Follicular Dendritic Cell Tumor of the Liver: A Clinicopathologic and Epstein-Barr Virus Study of Two Cases

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Two cases of hepatic follicular dendritic cell (FDC) tumor are described. Both patients were female, aged 57 and 51 years. They presented with epigastralgia or abdominal fullness and weight loss. The first patient refused surgical resection. She developed progressive polyclonal gammopathy and then bilateral purpura over the legs. Skin biopsy revealed leukocytoclastic vasculitis with granular vascular deposits of IgA and C_3. The second patient had marked peripheral blood and tissue eosinophilia. The histological diagnosis was confirmed by positive staining for CD21 and CD23. The stromal lymphocytes were predominantly composed of CD3(+) and CD8(+) cells. *In situ* hybridization for EBER showed a positive nuclear signal in tumor cells but not in inflammatory cells. Polymerase chain reaction amplification for Exon 3 of the latent membrane protein-1 (LMP-1) gene showed a characteristic 30-bp deletion between nucleotides 168282 and 168253, corresponding to the B95-8 sequence. The unique clinicopathological features of our cases have not been reported for FDC tumors before. The clinical significance of the 30-bp deletion in Exon 3 of the LMP-1 gene in FDC tumor of the liver warrants further investigation.

KEY WORDS: Eosinophilia, Epstein-Barr virus, Follicular dendritic cell, Latent membrane protein-1 gene, Liver, Leukocytoclastic vasculitis.

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The follicular dendritic cell (FDC) tumor was first reported by Monde *et al.* (1) in 1986. Since then, approximately 50 cases of FDC tumor have been reported (1–12). In most of these cases, the sites affected were the lymph nodes. Extranodal cases were uncommon. Approximately 12% of FDC tumors were demonstrated to contain the Epstein-Barr virus (EBV) sequence (7). Therefore, EBV may play a role in the pathogenesis of the FDC tumor. In this report, we describe two FDC tumors of the liver with unique clinical features and a unique EBV subtype.

MATERIALS AND METHODS

Two cases of hepatic FDC tumor were retrieved from the surgical pathology files of Chang Gung Memorial Hospital from the period 1996 to 1999. Formalin-fixed and paraffin-embedded tissues were used for histopathologic, immunohistochemical, and *in situ* hybridization studies. The immunohistochemical studies were performed using the avidin-biotin-peroxidase complex method. A panel of antibodies was used, and the antibodies are listed in Table 1. A fresh skin biopsy specimen (Case 1) was also subjected to direct immunofluorescence studies for IgG, IgM, IgA, C_3, and C_4. EBV-encoded nuclear RNA (EBER) *in situ* hybridization studies were performed with a DAKO fluorescein-conjugated EBV (EBER) PNA probe (complementary to two nuclear EBER RNAs encoded by EBV; DAKO A/S, Glostrup, Denmark) on 5-μm-thick, deparaffinized, and proteinase K–pretreated tissues for 1.5 hour at 55°C. After washing, the reaction was detected by the DAKO PNA ISH detection Kit (DAKO A/S).

Latent Membrane Protein-1 Gene Study

Fresh tumor tissue obtained from Case 2 was frozen at −80°C until analyzed. High–molecular weight DNA was purified by phenol/chloroform ex-

TABLE 1. Antibodies Used in this Study

Antibody	Source	Dilution	Pretreatment
CD21	DAKO	1:50	TP
CD23	The Binding Site	1:50	PC
CD35	DAKO	1:20	TP
CD3	DAKO	1:800	TP
CD20	DAKO	1:1600	PC
CD4	Novocastra	1:10	PC
CD8	DAKO	1:100	PC
HHF-35	DAKO	1:400	TP
κ light chain	DAKO	1:1000	TP
λ light chain	DAKO	1:1000	TP

PC, pressure cooking in EDTA for 2 minutes or in citrate buffer for 3 minutes; TP, trypsinization for 20 minutes.

