenomas. [39] Specifically, an activating mutation (L205R) in the p+1 loop of PKA C has been identified in 70% of these tumors; [43] this loop is known to be involved in cross talk between the C subunit and its substrates. PKA C with the L205R mutation has increased *in vitro* kinase activity, suggesting that kinase overactivity contributes to the pathogenesis of adrenal adenomas. [44] Further, overexpression of the C subunit of PKA has been demonstrated in prostate cancer; in fact extracellular PKA C subunit has been detected at a higher concentration in the serum of prostate cancer patients than in normal serum, and thus could be used as a biomarker. [45]

Another finding that implicates PKA in carcinogenesis is that mutations in the PKA RI α subunit that result in a reduction in RI α function have been linked to Carney complex. [46] Patients with this disease suffer from excess cortisol secretion and develop pigmented adenomas, cutaneous neuromas, and cardiac myxomas. Mechanistic analysis of this phenomenon has been provided by mouse models of Carney complex, as *PRKAR1a* heterozygous mice and mice with inactivating mutations in *PRKAR1a* develop schwannomas along with adrenal, pancreatic, liver, and lung tumors. [47]

It is thus speculated that any disruption in the regulation or stoichiometry of the R or C subunits can lead to tumor formation. The aforementioned information regarding PKA signaling in cancer led to the hypothesis that the *DNAJBI-PRKACA* fu-

sion product drives FL-HCC tumorigenesis through increased PKA signaling in hepatocytes.

DNAJBI-PRKACA MUTATION IS UNIQUE TO FL-HCC

Following the discovery of the *DNAJBI-PRKACA* mutation in FL-HCC, it was important to determine whether this mutation is unique to FL-HCC or if it is present in other liver tumors. We evaluated a panel of 10 classic HCCs and two cholangiocarcinomas (CCs) by RT-PCR and immunoblotting, and found no evidence of the *DNAJBI-PRKACA* transcript or the resultant fusion protein in any of these tumors (manuscript in press). Additionally, Torbenson's group evaluated 25 FL-HCCs, 25 HCCs, 25 CCs, and five hepatic adenomas by RT-PCR and found the *DNAJBI-PRKACA* transcript in all FL-HCC samples, but not in the other tumors. [35] Further, Darcy and colleagues performed mutational analysis on 10 typical HCCs and found no evidence of the *DNAJBI-PRKACA* mutation. [34] The unique and near universal presence of the *DNAJBI-PRKACA* fusion in FL-HCC strongly supports the hypothesis that this mutation plays a key role in FL-HCC tumorigenesis.

CHARACTERISTICS OF THE HSP40-PKA C FUSION PROTEIN

To further elucidate the mechanism of tumorigenesis in FL-HCC, our lab and others have characterized the HSP40-PKA C fusion protein in human tumors and in cell culture systems. Multiple groups have found that cells overexpressing the fusion protein retain full kinase activity, and studies by Xu et al. demonstrated that in a transformed HCC cell line (HepG2 cells) transfection with the mutant transcript leads to increased colony formation, suggesting that the mutant functions as an oncogene. [7,9] We further characterized the function of the fusion protein in human FL-HCCs and found that *baseline* PKA activity is low and unchanged in FL-HCCs compared to paired, normal livers. Thus unlike the PKA C(L205R) mutation associated with adrenocortical tumors, the mutant PKA C in FL-HCC is *not* constitutively activated. In contrast, we found that FL-HCCs have significantly greater capacity for PKA activation in the presence of cAMP (manuscript in press). These findings are consistent with *in vitro* work by Cheung et al., which demonstrates the ability of the purified mutant PKA C protein to interact with the R subunits to create a functional holoenzyme. [44] They also found that the intrinsic kinase activity of purified mutant PKA C is similar to wild-type PKA C. [44] We have confirmed these results *in vivo* by determining that cAMP sensitivity as measured by enzymatic K_m is similar in FL-HCCs and normal livers, and that the mutant HSP40-PKA C in FL-HCC tumor lysates associates with both RI α and RII α subunits. Honoyman et al. also reported no difference in the kinase activities of the mutant and WT proteins when overexpressed in HEK 293T cells. [7]

One potential mechanism for the increased PKA activity seen in FL-HCCs is overexpression of the C subunit, given that expression of the fusion transcript is driven by the *DNAJBI* promoter, which has a higher basal transcription rate than the native *PRKACA* promoter. Accordingly, we have found that in human FL-HCCs, PKA C is significantly overexpressed at the mRNA and protein levels (10.5- and 2.6-fold, respectively (manuscript

in press)). Since the R and C subunits of PKA are tightly co-regulated,[36] we also found compensatory overexpression of RI α in the tumors. As noted by Xu et al., increased PKA activity could promote additional transcriptional activation through the mobilization of transcription factors, such as the cAMP responsive binding element CREB.[9] Finally, based on transcriptome analyses, Simon's group highlighted putative downstream effector pathways including upregulation of aurora kinase A,[48] and a new trial is currently underway to test the efficacy of an aurora kinase inhibitor, ENMD-2076, in FL-HCC patients (NCT02234986).

SUMMARY

The mechanism of tumorigenesis in FL-HCC has been a mystery since the initial identification of this cancer in 1956. Major strides have been made over the past year, however, following the discovery of the *DNAJB1-PRKACA* mutation by Honeyman et al. [7] The prevalence of this mutation in virtually all FL-HCC samples, coupled with an otherwise relatively stable genome, strongly suggests that the fusion protein is a primary driver of FL-HCC. The fusion protein is overexpressed, retains its kinase activity, and has significantly greater cAMP-stimulated PKA activity compared to wild-type PKA C. Combining this new molecular information with the epidemiological and clinical characteristics of FL-HCC, it is clear that this is a unique primary liver tumor that mechanistically differs from typical HCC. Many questions remain regarding the role of the chimeric protein in liver oncogenesis, however. While it is tempting to implicate PKA as an oncogene, and thus a potential therapeutic target, there exists a body of literature showing an antiproliferative effect of cAMP on hepatocytes *in vitro*, and ethyl-ester of cAMP has been shown to inhibit tumors *in vivo*. [49,50] With our knowledge of the structural differences between the mutant and wild-type PKA C, it can be expected that HSP40-PKA C phosphorylates a unique set of substrates that play a role in tumorigenesis. Future studies will focus on defining PKA substrates that are unique to FL-HCC, and designing specific inhibitors targeting the mutant kinase. Also important to our understanding of this cancer is the identification of the factors that may be promoting cAMP/PKA activity in the tumors, and the role of the first exon of *DNAJB1* in the fusion protein. With the genetic basis of FL-HCC defined, mechanistic studies will now allow for meaningful translation of new scientific knowledge to patient care, and give us a reason to be hopeful for a cure for this deadly cancer.

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