

Case Report

Primary mixed lymphoepithelioma-like carcinoma and intra-hepatic cholangiocarcinoma: a case report and review of literature

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Abstract: Primary lymphoepithelioma-like carcinomas (LELC) of the hepatobiliary tract are quite rare and the majority are associated with the Epstein-Barr virus (EBV). Here we report an unusual case of intrahepatic cholangiocarcinoma (ICC), admixed with LELC in a 63 year-old Filipino woman who presented clinically with right flank and back pain. Histologically, the tumor showed a dense lymphocytic infiltrate, predominantly composed of CD3 (+) T cells, and two components: an undifferentiated carcinoma, morphologically similar to nasopharyngeal carcinoma, and a poorly differentiated ICC intimately admixed. Immunohistochemical studies revealed that both components were immunoreactive for AE1/AE3, cytokeratin 7 and, focally, for monoclonal CEA. Both components were negative for cytokeratin 20 and HePar 1. EBER-1 in situ hybridization was uniformly positive in the tumor cells. The presence of EBV in ICC and LELC suggests that the virus may be linked to the pathogenesis of both components of the tumor. The mechanism of virus-driven neoplastic transformation needs further study.

Keywords: Liver, lymphoepithelioma-like carcinoma, cholangiocarcinoma, review

Introduction

Lymphoepithelioma-like carcinomas (LELC) are tumors composed of undifferentiated carcinoma with an intense lymphocytic infiltrate, morphologically reminiscent of undifferentiated nasopharyngeal carcinoma. These tumors have been reported in various anatomic sites such as the stomach, salivary gland, and thymus [1]. Undifferentiated nasopharyngeal carcinoma has been strongly linked to Epstein-Barr virus (EBV). Similarly, the majority of LELCs have also been reported to be associated with EBV [1]. The occurrence of LELC in the liver is rare. Only twelve cases of LELC arising in the hepatobiliary tract have been reported [2-9]. Most of these tumors were positive for EBV by EBER-1 in situ hybridization. In this report, we describe an unusual case of mixed intrahepatic lymphoepithelioma-like carcinoma (LELC) and cholangiocarcinoma (ICC) positive for EBV by in-situ hybridization. We also provide a review of the current literature on this extremely rare primary hepatic malignancy.

Case Report

A 63 year-old Filipino woman presented clinically with the chief complaint of right-sided flank and back pain. The patient had a PET scan performed that revealed a dominant lesion in the left lobe of the liver with increased metabolic activity. A liver core biopsy revealed a poorly differentiated LELC. Her past medical history was significant for sick sinus syndrome (requiring pacemaker placement), hypertension, atrial fibrillation, and diabetes mellitus type II. Serum tumor markers, including alpha-fetoprotein, carcinoembryonic antigen, CA 125 and CA 19-9, were all within reference range. Serum hepatitis viral markers were negative. A CT of the abdomen revealed a 3.8 x 3.3 cm hypodense mass in the medial segment of the left lobe of the liver, and a 1.4 x 1.6 cm hypodense lesion in the inferior segment of the right lobe of the liver. The lesion was resected and submitted for pathologic evaluation. There was no evidence of extrahepatic disease. The patient was discharged home on postoperative day 10.