traction, followed by ethanol precipitation. DNA pellets were resuspended in distilled water. To examine the 30-bp deletion in Exon 3 of the latent membrane protein-1 (LMP-1) gene, a set of primers, 9/11 described by Knecht et al. (13), were used. The amplification reaction mixture contained 10 mM Tris HCL (pH 7.5), 50 mM sodium chloride, 10 mM magnesium chloride, 1.5 mM dNTP, 2 primers (0.2 μM each), and 2.5 units of Taq DNA polymerase. DNA preparations from B95-8 cells and pT7 strain were used as controls. The amplification cycle included 1 minute of DNA denaturation at 95°C followed by 1 minute of annealing at 55°C and 1 minute of DNA extension at 72°C. The amplification was repeated for 30 cycles. After the reaction, one tenth of the reaction mixture was fractioned electrophoretically in 6% polyacrylamide gel and visually inspected under ultraviolet light for the presence of DNA bands of appropriate size after ethidium bromide staining. The PCR products were directly analyzed on the gel by size difference.

DNA sequencing for the PCR products of Exon 3 of the LMP-1 gene was carried out using an ABI PRISM dRhodamine terminator cycle-sequencing ready reaction kit. Reaction products were analyzed on the ABI PRISM 377 DNA sequencer using ABI PRISM sequencing 3.0 software.

RESULTS

Clinical Histories

Case 1

A 57-year-old woman was admitted to Chang Gung Memorial Hospital because of intermittent epigastralgia of 2 weeks' duration. A 9.5 × 7.6 cm hepatic left lobe tumor was found after a series of imaging studies. Laboratory investigations showed a mild anemia and slightly increased serum globulin. She was positive for serum hepatitis B surface antigen (HBsAg) and negative for anti-hepatitis C virus (HCV) antibody. Serum α-fetoprotein was 6 ng/mL. Initial protein electrophoresis only showed slightly increased α1-globulin. The patient refused

surgical resection. During follow-up, serumγ globulin began to increase (from 1.5 to 2.6 gm%), and repeat protein electrophoresis showed a chronic inflammation pattern with a polyclonal increase of γ-globulin. Later, she developed lower limb purpura bilaterally. A skin biopsy was taken. Immunological investigations revealed the following levels: serum IgA, 578 mg% (normal, 74.7–373.5); C_3, 8.4 mg% (normal, 82.5–180); and C_4, 13.3 mg% (normal, 18.2–45.5). Antinuclear antibody was negative. The liver also progressively enlarged to a maximum span of 15 cm in a 3-year period. No metastases were found. The patient is now receiving steroid treatment.

Case 2

A 51-year-old Taiwanese woman presented with abdominal fullness and weight loss of 6 kg within 3 months. Abdominal ultrasound revealed a mixed echoic mass in the left hepatic lobe. She had no fever. Liver function tests were within normal ranges. Hematological investigations produced the following results: hemoglobin, 10.7 g%, white blood cell, 12.3 × 10^9/L with 46% polymorphs, 38% eosinophils, 10% lymphocytes, 4% monocytes, and 1% basophils. Serum HBsAg and anti-HCV antibody were negative. Serum α-fetoprotein was less than 3 ng/mL (normal, less than 3), and carcinoembryonic antigen was 0.78 ng/mL (normal, less than 5). An abdominal computed tomographic scan showed a 15 × 12 × 10-cm heterogenous mass in the right hepatic lobe. An angiographic study showed that the tumor was hypervascular. She underwent left lobectomy. Her blood eosinophil count returned to the normal range, and she was well, with no evidence of disease 1 year after the operation.

Pathologic Findings

The liver biopsy specimen from Patient 1 measured 1.7 cm in length. Microscopically, it showed a spindle cell tumor arranged in irregular pattern or in short fascicles with dense infiltrates of small lymphocytes and plasma cells (Fig. 1). The spindle cells had vesicular nuclei and small basophilic nucleoli with indistinct cell borders. The skin biopsy from the leg of Patient 1 showed thickened dermal vessels with fibrinoid necrosis and surrounded by mononuclear cells, neutrophils, and eosinophils undergoing karyorrhexis (Fig. 2). Extravasated red blood cells were diffusely present.

