Pathology International 1996; 46: 84–90

Case Report

Fibrolamellar hepatocellular carcinoma: A case report of a resected with an electron microscopic and flow cytometric analysis

Akio Hasegawa

The Department of Pathology and Laboratory Medicine, Odawara Municipal Hospital, Odawara, Japan

A resected case of fibrolamellar (FLC) and hepatocellular (HCC) combined carcinoma arising in a non-cirrhotic liver of a 29 year old female is reported, including results of the preoperative percutaneous aspiration biopsy, which suggested FLC, and postoperative electron microscopic and flow cytometric analysis. Sections of the resected massive tumor of the left lobe of the liver showed hepatocellular carcinoma accompanying the fibrolamellar carcinoma element which was composed of tumor cells with eosinophilic granular cytoplasm and unique cytoplasmic vacuoles (pale bodies). Lamellar fibrosis was present in the stroma, while no macroscopic central scar was demonstrated. Electron microscopy showed typical features of FLC and flow cytometric DNA analysis indicated diploid DNA pattern with a low proliferation rate. A common HCC element with trabecular structure also existed at the periphery of the tumor. No apparent etiologic agent for the development of hepatic neoplasm was indicated in the history of this patient. She had been without recurrence for about 3 years after extended left lobectomy, when local recurrence was revealed. The recurrence has been treated with chemoembolization and percutaneous ethanol infusion several times up till the present. This case reconfirms the importance of the pathological diagnosis of FLC to promote surgical intervention.

Key words: electron microscopy, flow cytometry, hepatocellular carcinoma, liver

Among hepatocellular carcinomas (HCC), the special subtype fibrolamellar hepatocellular carcinoma (FLC) has been characterized by the following: (i) it originates from non-cirrhotic livers of adolescents or young adults, (ii) it grows slowly and has an indication for surgical treatment with rather better prognosis, (iii) histologically, the parenchyma composed of tumor cells with polygonal, eosinophilic and granular cytoplasm is surrounded by thick and lamellar fibrous stroma.[1,2,3,4,5,6] This unique entity is variously designated by hepatopathologists and suggested as an intermediate lesion between focal nodular hyperplasia and HCC.[4,6] In Japan, the Far East and Africa, the incidence of this subtype among hepatic tumors is supposed to be very rare, although HCC is much more common in these countries than in the countries of North America and Europe.[7,8]

This paper reports clinical and pathologic features of FLC arising in a non-cirrhotic liver of a Japanese adult female, and the patho-epidemiological implications are discussed.

CLINICAL SUMMARY

This 29 year old Japanese female was asymptomatic until June 1990, when she experienced edema of eyelids and laboratory examination revealed leukocytosis, elevated erythrocyte sedimentation rate and elevated serum liver enzymes (aspartate aminotransferase [ASAT] 78 IU, alanine aminotransferase [ALAT] 81 IU). She was then referred to our hospital where a large mass was revealed in the left lateral and medial segment by abdominal ultrasound and CT scan (Fig. 1), measuring 14 × 8 × 7 cm. Past medical history and family history was unremarkable. There was no history of oral contraceptive use or alcohol excess. The hematocrit was 35.9%, the white blood cell count was 13350, and the platelet count was 45400. The erythrocyte sedimentation rate was 99.0 mm per hour. The total bilirubin was 0.4 mg per 100 mL, ASAT 105 IU, ALAT 94 IU, lactic dehydrogenase (LDH) 610 IU, γ-GTP 63 IU/L, hepatitis C virus antibody (HCV Ab) absent, hepatitis B surface antigen (HBsAg) absent, and alpha-fetoprotein (AFP) was not elevated. A percutaneous aspiration needle biopsy of the hepatic mass was performed,

Correspondence: Akio Hasegawa, MD, PhD, Department of Pathology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.

Received 6 July 1995. Accepted for publication 7 September 1995.

