Environmental exposures as a risk factor for fibrolamellar carcinoma

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Fibrolamellar carcinoma was first described in 1956. Subsequent large studies failed to identify cases before 1939 (the start of the World War II). This finding, combined with the presence of aryl hydrocarbon receptors on the tumor cells, have suggested that fibrolamellar carcinomas may be caused by environmental exposures that are new since World War II. To investigate this possibility, the surgical pathology files before 1939 were reviewed for hepatocellular carcinomas resected in young individuals. Two cases of fibrolamellar carcinoma were identified, from 1915 to 1924. The diagnosis of fibrolamellar carcinoma was confirmed at the histologic, ultrastructural and proteomic levels. These two fibrolamellar carcinoma cases clarify a key aspect of fibrolamellar carcinoma biology, reducing the likelihood that these tumors result exclusively from post World War II environmental exposures.
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Fibrolamellar carcinoma is a unique primary hepatic malignancy characterized by a predilection for young individuals without underlying liver disease.[1–3] The distinct histologic findings include large eosinophilic cells, which frequently have pale bodies and hyaline bodies. The tumor also characteristically shows abundant intratumoral fibrosis. The distinctive nature of fibrolamellar carcinoma extends to the protein level. Fibrolamellar carcinomas are routinely positive for CK7 (ref. 4) and their increased lysosomal content leads to positive CD68 immunostaining.[5] Fibrolamellar carcinomas also overexpress anterior gradient-2 (ref. 6) and ornithine aminotransferase,[7] while showing reduced or absent LFABP staining.[8] At the molecular level, fibrolamellar carcinomas show a specific *DNAJB1–PRKACA* fusion transcript in almost all *bona fide* cases.[7,9]

The etiological risk factors for fibrolamellar carcinomas are unknown, as they arise in livers without underlying liver disease and without fibrosis. However, as one possible clue, Craig and Edmonson noted that no cases of fibrolamellar carcinoma were identified in large autopsy studies[10,11] or in the consultation practice of Dr Edmonson before World War II (1939-1945). Furthermore, Oikawa *et al*[12] suggested that the incidence of fibrolamellar carcinoma is increasing, perhaps related to the development of the plastics industry during and after World War II.

The goal of this study is to further investigate the presence/absence of fibrolamellar carcinomas before 1939, using morphological findings and additional testing to confirm cases.

Materials and methods

With IRB approval, the pathology archives at Mayo Clinic from 1905 to 1939 were reviewed for surgical resections of hepatocellular carcinoma in individuals < 50 years of age. Original slides were examined (RPG and MST) and cases that were likely fibrolamellar carcinoma based on the original slides were selected for further study. The clinical charts were reviewed. Immunohistochemistry for HepPar1 (clone OCH1E5; predilute; Ventana, Tucson, AZ, USA), CK7 (clone OV-TL 12/30; 1:100; Dako, Santa Clara, CA, USA) and CD68 (clone KP1; 1:100; Dako) was performed using heat-induced epitope retrieval and standard laboratory methods.

Ultrastructural examination was performed on both cases using formalin-fixed paraffin-embedded tissues. The sections were reviewed on a FEI Tecnai 12 model electron microscope. Fluorescent *in situ* hybridization for *PRKACA* rearrangement and

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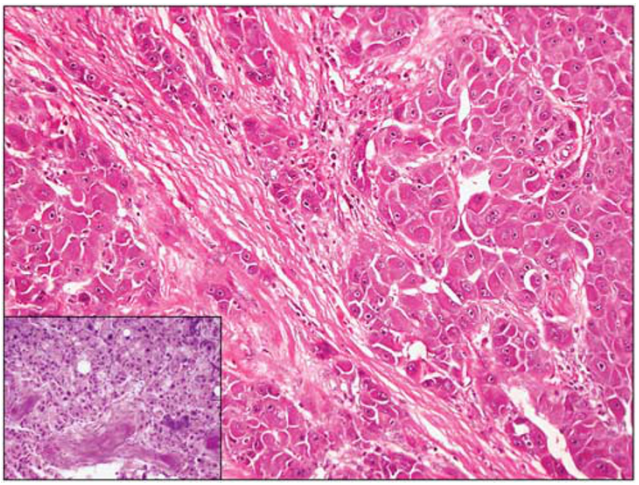

Figure 1 The photomicrograph shows a typical fibrolamellar carcinoma composed of oncocytic neoplastic cells with prominent macronucleoli. The neoplastic cells form trabecula, which are separated by bands of fibrosis (original magnification × 200). Inset: the original toluidine stained slide from 1915 revealed the same histologic features and focal intratumoral steatosis.