The resected liver from Patient 2 measured 17 × 17 × 9 cm and weighed 1150 g. There was a well-circumscribed but unencapsulated tumor measuring 12 × 11 × 9 cm. It was tan colored and soft with areas of necrosis and hemorrhage (Fig. 3). No daughter nodules were present. Histologic features were generally similar to that observed in Patient 1.
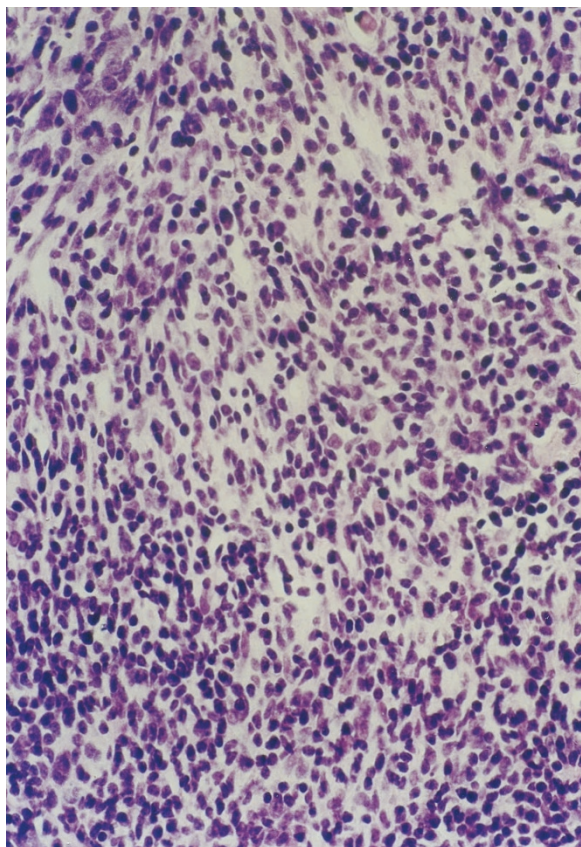
FIGURE 1. Liver biopsy (Patient 1) showing a spindle cell tumor with dense infiltrates of plasma cells and lymphocytes.
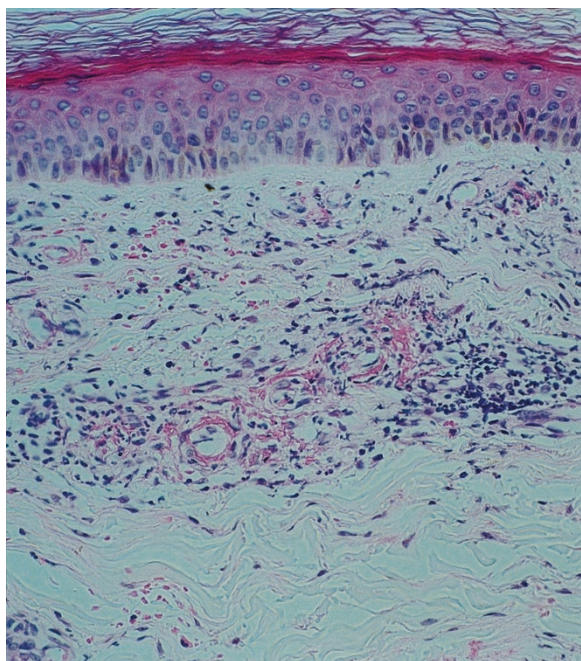

FIGURE 2. Skin biopsy (Patient 1) showing thickened dermal vessels with fibrinoid necrosis and surrounded by mononuclear cells, neutrophils, and eosinophils undergoing karyorrhexis.

In addition, the tumor cells were arranged in storiform, whorled, and interlacing fascicle patterns
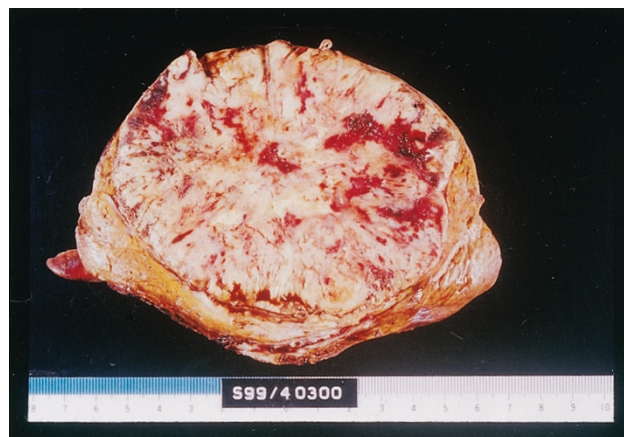

FIGURE 3. A well-circumscribed but unencapsulated tumor in the liver with areas of necrosis and hemorrhage (Patient 2).