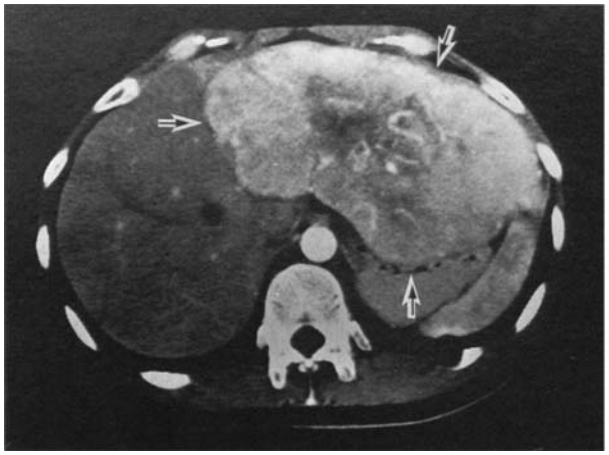

Figure 1 Abdominal CT scan showing a large mass in the left lobe of the liver (arrows).

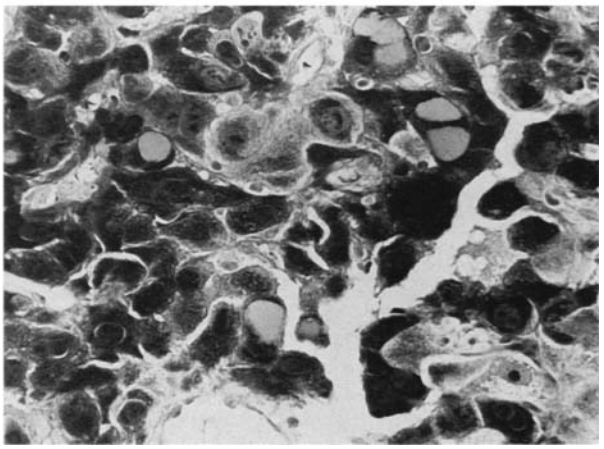

Figure 2 Section of aspiration biopsy specimen demonstrating polygonal tumor cells with distinct cytoplasmic pale bodies (Azan-Mallory).

which showed hepatocellular carcinoma, Grade II, suggestive of FLC (Fig. 2). Extended left lobectomy was performed on 22 August. The postoperative period was uneventful for about 3 years until June 1993, when local recurrence of the tumor at the surgical stump was revealed by CT-scan, measuring $7.0 \times 2.7 \times 3.8$ cm. The patient was admitted for chemoembolization from July until October 1993, for percutaneous ethanol infusion (PEI) in December 1993, and chemoembolization and PEI in January 1995. She has been under strict follow-up check until now.

PATHOLOGICAL FINDINGS

On gross examination the tumor measured $15 \times 10 \times 7$ cm, divided by thin fibrous tissue into a multinodular appearance at the cut surface (Fig. 3). In the central part of the tumor many small blood-filled cavities were present and there was no discernible central depression of scar tissue. The resected left lobe of the liver weighed 870 g and measured $16 \times 10 \times 8$ cm externally, showing a smooth surface. The peritumoral uninvolved liver tissue showed compression by the sharply demarcated expanding margin of the tumor without intervening encapsulation. Liver cirrhosis was not present.

Microscopic analysis examining the largest cut section of the tumor (circa 20 paraffin-embedded blocks per section) demonstrated an admixture in a complex manner of the fibrolamellar hepatocellular carcinoma and the common hepatocellular carcinoma. Fibrous strands, focally arranged in a lamellar fashion, were interspersed between the parenchyma of the tumor, which contained no bile ducts (Element A, Fig.4). The nuclei of tumor cells were hyperchromatic and pleomorphic with prominent nucleoli, and cytoplasmic invaginations into the nuclei were common. The cytoplasm was relatively abundant, polygonal or ovoid, finely granular without bile production, and conspicuously eosinophilic in localized areas, which were immunohistochemically positive for α-1-antitrypsin (AAT; Nichirei, Tokyo, Japan) throughout the cytoplasm, and negative for α-smooth muscle actin (SMA; Nichirei). Distinct cytoplasmic pale bodies of variable size occurred in a large proportion of tumor cells which were negative for PAS stain, and immunohistochemically negative for AAT and factor VIII (Dako, Kyoto). Stromal cells with spindle-shaped, rather small nuclei in the fibrous stroma were immunohistochemically positive for vimentin (Nichirei), which was localized around the nucleus, and negative for desmin (Dako). SMA was detected throughout the fibrous strands. Intermingled in a transitional fashion with Element A, without forming a sharp border, the tumor cells with the same cytological features were arranged in thin trabecular and adenoid structures, accompanying diffuse pericellular fibrillosis of various degrees (Element B). In the central portion of the tumor, the hemorrhage into the lumen of adenoid structures appeared like a peliosis. At the outer margin of the expanding tumor composed of Elements A and B, common HCC remained, forming thin and thick trabecular structures with vascular-rich stroma which was devoid of fibrosis, involving the whole circumference discontinuously and being less than 5 mm thick (Element C). The boundary between Elements C and A/B was distinct. The cytological features of Element C were quite different from those of Elements A and B, the nucleus was rather uniform in size and shape without