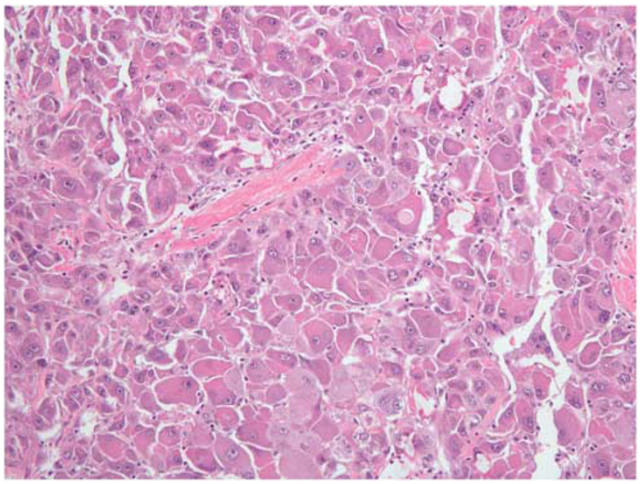

Figure 2 This case from 1924 showed the same histologic features and is characteristic of fibrolamellar carcinoma. A pale body is shown in the center of the image. Pale bodies are seen in almost half of fibrolamellar carcinomas.

RT-PCR for *DNAJB1-PRKACA* were performed using formalin-fixed, paraffin-embedded tissues, as previously described.[9] Finally, laser capture microdissection and mass spectrometry-based proteomic analysis were performed on formalin-fixed, paraffin-embedded tissue sections as previously described.[8]

Results

Case 1 A 39-year-old man who worked as a fence builder presented with a history of abdominal pain in 1915. Clinical examination revealed only right upper quadrant tenderness. Gynecomastia was not present. The persistence of the pain led to surgical intervention. From the operative notes, a hen egg-sized mass was found in the right lobe of the liver, close to the fundus of the gallbladder. The mass was excised and sent for pathological examination. The patient was followed for 9 years after surgery, during which no recurrence or metastatic disease was documented. He was lost to follow-up thereafter.

On review of the original slides, the tumor was composed of large eosinophilic cells with macronucleoli, separated by parallel arrays of lamellar fibrosis. Focally, the neoplastic cells showed steatosis (inset of Figure 1). A recut H&E confirmed the classic histological findings of fibrolamellar carcinoma (Figure 1). Immunostains were focally positive for HepPar1 and CK7 but the CD68 immunostain failed, as evidenced by no staining of Kupffer cells. FISH and RT-PCR were unsuccessful because of failure of hybridization and poor quality RNA, respectively. However, mass spectrometry-based proteomics was successful and confirmed that the tumor was hepatocellular, with detection of Arginase. Importantly, multiple classic markers of fibrolamellar carcinoma were also detected: CK7, anterior gradient-2 and ornithine aminotransferase, confirming the diagnosis of fibrolamellar carcinoma.

Case 2 A 68-year-old man who worked as a banker presented in 1924 with a clinical history of abdominal pain and a liver mass excision, 29 years prior, which was not available for review. At laparotomy, an advanced liver mass was identified, with extension to the lesser curvature of the stomach, adenopathy and ascites. An incisional biopsy was performed. On clinical follow-up, the patient died of disease < 11 months after presentation.

Histologic sections showed the typical elements of fibrolamellar carcinoma: monotonous large neoplastic cells with granular eosinophilic cytoplasm, prominent macronucleoli and bands of intratumoral fibrosis (Figure 2). Occasional pale bodies and pink bodies were identified. CK7 was focally positive. RT-PCR and FISH were unsuccessful because of nucleic acid degradation. However, mass spectrometry-based proteomics was informative, detecting arginase, CK7, anterior gradient-2 and ornithine aminotransferase.

Electron Microscopy Findings

Ultrastructural analysis of both cases showed similar features. The tumor cells showed abundant cytoplasm rich in mitochondria (Figure 3a). The membranes of the endoplasmic reticulum were abundant and formed concentric whorls (Figure 3b) with geometric/fingerprint-like (Figure 3c) structures. In case 2, internalized canaliculi lined by long microvilli were seen within the cytosol of neoplastic hepatocytes and were surrounded by thick ectoplasm (data not shown). These findings are not entirely specific, but are in line with previous reports