(Fig. 4). A few multinucleated giant cells were observed. There was no obvious cellular atypia. Mitotic figures averaged 1 per 10 high-power fields. There was multifocal geographic necrosis and hemorrhage. The non-tumor area contained scattered infiltrates of neoplastic FDCs in portal tracts and hepatic lobules.

Immunohistochemical Study

The tumor cells in both cases were positive for CD21 (Fig. 5), CD23, and HHF-35 (muscle-specific actin). They were negative for CD35. The stromal small lymphocytes were predominantly composed of CD3 (+) T cells, among which CD8 (+) T cells clearly outnumbered CD4 (+) T cells (Fig. 6). The plasma cells were polyclonal as evidenced by a mixture of both $\kappa(+)$ and $\lambda(+)$ cells.

EBER *In Situ* Hybridization

The spindle cells in both cases showed positive nuclear EBER signals (Fig. 7), whereas the inflammatory cells in the background were negative.

LMP-1 Gene Study

The presence of the EBV genome was confirmed by polymerase chain reaction with the LMP-1 gene in Patient 2. In addition, the LMP-1 gene also had a 30-bp deletion in Exon 3 as compared with the products from B95-8 cells (Fig. 8). We further sequenced the PCR product and confirmed a 30-bp deletion between nucleotides 168282 and 168253 (corresponding to the B95-8 sequence). There were also point mutations at positions 168367 (C→A), 168320 (A→G), 168308 (T→C), 168295 (A→T), 168285 (C→G), and 168225 (T→A).

DISCUSSION

FDC tumor is a rare disease. Approximately two thirds of documented cases occurred in the lymph
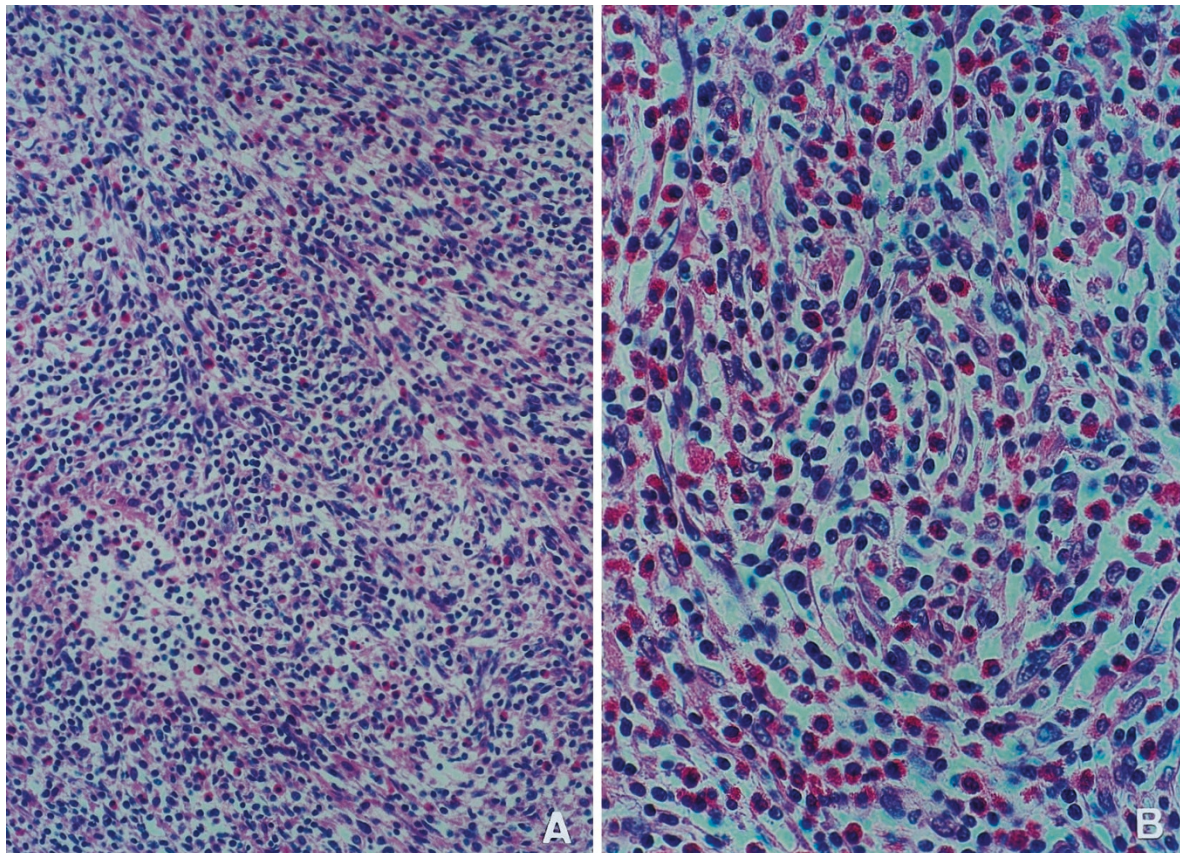
FIGURE 4. The spindle tumor cells showed vesicular nuclei, small nucleoli, and indistinct cytoplasmic borders. They were arranged in fascicles (**A**) and whorled (**B**) patterns. Note the marked eosinophilic infiltration (Patient 2).
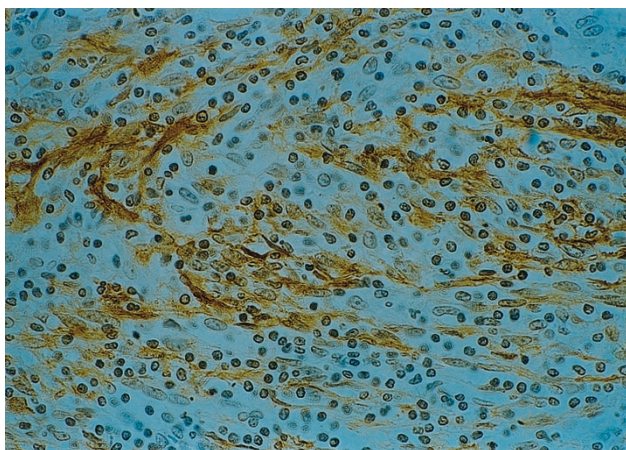

