Intrahepatic Cholangiocarcinoma with Lymphoepithelioma-Like Component

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We present two cases of intrahepatic cholangiocarcinoma with lymphoepithelioma-like component. The patients included one woman and one man, aged 67 and 41 years, respectively. They presented with right upper quadrant pain and epigastralgia. Histologically, both tumors showed two distinct histological patterns with dense lymphoplasma cell infiltration. The first pattern was a well to moderately differentiated adenocarcinoma; the second component showed a feature similar to lymphoepithelioma-like carcinoma. Granulomatous reaction was noted in one case. Immunohistochemical study revealed that both tumors were immunoreactive with AE1/AE3, cytokeratin 7, and cytokeratin 19 but negative for carcinoembryonic antigen and cytokeratin 20. The stromal lymphocytes were composed of predominantly CD3(+) T cells. *In situ* hybridization for Epstein-Barr virus (EBV)–encoded RNA (EBER) showed positive nuclear signal in tumor cells but not in inflammatory cells in one case. The presence or absence of EBV genome was confirmed by polymerase chain reaction of LMP-1 gene in both cases. The LMP-1 gene also had a 30-bp deletion in Exon 3 as compared with the products from B95–8 cells. We further sequenced the PCR product and confirmed a 30-bp deletion between Nucleotide (nt) 168,282 and nt 168,253 corresponding to the B95–8 sequence. The clinical significance of 30-bp deletion in Exon 3 of the LMP-1 gene in lymphoepithelioma-like carcinoma of the liver warrants further investigation.

KEY WORDS: Epstein-Barr virus, Latent membrane protein-1 gene, Liver, Lymphoepithelioma-like carcinoma.

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Intrahepatic cholangiocarcinoma is a malignant tumor arising from biliary epithelium. Histologically, it is characterized by sclerotic stroma with rare prominent lymphocytic infiltration. Recently, three cases of hepatic lymphoepithelioma-like carcinoma (LELC) were reported (1–3). Two of the reported cases have been shown to have Epstein-Barr virus (EBV) sequence (1, 2). In this report, we describe two additional cases of intrahepatic cholangiocarcinoma with lymphoepithelioma-like component with immunohistochemical and EBV study.

MATERIALS AND METHODS

Two cases of intrahepatic cholangiocarcinoma with lymphoepithelioma-like component were retrieved from the surgical pathology file of Chang Gung Memorial Hospital from 1999 to 2000. Formalin-fixed and paraffin-embedded tissues were used for histopathologic, immunohistochemical, and *in situ* hybridization studies. The immunohistochemical studies were performed by using avidin-biotin-peroxidase complex method. A panel of antibodies was used and listed in Table 1. EBV-encoded RNA (EBER) *in situ* hybridization studies were performed with 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 hours 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 was frozen in −80°C until they were used. High molecular DNA was purified by phenol/chloroform extraction and 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.* (4), were used. The amplification reaction mixture contained 10 mM Tris HCL (pH 7.5), 50 mM

TABLE 1. Antibodies Used in This Study

Antibody	Source	Dilution	Pretreatment
AE1/AE3	BioGenex	1:202	TP
CEA	DAKO	1:50	TP
CK7	BioGenex	1:50	PC
CK19	BioGenex	1:200	PC
CK20	BioGenex	1:50	PC
CD3	DAKO	1:800	TP
CD20	DAKO	1:1600	PC
CD21	DAKO	1:50	TP

CEA, carcinoembryonic antigen; CK, cytokeratin; PC, pressure cooking in EDTA for 2 minutes or in citrate buffer for 3 minutes; TP, trypsinization for 20 minutes.

sodium chloride, 10 mM magnesium chloride, 1.5 mM dNTP, two primers (0.2 μM each), and 2.5 units of *Taq* DNA polymerase. 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 preparations from B95–8 and pT7 strains were used as controls.

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

RESULTS

Clinical Histories

Case 1

A 67-year-old female patient presented with right upper quadrant pain for several months. Imaging studies, including abdominal ultrasound and angiography, revealed a mixed echoic and hypervascular tumor in Segment 8. The liver tumor measured 5 $\times$ 4.5 cm. Serum anti-hepatitis virus C (HCV) antibody was positive, but hepatitis B surface antigen was negative. Serum tumor markers, including α-fetoprotein and carcinoembryonic antigen, were within the normal range. A hepatectomy was performed. At operation, an enlarged lymph node was noted at the hepatoduodenal ligament. No tumor was detected in any other organs. The patient died of postoperative pancreatitis.