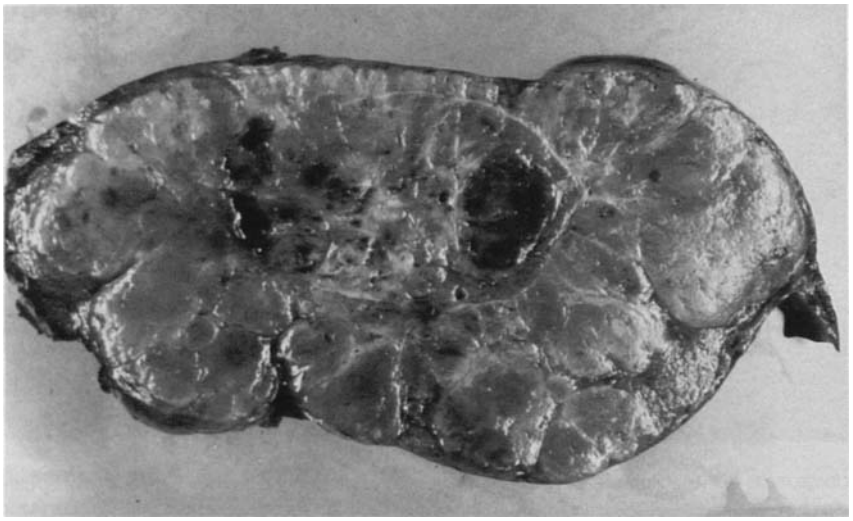

Figure 3 Gross appearance of the resected specimen. The marginally compressed liver tissue surrounds a multinodular solid tumor. Peliosis-like change is present in the central part.