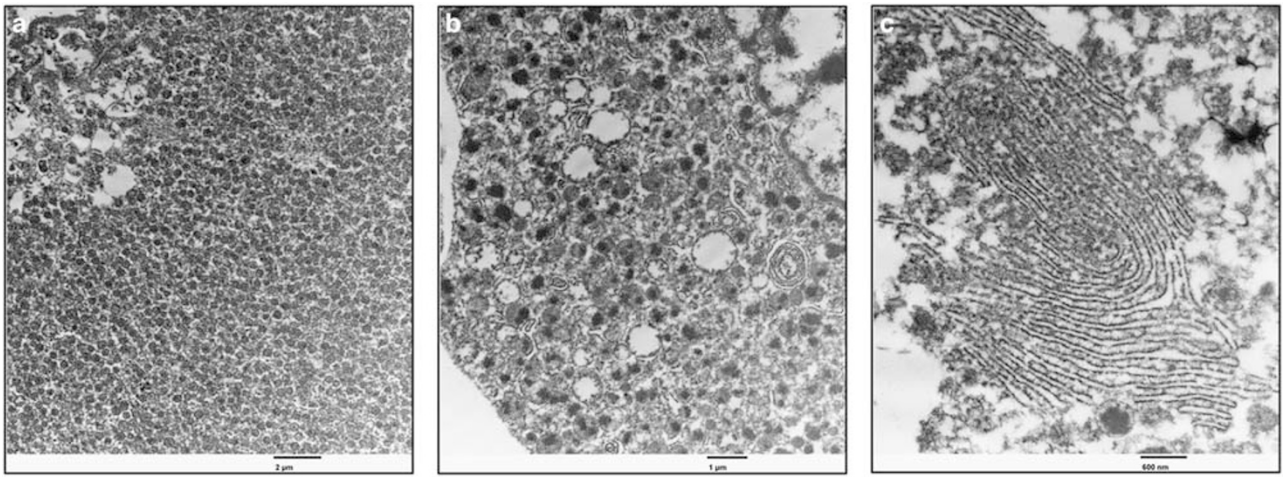

Figure 3 (**a**) Numerous mitochondria were seen in the cytoplasm of tumor cells from both cases. (**b**) Concentric whorls of the endoplasmic reticulum are shown in this image. This is a feature reported in fibrolamellar carcinoma. (**c**) Geometric structures formed by the abundant endoplasmic reticulum are another ultrastructural characteristic of fibrolamellar carcinoma and was seen in these cases.

of ultrastructural findings in fibrolamellar carcinoma from paraffin-embedded tissues.[1,2,13–17]

Discussion

Fibrolamellar carcinoma was first described by Edmondson[18] in 1956 as a neoplasm affecting a 14-year-old girl. Craig *et al*[1] refined the unique clinical and histologic characteristics of fibrolamellar carcinoma, including the major histological findings of large polygonal cells with abundant eosinophilic cytoplasm, large nucleoli and prominent intratumoral fibrosis. Craig *et al*[1] also provided the name fibrolamellar carcinoma and described the propensity for fibrolamellar carcinoma to affect individuals between 5 and 35 years of age. The distinctive histologic features of fibrolamellar carcinoma were further emphasized by Berman *et al*[19] in 1980 and others in review articles since then.[20–24]

The etiology for fibrolamellar carcinoma continues to be a major unresolved question. Early studies of thousands of autopsy and consultation cases found no examples of fibrolamellar carcinoma before World War II.[1,10,11] This suggested that fibrolamellar carcinoma may be associated with environmental risk factors introduced after the World War II.[12] This interesting finding was followed by a subsequent observation that tumor cells expressed aryl hydrocarbon receptors,[12] suggesting a potential role for the plastics industry, which expanded rapidly after 1945. However, the findings in the current study significantly reduce the likelihood that fibrolamellar carcinomas are driven by environmental exposures unique to the post World War II economy, as we describe the first known cases of fibrolamellar carcinoma occurring before World War II, in 1915 and 1924. As a result of the importance of this observation for understanding potential risk factors, the diagnosis was carefully validated by both morphological findings and protein-based studies with supporting ultrastructural examination results. However, these findings do not exclude a role for other potential environmental risk factors. In addition, our results are not able to address the question of whether the frequency of fibrolamellar carcinomas has changed before and after World War II.