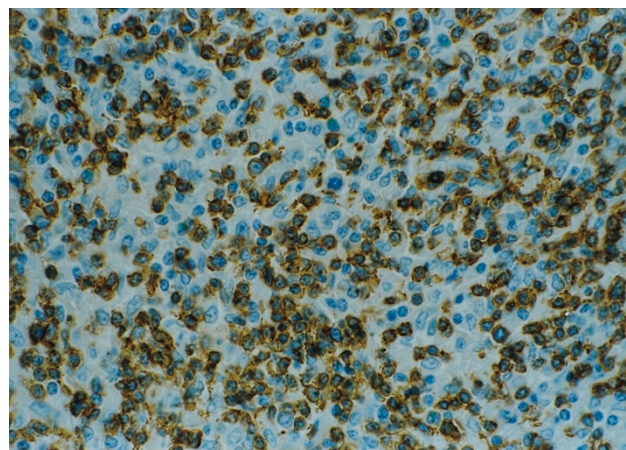**FIGURE 5.** The spindle cells were immunoreactive with CD21.

FIGURE 6. The small lymphocytes in the background were predominantly CD8 (+) cells.

nodes (6). It has also been reported in extranodal sites, including the palate (2), tonsils (5–7), pharynx (6), intraabdominal soft tissue (mesentery and mesocolon; 3, 4), ampulla of Vater (12), pancreas (7), spleen (8), and liver (10–12). To our knowledge, three cases have previously been reported with liver involvement (Table 1; 8–10). All patients were female, aged from 35 to 57 years. The presenting symptoms were nonspecific, including abdominal fullness, epigastralgia, low-grade fever, weight loss,