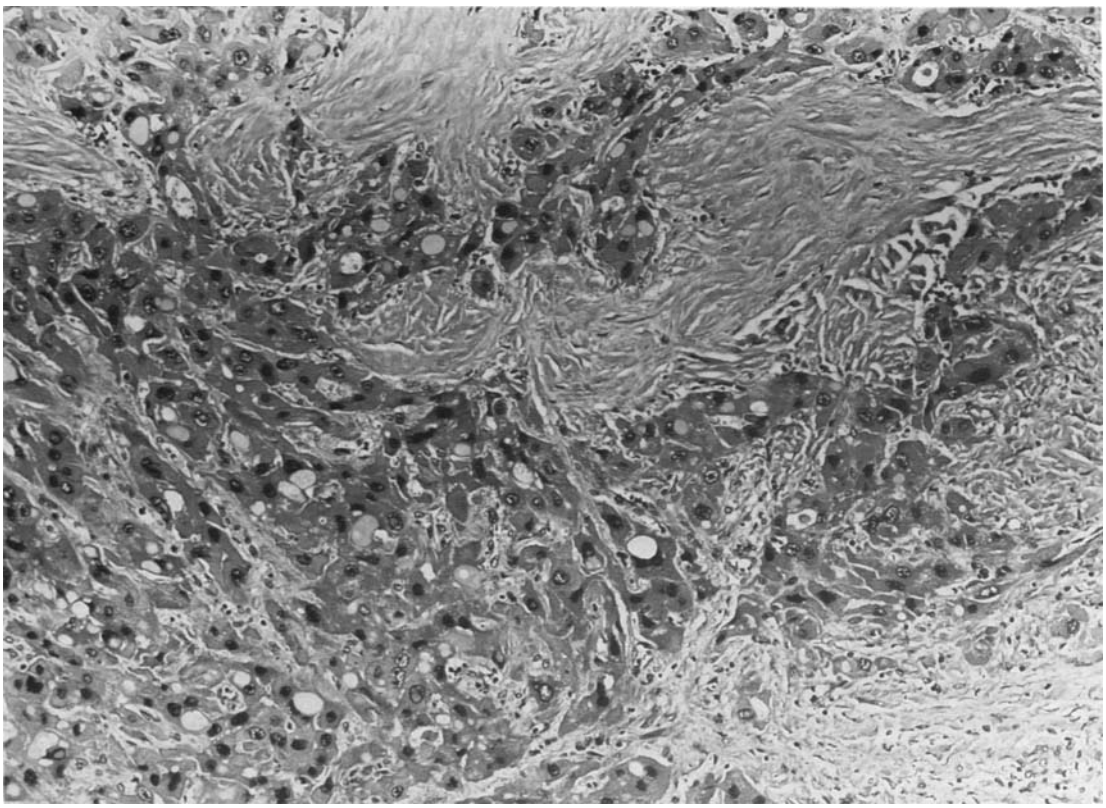

Figure 4 Histology of the resected specimen. Fibrous strands extend between trabeculae of tumor (Element A), while the left lower part shows Element B (HE).