The etiology of fibrolamellar carcinomas remains one of the key unsolved questions regarding this tumor. There is a defining molecular feature found in almost all fibrolamellar carcinomas but not in conventional hepatocellular carcinomas, as fibrolamellar carcinomas have deletions on the short arm of chromosome 19 that leads to a *DNAJB1–PRKACA* fusion gene.[7,9,12] The etiology for fibrolamellar carcinoma must cause this novel oncogenic genomic event. In general, the known mechanisms for the formation of oncogenic gene fusions include interchromosomal rearrangements, inversions and deletions.[25–27] The two main prerequisites for formation of a fusion gene by any of these mechanisms are close spatial proximity of the gene partners in the nucleus and the formation of double-stranded DNA breaks.[25,26] In the case of fibrolamellar carcinoma, the 400-kb separation between the two genes meets the former prerequisites. Double-strand DNA breaks may occur due to homologous recombination errors, the presence of the partner genes at recombination hot spots in the genome, topoisomerase II toxins/poisons or failure of mechanisms of repair of double-strand DNA breaks.[25,26] Patients with inherited syndromes associated with the failure to repair double-stranded DNA breaks (eg, Nijmegen syndrome) have not been reported with fibrolamellar carcinoma. Furthermore, although recipients of topoisomerase inhibitors are at increased risk for translocation-associated leukemias,[25] no fibrolamellar carcinomas have been reported in this clinical

context. Study of chromosome 19 has revealed no recombination hot spots, making this possible mechanism seem unlikely for fibrolamellar carcinoma.[28] However, chromosome 19 does show increased levels of homology relative to other chromosomes in the genome, which may make it susceptible to homologous recombination errors.[28] These errors underlie deletion and duplication syndromes[29] and would be concordant with the formation of *DNAJB1-PRKACA* by an interstitial deletion. However, this possibility would need to be tested before making a conclusion concerning the mechanism for double-strand breakage in *DNAJB1–PRKACA* formation. Nevertheless, existing data suggest that recurrent fusion genes are not random events.[27]

The environmental agent(s) responsible for the genomic events giving rise to the formation of *DNAJB1-PRKACA* remain(s) to be identified but there are some etiologies which have been excluded. Fibrolamellar carcinomas are not associated with chronic viral hepatitis C or B, alcohol use, iron overload, aflatoxin, or other factors that drive risk in conventional hepatocellular carcinoma. In keeping with this, the background livers are essentially normal at the microscopic level, lacking significant inflammation or fibrosis.[30] There have been occasional reports documenting the presence of hepatitis B viral proteins or DNA in fibrolamellar carcinoma,[31–33] but this finding appears to be one of chance, given the high world-wide prevalence of chronic hepatitis B infection, and overall there are no data to suggest hepatitis B as an etiological agent.

No histological precursor lesions to fibrolamellar carcinoma have been identified and there is no evidence for a 'field effect', as can be seen in conventional hepatocellular carcinoma where the background liver shows chronic disease. Fibrolamellar carcinomas can rarely co-occur with type 1 hepatocellular adenomas (*HNF1α*-inactivated), but they clearly do not develop out of hepatic adenomas.[8] Similarly, old notions of possible malignant transformation of focal nodular hyperplasia[34] have been disproven.

Fibrolamellar carcinomas are well known for their propensity to occur in younger individuals, with 80% of all cases presenting between ages 10 and 35.[21] Yet, fibrolamellar carcinomas do not occur in neonates or toddlers, making *in utero* exposures leading to the tumor less likely.

Animal models provide an informative avenue into our understanding of disease processes and can provide important insights into tumorigenesis. Unfortunately, there are no animal models of fibrolamellar carcinoma. This is in contrast to conventional hepatocellular carcinomas, for which there are dozens of animal models.[35–37] In these models, exposure to chemicals or chronic viral infection induces development of conventional hepatocellular carcinoma. However, no mutagen exposure has yielded a fibrolamellar carcinoma to date. Nonetheless, rare spontaneous fibrolamellar carcinoma has been reported in other mammals,[38] suggesting models could yet be developed which may advance our understanding of the etiologic event(s) in fibrolamellar carcinoma.

In conclusion, fibrolamellar carcinoma is a unique translocation-associated hepatocellular carcinoma, which was first recognized in 1956.[18] Early large studies failed to identify fibrolamellar carcinomas from before World War II.[10,11] These observations and the finding of aryl hydrocarbon receptors on the tumor cells suggested the possibility that environmental exposures after World War II could be responsible for causing fibrolamellar carcinoma.[12] The two cases of fibrolamellar carcinoma characterized in this study confirm that the genomic events responsible for formation of the oncogenic *DNAJB1–PRKACA* fusion gene in fibrolamellar carcinoma preceded World War II. Further work is needed to identify the agent(s) responsible for genomic events leading to the formation of the oncogenic *DNAJB1–PRKACA* fusion gene.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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