Case 2

A 41-year-old male patient, with a history of IgA nephropathy for 10 years, complained of epigastralgia that he reported having had for 2 weeks. He was also a hepatitis B virus carrier. Abdominal ultrasound examination revealed a 3 $\times$ 2–cm hypoechoic tumor in segment 2. Angiographic study showed it to be a hypervascular tumor. Serum α-fetoprotein was within the normal range. Serum anti-HCV antibody was negative. The liver nodule was resected. No evidence of recurrence or distant metastasis was found within an 8-month follow-up period.

Pathologic Findings

Both tumors were gray-white, rubbery, and nonencapsulated. Microscopically, both tumors shared similar histological features. They consisted of two distinct patterns and merged together. The first component consisted of irregular large and small glandular structures (Fig. 1A). The second pattern is composed of solid nests or irregular cords of large undifferentiated cells with vesicular nuclei, prominent nucleoli, and indistinct cell borders (Fig. 1B). This component was identical to LELC in morphology. Both components were associated with dense lymphoplasma cell infiltrations. Granulomatous reaction was also present with some multinucleated giant cells in Case 1 (Fig. 2). The group 12 lymph node taken from Case 1 was metastasized by both ordinary adenocarcinoma and lymphoepithelioma-like components. The background liver of Case 2 showed cirrhotic change without obvious activity.

The neoplastic cells from both ordinary adenocarcinoma and LELC component were strongly positive for AE1/A3. They were also immunoreactive with cytokeratin (CK) 7 and CK 19 (Fig. 3) but negative for carcinoembryonic antigen (CEA) and CK 20. The tumor cells were also not stained by CD21. The lymphoid stroma was composed of a mixture of CD3(+) and CD20(+) cells, with a predominance of CD3(+) T-cells.

EBER *In Situ* Hybridization

Case 1 showed positive nuclear EBER signals in neoplastic epithelial cells of both ordinary adenocarcinoma and LELC components (Fig. 4), whereas the inflammatory cells in the background were negative. In contrast, the neoplastic cells and the inflammatory cells in the background in Case 2 were all negative.

LMP-1 Gene Study

The presence or absence of EBV genome was confirmed by PCR of LMP-1 gene in both cases. In addition, the LMP-1 gene had a 30-bp deletion in Exon 3 as compared with the products from B95–8 cells (Fig. 5) in Case 1. We further sequenced the PCR product and confirmed a 30-bp deletion be-