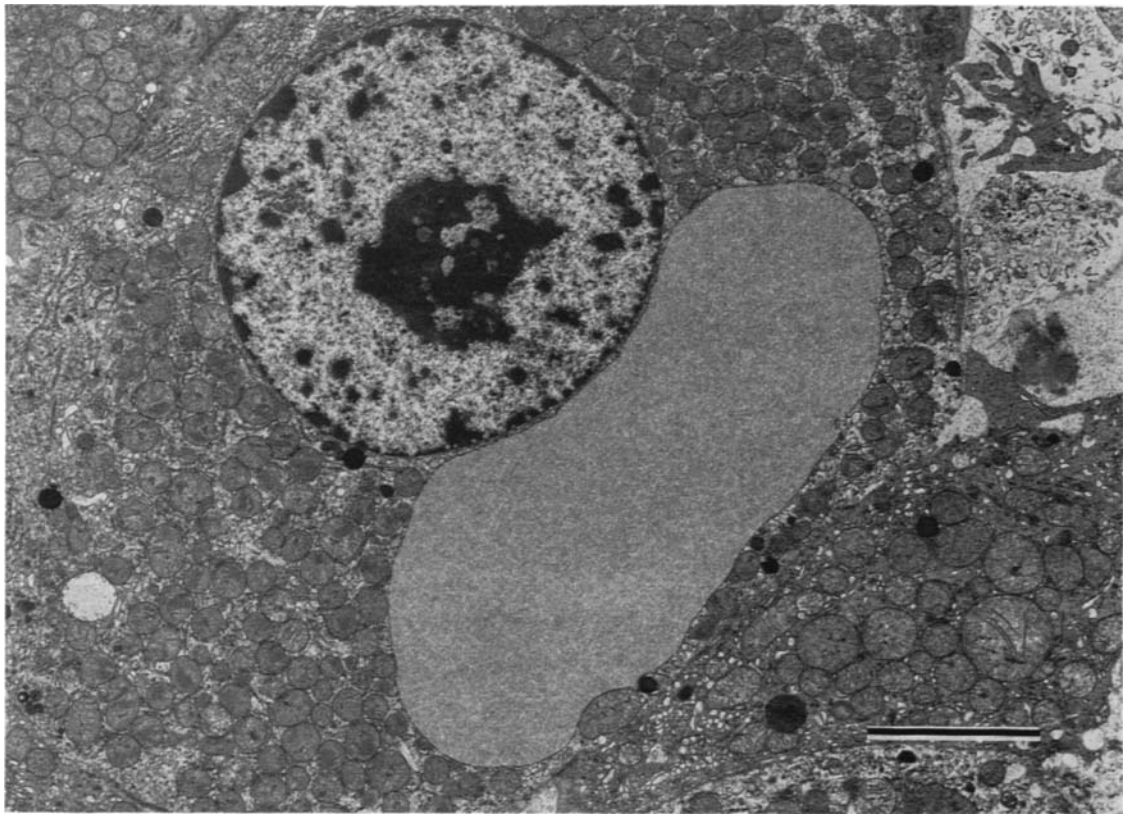

Figure 5 Electron micrograph of tumor cell with a round nucleus and cytoplasm containing abundant mitochondria, electron-dense bodies and a large endoplasmic vacuole (pale body). Residual sinusoid-like structure in the right upper corner (× 5200). Bar = 5 μm.

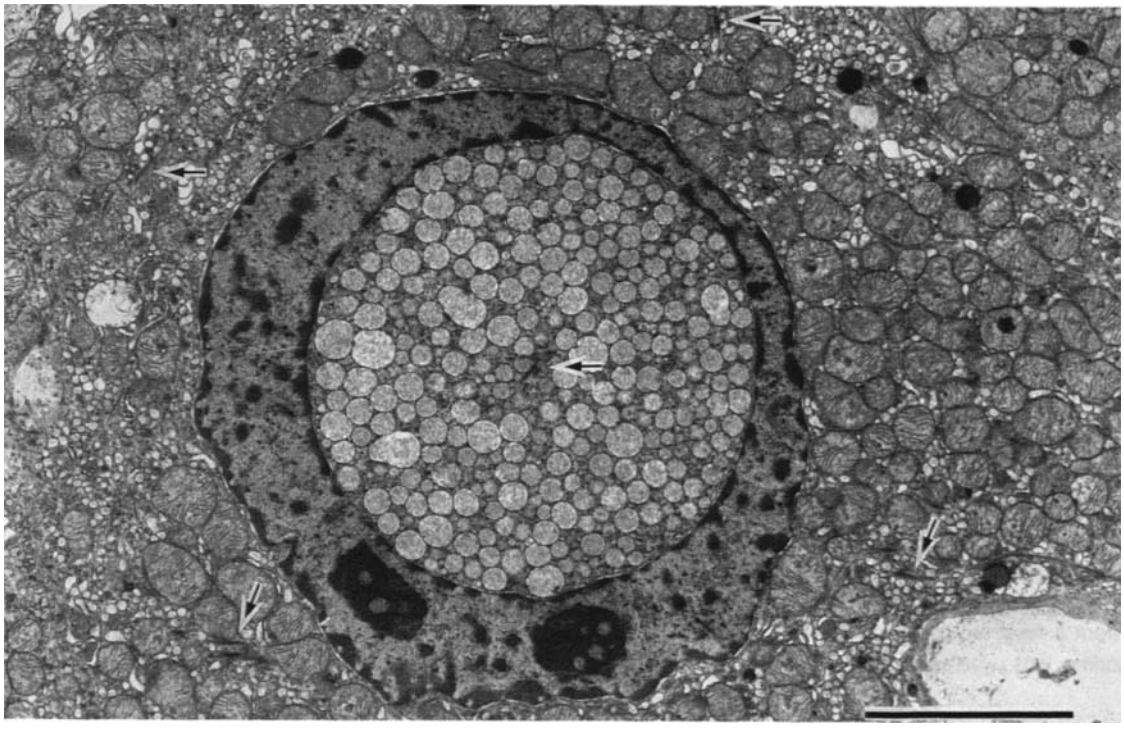

Figure 6 Electron micrograph of a tumor cell with a cytoplasmic invagination into the nucleus. Arrows show intracytoplasmic distribution of parallel filaments (× 6300). Bar = 5 μm.