slight anemia, and hypergammaglobulinemia. Of these three reported cases, in two cases, the tumors were located in the right lobe, and in the third case, the left lobe. Tumor size usually measured more than 10 cm in greatest diameter. Over the past 3 years, we have encountered two cases in Taiwan. As reported previously, the hepatic tumors were bulky and occurred in older females. In both cases, the tumors were located in the left lobe. The first pa-
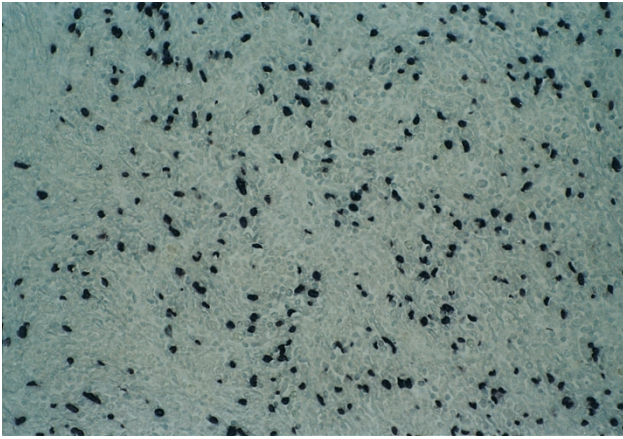
FIGURE 7. Positive Epstein-Barr virus–encoded nuclear RNA signal was confined to the nuclei of neoplastic follicular dendritic cells.
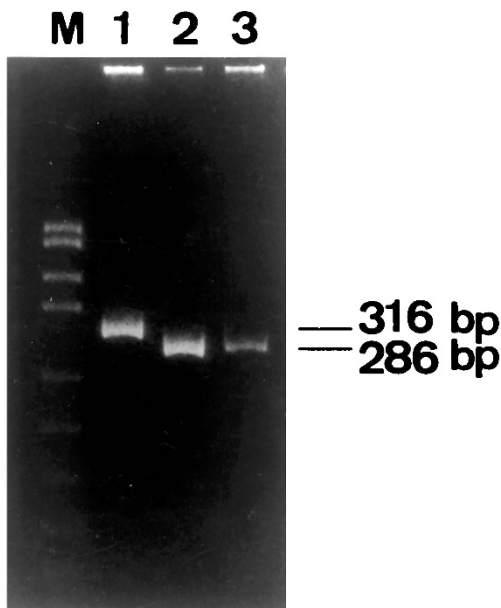

FIGURE 8. Analysis of PCR products from Exon 3 of the latent membrane protein-1 gene. DNA fragments of 316 bp and 286 bp were generated with the B95-8 strain (*Lane 1*) and plasmid pT7 (*Lane 2*). Case 2 (*Lane 3*) had a 286-bp product with a characteristic 30-bp deletion as compared with the B95-8 strain and was the same as that from plasmid pT7.

tient refused surgical resection, which gave us the opportunity to observe the natural course of a hepatic FDC tumor. Over a 3-year follow-up period, hypergammaglobulinemia occurred insidiously, with the level progressively increasing but remaining polyclonal. The patient then developed lower-limb leukocytoclastic vasculitis bilaterally with granular IgA and C_3 deposits. Marked eosinophilia was found in the second patient's peripheral blood. The inflammatory cells in FDC tumor also demonstrated marked eosinophilic infiltration in addition to small lymphocytes and plasma cells. The peripheral blood eosinophil count returned to within a normal range after resection of the liver tumor. To

our knowledge, these observations have not been reported before for patients with FDC tumors.

FDC tumors have been considered to have a malignant potential (7, 9). In a review article dealing with 47 well-documented cases of FDC tumors (9), 28 (59%) patients were alive without disease, 6 (13%) were alive with recurrent disease or metastasis, and 7 (14%) had died with recurrent disease at the time of last follow-up. Adverse clinical outcome was correlated with intraabdominal location, large tumor size (more than 6 cm), high mitotic count (more than 5 per 10 high-power field), coagulative necrosis, and cellular atypia. On the basis of our experience and three previously reported cases (10–12), FDC tumors of the liver might not be as aggressive as the reported intraabdominal cases because local recurrence only occurred in one of the five cases over 1- to 5-year follow-ups. Although the tumor in Case 1 of our series was progressively growing in the absence of surgical resection, no metastases were found over a 3-year period. Therefore, FDC tumors of the liver may be a low-grade malignancy.