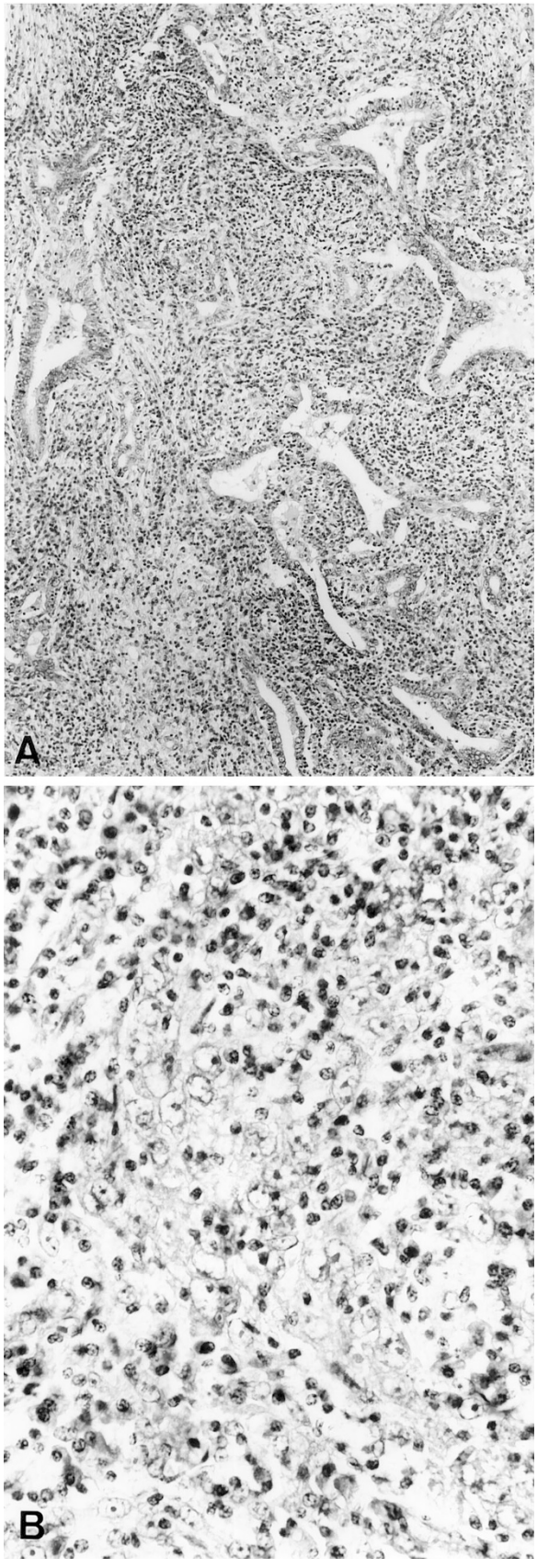

FIGURE 1. Both liver tumors showed large and small neoplastic glands (**A**) and lymphoepithelioma-like features (**B**) with dense infiltrates of plasma cells and lymphocytes.

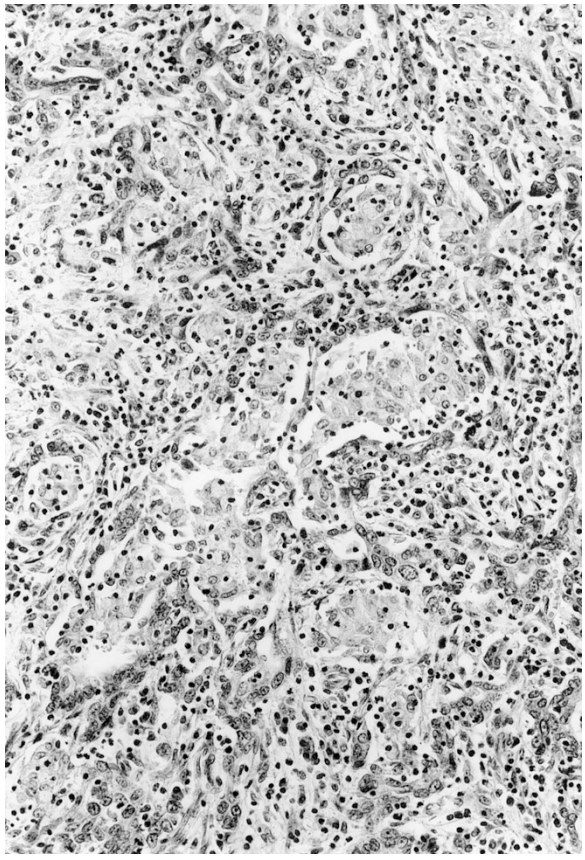

FIGURE 2. Granulomatous reaction was noted in Case 1.

tween Nucleotides 168,282 and 168,253 (corresponding to the B95–8 sequence). There were also five point mutations at positions 168320 (A→G), 168308 (T→C), 168295 (A→T), 168285 (C→G), and 168225 (T→A).

DISCUSSION

LELC is a tumor with morphological features identical to undifferentiated nasopharyngeal carcinoma that occurs outside the nasopharynx (5). The tumor has been reported in a variety of organs, including stomach (6), salivary gland (7, 8), lung (9), thymus (10, 11), skin (12), uterine cervix (13), trachea (14), ureter (15), urinary bladder (16), vagina (17), and vulva (18). To the best of our knowledge, only three cases of LELC have been reported in the liver. In the present report, we present two additional cases. The summary of the reported cases of hepatic LELC is listed in Table 2. The patients included three women and two men. Their ages ranged from 41 to 71 years. They presented with liver nodules, abdominal fullness, or epigastralgia. Three of the cases metastasized to lymph nodes in the portal region at the first operation or during the follow-up, and three of the five cases were demonstrated to have the presence of EBV. Of the five cases, one was a pure LELC, and the other four