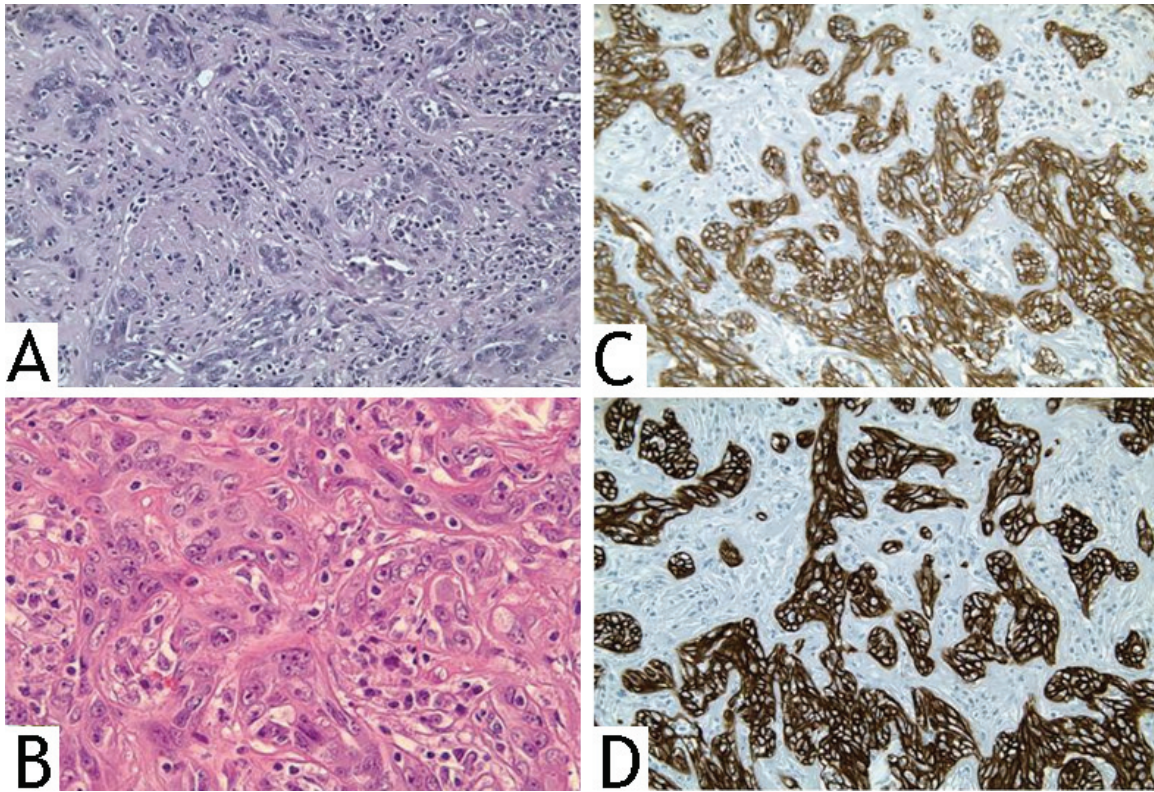


Figure 1. (A). The ICC component of the tumor is shown with the associated lymphoplasmacitic infiltrate (H&E X200). (B). The LELC component showing cohesive sheets and clusters of undifferentiated and large epithelial cells, with eosinophilic cytoplasm. The nuclei are ovoid and vesicular and exhibit nuclei prominent nucleoli (H&E X 400). (C). Diffuse cytoplasmic staining for cytokeratin AE1/AE3 in the LELC component (IHC X 200). (D). Diffuse cytoplasmic staining for CK7 in LELC component (IHC X 200).

She is doing well with no complications and no evidence of tumor, 6 months after her discharge from the hospital.

Pathologic Findings

A 16.5 x 8.5 x 5.0 cm segment of liver was resected. A 4.0 x 3.8 x 3.2 cm tan, well-defined, non-encapsulated solid mass was grossly identified, 0.2 cm from the nearest resection margin. Microscopically, the neoplasm was composed of two components: one characterized by a poorly differentiated adenocarcinoma composed of atypical glands infiltrating a desmoplastic stroma, and consistent with cholangiocarcinoma (**Figure 1A**); the second characterized by an undifferentiated carcinoma composed of large neoplastic epithelial cells showing an ill-defined syncytial arrangement. The tumor cells of the second component had eosinophilic cytoplasm and vesicular nuclei with prominent

nucleoli (**Figure 1B**). In some areas a dense lymphoid infiltrate, admixed with the tumor cells, was present (**Figure 2**). The two components

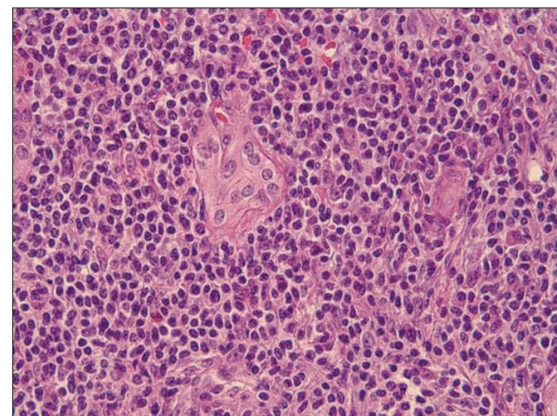


Figure 2. A high power view of a lymphocytic infiltrate within the LELC component. (H&E X400)