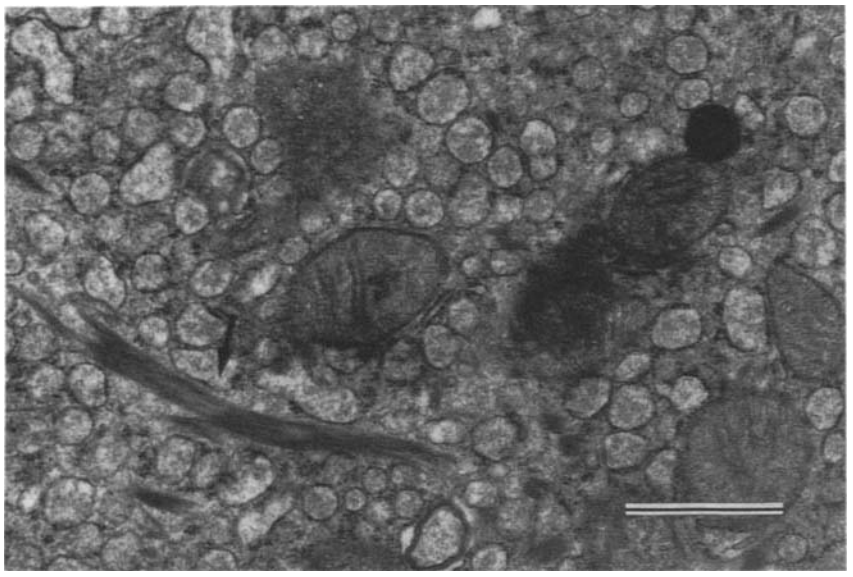

Figure 7 Electron micrograph showing cytoplasm of a tumor cell containing mitochondria, endoplasmic reticulum, sparse electron-dense body and parallel filaments (arrow; × 22 000). Bar = 1 μm.

conspicuous cytoplasmic invaginations, and the cytoplasm was less eosinophilic, partly clear, accompanying mild bile production (Edmondson grade I). AAT was observed throughout the cytoplasm. The Victoria blue and Orcein stains of the cells of FLC, common hepatocellular carcinoma and peritumoral uninvolved liver tissue were negative for HBsAg and granular substances (copper binding protein).[9] Permeation into the portal vein or hepatic vein was not present and lymph nodes of the hepatic hilum were negative for metastasis.

Electron microscopy demonstrated the cytoplasm of tumor cells filled with abundant mitochondria, rough surfaced endoplasmic reticulum, and large endoplasmic vacuoles corresponding to the pale bodies shown in photomicroscopy (Fig. 5).[3] Sparse electron-dense bodies with a diameter of 0.5–1.0 μm were present, and were membrane-bound.[18] Parallel filaments were also observed scatteringly and individually between endoplasmic reticula in the cytoplasm, without formation of the condensed area (Figs. 6, 7).[18]

Flow cytometric analysis of the fresh tumor specimen using an Ortho CYTORON (Ortho, Tokyo, Japan) indicated that this tumor is composed of a single diploid population of cells with a single G0/G1 peak (DNA index of 1.0) and a proliferation index of about 4% (S + G2 + M phase fraction/total) (Fig. 7). This histogram was prepared using about 10 000 cells and the coefficient of variance (CV) of the G0/G1 peak was 3.9%. Tissue section taken from the area facing the sampling site was histologically checked. Human lymphocytes from a resected and frozen tonsil, stained with propidium iodide, was used as a standard.

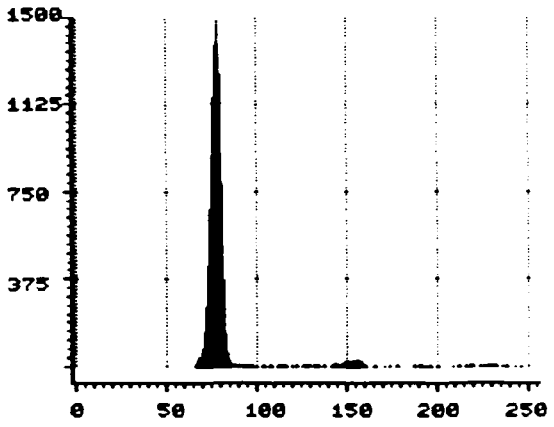

Figure 8 Flow cytometric analysis of the resected tumor.