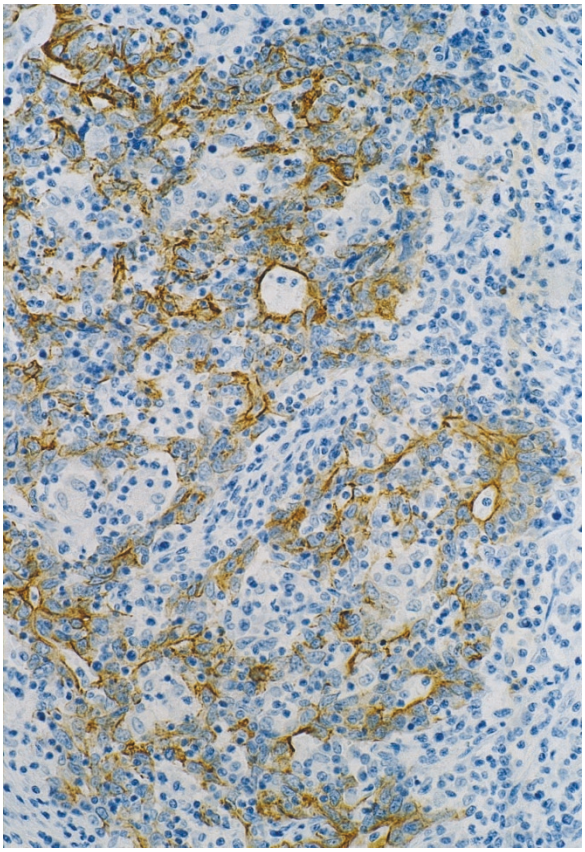

FIGURE 3. The tumor cells were immunoreactive with cytokeratin 19.

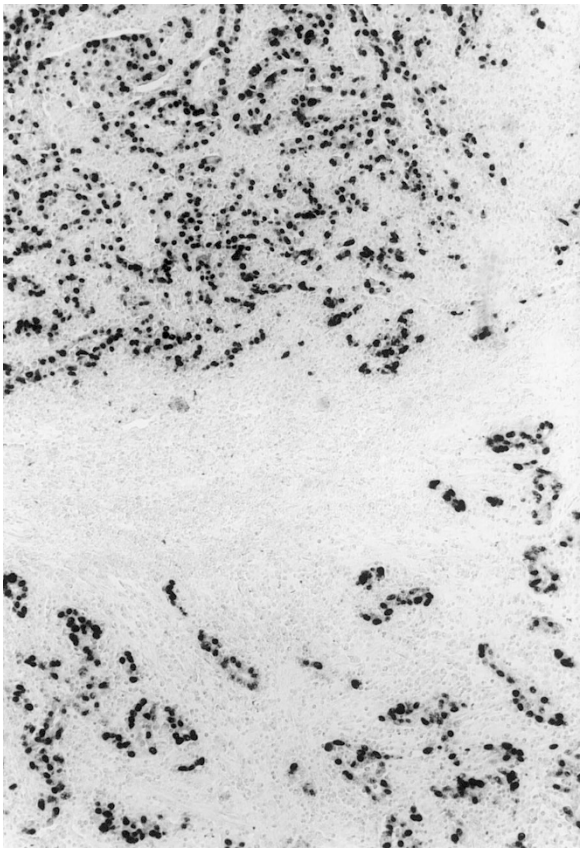

FIGURE 4. Positive nuclear EBER signal was seen in both ordinary adenocarcinoma and lymphoepithelioma-like carcinoma (Case 1). The stromal inflammatory cells were negative.

cases were mixed with ordinary adenocarcinoma. Both components merged together, indicating that the lymphoepithelioma-like component was transforming from the ordinary adenocarcinoma. In the present series, Case 1 also showed a marked granulomatous reaction. It was not uncommon in the LELC of other sites. But it had not been described in the LELC of the liver before.

Immunohistochemical study has been done on only 2 of the 3 previously reported hepatic LELCs (2, 3). The case reported by Vortmeyer *et al.* (2) was negative for CEA but positive for AE1/AE3 and EMA. The case reported by Kim *et al.* (3) was immunoreactive with CK 19 but negative for CEA and AE3. Although most cholangiocarcinomas are positive for CEA (19), our cases were not immunoreactive with CEA. They were immunoreactive with CK 7 and CK 19–9 but unstained by CK20. The immunophenotypes were compatible with cholangiocarcinoma (20). No tumor was also detected in any other sites. The stromal lymphoid cells were predominantly composed of CD3(+) T-cells. It was consistent with LELCs of other sites (5). The neoplastic cells were negative for CD21, which is the EBV receptor. The finding was identical to the previous report by Vortmeyer *et al.* (2).