The major differential diagnoses included Hodgkin's disease and inflammatory pseudotumor (9, 11). Because FDC tumor may contain some Reed-Sternberg–like cells, it may be confused with Hodgkin's disease, especially in needle biopsy specimens (11). It is also difficult to distinguish FDC tumor from inflammatory pseudotumor. In fact, inflammatory pseudotumor represents a family of lesions diverse in nature and etiology, including reparative lesions, infective lesions, myofibroblastic neoplasms, and FDC tumors (14). The diagnosis of FDC tumor should be based on the recognition of FDCs, which exhibit distinctive histologic features. However, a definite diagnosis requires confirmation with immunohistochemical studies. FDCs are immunoreactive with the antibodies CD21, CD35, CD23, R4/23, and KiM4 (9, 10, 14). Our cases were confirmed by immunoreactivity with CD21 and CD23. But they were unstained with CD35. The staining for CD35 is highly variable on formalin-fixed and paraffin-embedded tissue, even with microwave pretreatment (9, 10). This experience has been documented. The staining for muscle-specific actin (HHF-35) was also positive in our cases. The finding has been described in some FDC tumors by Chan *et al.* (7). It should not lead to misdiagnose other mesenchymal tumors. The small lymphocytes in the background were found to be composed predominantly of CD3(+) cells, among which CD8(+) cells outnumbered CD4 (+) cells. The plasma cells in our study were a mixture of λ(+) and κ(+) light-chain cells. These results are consistent with those of previous reports (10–12).

EBV has been detected in FDC tumor previously. This is not surprising because CD21 is the EBV

TABLE 2. Summary of Reported Cases of Follicular Dendritic Cell Tumors of the Liver

Case	Authors (Citation No.)	Age/Sex	Site/Size (cm)	Symptoms	EBV	Outcome	Remarks
1	Shek et al. (8)	35/F	R/20	Epigastric discomfort, low-grade fever, weight loss	+	Recurrent at 30 mo	
2	Selves et al. (9)	68/F	L/11	Malaise, weight loss, mild anemia	+	Alive and well at 2.5 y	
3	Shek et al. (10)	37/F	R/15	Malaise, weight loss	+	Alive and well at 2 y	
4	Chen et al. (this study)	57/F	L/9.5	Intermittent epigastralgia	+	Alive and well at 3 y	Leukocytoclastic vasculitis
5	Chen et al. (this study)	51/F	L/12	Abdominal fullness, weight loss	+	Alive and well at 1 y	Blood and tissue eosinophilia

EBV, Epstein-Barr virus; F, female; R, right; L, left.

receptor. Approximately 12% of all reported cases of FDC tumors are associated with EBV (9). The association of FDC tumor with EBV seems to vary in different organs. FDC tumors of the liver, including the cases from Hong Kong and France, were all identified as being EBV related (10–12). The role of EBV nevertheless remains unclear in the pathogenesis of FDC tumors. Southern blot analysis demonstrated that it is a clonal proliferative disease (10, 11), indicating that the EBV infection occurred before the monoclonal proliferation of the neoplastic FDC. LMP-1 is an integral membrane protein containing 386 amino acids and is encoded by the BNLF gene (also called LMP-1 gene) of EBV (15–17). LMP-1 is considered to be a viral oncogene because of its capacity to transform rodent fibroblasts *in vitro* and render them tumorigenic in nude mice (18). LMP-1 also serves as a target for T-cell–mediated cytotoxicity (19). Any mutations in the LMP-1 gene that result in failure to be recognized by T cells would allow the LMP-1 variant to escape immunologically mediated elimination (20). DNA sequencing of the LMP-1 gene in hepatic FDC tumor reported by Selves *et al.* showed three point mutations in Exon 3 of the LMP-1 gene as compared with the standard sequence of the EBV strain B95-8. In our series, Case 2 had a 30-bp deletion in Exon 3 of the LMP-1 gene, which was different from that reported by Selves *et al.* In addition, six point mutations were also present in Exon 3. The significance of the 30-bp deletion and six point mutations of the LMP-1 gene remains unclear. The deletion strain is prevalent in Taiwan, is not restricted to nasopharyngeal carcinoma, and was also found in throat washings of healthy individuals (21). Therefore, the clinicopathologic significance of the 30-bp deletion of Exon 3 of the LMP-1 gene in FDC tumors warrants further investigation.

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