DISCUSSION

The case presented here fulfilled most of the criteria for a diagnosis of fibrolamellar hepatocellular carcinoma. It occurred in the non-cirrhotic liver of a young adult, the level of AFP was not elevated, it originated from the left lobe of liver, and it had not metastasized at the time of the left lobectomy despite the massive growth shown in the resected specimen. Clinical symptoms are usually vague and often of long duration.[1] Based on histological criteria Craig described that numerous blocks may be needed to disclose the lamellar fibrosis for the diagnosis of FLC.[1] Therefore diagnosis of FLC was made on the present tumor, where the lamellar

fibrosis was present in a relatively limited number of the blocks taken from the tumor, but was demonstrated conspicuously. The pattern of fibrous strands formation was very consistent with the previous description that the fibrous stroma subdivided the tumor cells into thin columns or grew as trabecula into the tumor, similar to the structure of splenic trabecula.[2] It was also reported that three out of 12 FLC cases contained significant mixtures of HCC with trabecular and adenoid patterns.[4] The true nature and significance of the increase in number of mitochondria, or oncocytic change, remains controversial,[3] and in my view it suggests that the adaptations of tumor cells to the micro-environment lacking O_2 supply are due to stromal fibrosis. In the current case, oncocytic change was not prominent in the area where the fibroplasia was not far advanced.

The incidence of FLC in primary hepatic carcinoma depends on the selection of patients, including age distribution and geography. Berman reported 12 cases of FLC out of 125 HCC cases (1%)[4] and Farhi reported 10 cases of FLC out of 23 HCC cases in patients less than 35 years of age (43%).[10] The incidence of FLC in Japan is not accurately known. The published case reports of FLC with typical clinico-pathologic features are very limited despite the prevalence of HCC here.[7] Exceptionally, three individual cases of FLC have been shown; Case 1 of a 56 year old non-cirrhotic Korean male with positive HBsAg, HBeAg and slight increase of AFP,[11] Case 2 of a 56 year old cirrhotic male with positive HCV Ab and slight increase of AFP level,[12,13] and Case 3 of a 36 year old cirrhotic female with positive HBsAg, increase of AFP level, and hypersplenism.[14] Compared with these three cases, the profile of the present subject is more similar to those described in the western countries. In Japan there are about 34 200 registered autopsy cases a year including circa 19 700 malignant neoplasms and 2700 HCC.[8] Resections of hepatic tumors are usually performed only at qualified hospitals and pathological examinations are principally undertaken by 1500 board certified pathologists. Considering this context, it seems true that FLC is an extremely rare form of HCC in Japan, although systematic re-examination of HCC cases in adolescents and young adults still remains necessary. Virtually, no information is available regarding specific etiologic factors of FLC. LeBrun discussed the relationship of the fibrolamellar carcinoma of the liver to Fanconi anemia and to anabolic steroid therapy in a 9 year old boy with FLC.[15] The present subject had no apparent risk factors for the development of hepatic neoplasms. It is interesting to study changes in the incidence of FLC in countries where the numbers of cases of post-transfusion hepatitis B and C are markedly decreasing due to recent advances in preventive measures. Through elucidation of its geographical pathology around the world and chronological changes in its incidence within each country, we may find a clue to the etiologic factor of FLC.

In this case, surgical resection of FLC was accomplished successfully at the time of the operation. A diploid pattern in the DNA histogram of this tumor revealed by flow cytometry reflects a high degree of differentiation, although the value of ploidy as predictive parameters for patients with certain malignant neoplasms including FLC is undetermined.[16] Considering the favorable behavior and prognosis of FLC[4,17] in spite of the remaining possibility of local recurrence, the definitive preoperative histologic diagnosis of FLC by fine needle aspiration specimens would be of clinical value to promote radical resection by surgeons. However, it is sometimes difficult to differentiate FLC from common HCC before obtaining multiple sections taken from resected or autopsied specimens. Even though a clear diagnosis of FLC cannot be made from the aspirated specimen during preoperative check-up, the aggressive surgical resection should still be considered as a possible choice in a case with a suspicion of FLC.