EBV has been shown in LELC, especially in stomach (6), salivary glands (7, 8), lung (9), and thymus

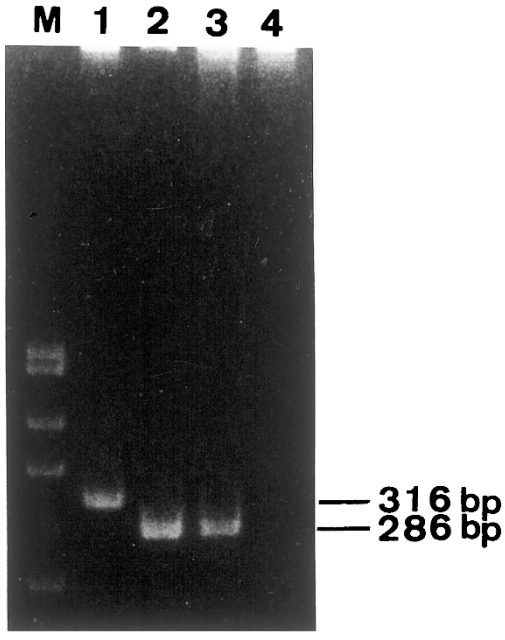

FIGURE 5. Analysis of polymerase chain reaction (PCR) products from Exon 3 of the LMP-1 gene. DNA fragments of 316 bp and 286 bp were generated with the B95–8 cells (*Lane 1*) and plasmid pT7 strain (*Lane 2*). Case 1 (*Lane 3*) had a 286-bp product, which was the same as that from plasmid pT7. But Case 2 (*Lane 4*) showed no PCR products.

TABLE 2. Summary of the Reported Cases of Lymphoepothelioma-like Carcinoma of the Liver

Case No.	Author(Ref. No.)	Age/Sex	Site/Size (cm)	Symptom	EBV	Outcome	Remarks
1	Hsu *et al.* (1)	47/F	L/10	Abdominal fullness mass	+	Lymph node, lung, rib, and spleen metastasis	Malaria infection
2	Vortmeyer *et al.* (2)	71/F	Porta hepatis/5	Liver nodule	+	Lymph node metastasis	
3	Kim *et al.* (3)	64/M	R/2	Liver nodule	−		
4	Chen *et al.* (this article)	67/F	R/5	Right upper quadrant pain	+	Lymph node metastasis	
5	Chen *et al.* (this article)	41/M	L/2.5	Epigastralgia	−	Alive and well at 8 months	

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

(10). Hepatic LELCs were also associated with EBV infection because two of the three previously reported intrahepatic cholangiocarcinoma with lymphoepithelioma-like component are associated with EBV in both ordinary adenocarcinoma and lymphoepithelioma-like components (1, 2). EBV was also identified in one of our cases. All EBV-related hepatic LELC occurred in women. But pure cholangiocarcinomas were not related to EBV infection (21). EBV has been long implicated in the pathogenesis of LELCs (5). Southern blot analysis has demonstrated that LELC of the liver is a clonal proliferative disease (1, 2), indicating that the EBV infection occurred before the monoclonal proliferation of the LELC. 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 (22–24). 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 (25). LMP-1 also serves as a target for T-cell–mediated cytotoxicity (26). Any mutations in the LMP-1 gene that result in failure of recognition by T cells would allow the LMP-1 variant to escape immunologically mediated elimination (27). DNA sequencing of the LMP-1 gene in hepatic LELC reported by Vortmeyer *et al.* (2) showed no deletion of the LMP-1 gene as compared with the standard sequence of the EBV strain B95–8. In our series, Case 1 had a 30-bp deletion in Exon 3 of the LMP-1 gene, which was different from that reported by Vortmeyer *et al.* (2). In addition, five point mutations were present in Exon 3. The significance of the 30-bp deletion and five 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 (28). Therefore, the clinicopathologic significance of the 30-bp deletion of Exon 3 of the LMP-1 gene in hepatic LELC warrants further investigation.

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