ACKNOWLEDGMENTS

The author thanks T. Iwasaki and H. Sato for technical assistance.

REFERENCES

1 Craig JR, Peters RB, Edmondson HA. Distinctive hepatocellular carcinoma variants. In: *Tumors of the Liver and Intrahepatic Bile Ducts*. Armed Forces Institute of Pathology, Washington, DC, 1989; 170–186.

2 Craig JR, Peters RB, Edmondson HA, Omata M. Fibrolamellar carcinoma of the liver. A tumor of adolescents and young adults with distinctive clinico-pathologic features. *Cancer* 1980; **46**: 372–379.

3 Altmann HW. Some histological remarks on the fibrolamellar carcinoma of the liver. *Pathol. Res. Pract.* 1990; **186**: 63–69.

4 Berman MM, Libbey NP, Foster JH. Hepatocellular carcinoma. Polygonal cell type with fibrous stroma — An atypical variant with a favorable prognosis. *Cancer* 1980; **46**: 1448–1455.

5 Lack EE, Neave C, Vawter GF. Hepatocellular carcinoma. Review of 32 cases in childhood and adolescence. *Cancer* 1983; **52**: 1510–1515.

6 Vecchino FM, Fabiano A, Ghirlanda G, Manna R, Massi G. Fibrolamellar carcinoma of the liver. The malignant counterpart of focal nodular hyperplasia with oncocytic change. *Am. J. Clin. Pathol.* 1984; **81**: 521–526.

7 Mori W, Machinami R, Tanaka K. Pathology of hepatocellular carcinoma. *Pathol. Res. Pract.* 1980; **169**: 4–20.

8 The Japanese Society of Pathology (ed). *Annual of the Pathological Autopsy Cases in Japan*, (Jan.–Dec. 1992). The Japanese Society of Pathology, Tokyo, 1993, **35**.

9 Tanaka K, Mori W, Suwa K. Victoria blue-nuclear fast red stain for HBs antigen detection in paraffin section. *Acta Pathol. Jpn.* 1981; **31**: 93–98.

10 Farhi DC, Shikes RH, Murari PJ, Silverberg SG. Hepatocellular carcinoma in young people. *Cancer* 1983; **52**: 1516–1525.
11 Yoshida K, Kobayashi S, Miyamoto S *et al.* Fibrolamellar carcinoma of the liver. A case report. *J. Jpn. Surg. Soc.* 1986; **87**: 1485–1489.
12 Hayashida Y, Nagahama J, Yokoyama S, Terao H, Tada I. A case of fibrolamellar hepatocellular carcinoma. *J. Jpn. Soc. Clin. Cytol.* 1991; **30**: 126–130.
13 Okada K, Kim YI, Nakashima K *et al.* Fibrolamellar hepatocellular carcinoma coexistent with a hepatocellular carcinoma of common type: Report of a case [Review]. *Surgery Today* 1993; **23**: 626–631.
14 Imai T, Yokoi H, Noguchi T, Kawarada Y, Mizumoto R. Fibrolamellar carcinoma of the liver. A case report. *Gastroenterol Jpn.* 1991; **26**: 382–389.
15 LeBrun DP, Silver MM, Freedman MH, Philips MJ. Fibrolamellar carcinoma of the liver in a patient with Fanconi anemia. *Hum. Pathol.* 1991; **22**: 396–398.
16 Frierson HF. The need for improvement in flow cytometric analysis of ploidy and S-phase fraction [Editorial]. *Am. J. Clin. Pathol.* 1991; **95**: 439–441.
17 Stevens WR, Johnson CD, Stephens DH, Nagorney DM. Fibrolamellar hepatocellular carcinoma: Stage at presentation and results of aggressive surgical management. *Am. J. Roentgenol.* 1995; **164**: 1153–1158.
18 Caballero T, Aneiros J, Lopez-Caballero J, Gomez-Morales M, Nogales F. Fibrolamellar hepatocellular carcinoma. An immunohistochemical and ultrastructural study. *Histopathology* 1984; **9**: 445–456.