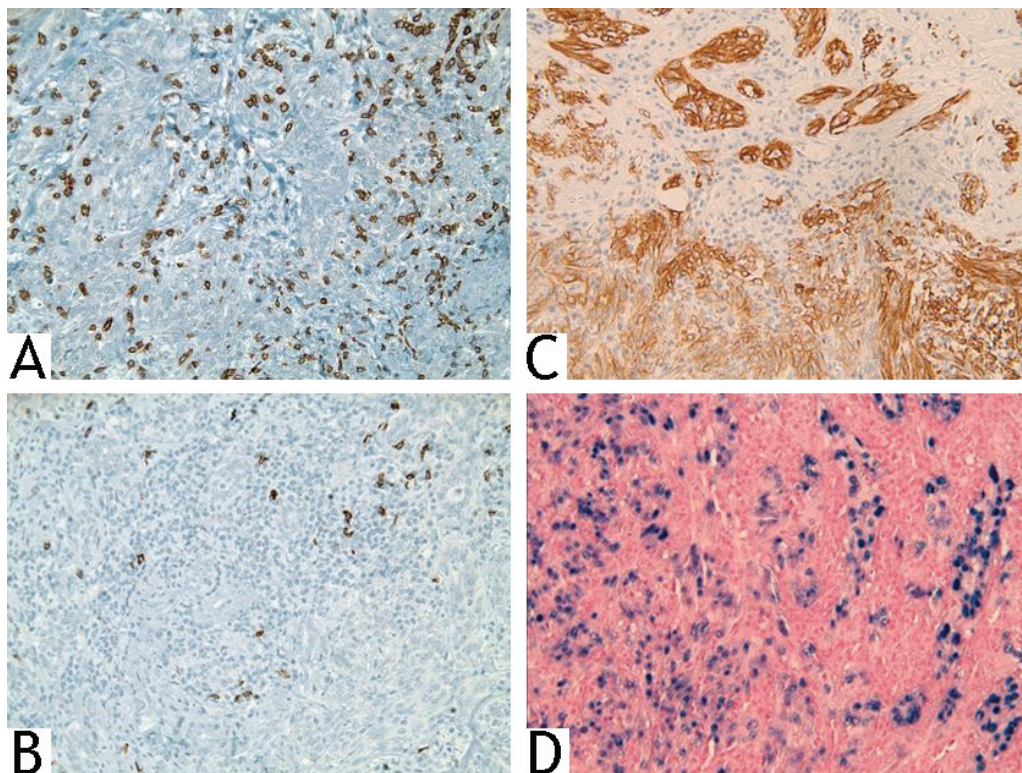


Figure 3. (A). The lymphocytic infiltrate intimately admixed with the ICC component showing a predominance of CD3+ T-lymphocytes (IHC X 200). (B). CD20+ B-lymphocytes were sparse in the lymphoepithelioma-like carcinoma (LELC) component (IHC X 200). (C). The cyokeratin 19 stain is stronger in the ICC component as compared to the LELC portion of the tumor (IHC X200). (D). Both LELC as well as the ICC component of the tumor exhibit positivity for Epstein Barr virus (EBV) by in situ-hybridization. The tumor cells reveal blue positivity, while the surrounding lymphoid cells are negative (ISH X400).

were intimately admixed. A mucicarmin stain showed intracellular mucin within the adenocarcinoma component. The non-neoplastic liver parenchyma did not show any evidence of cirrhosis.

Immunohistochemistry

Representative tumor sections, containing both components of the tumor, were immunostained for cyokeratin AE1/AE3, monoclonal carcinoembryonic antigen (mCEA), cyokeratin 7 (CK7), cyokeratin 20 (CK20), cyokeratin 19, HepPar 1, CD20, and CD3. Adequate positive and negative controls were included for each antibody. The neoplastic cells in both components were strongly immunoreactive for AE1/AE3 (**Figure 1C**) and CK7 (**Figure 1D**), and exhibited focal positivity for monoclonal CEA. They were negative for CK20 and HePar 1. The dense lymphocytic infiltrate consisted predominantly

of T-lymphocytes (CD3 positive) (**Figure 3A**), with rare CD20 positive B cells (**Figure 3B**). Cyokeratin 19, a marker for cholangiocarcinoma, stained strongly the glandular component, and weakly the LELC component (**Figure 3C**).

Epstein-Barr virus-encoded RNA-1 (EBER-1)

In situ hybridization for EBV-encoded RNA (EBER-1) revealed strong positivity in both cholangiocarcinoma and LELC components of the tumor (**Figure 3D**). The adjacent lymphoid cells were EBER-1 negative.

Discussion

In the present report, we describe a case of intrahepatic cholangiocarcinoma (ICC) with areas of LELC, exhibiting positivity for EBV-encoded RNA (EBER-1), by in-situ hybridization. The major differential diagnoses considered were me-

Primary mixed LELC and ICC carcinoma

Table 1. Primary mixed lymphoepithelioma-like carcinoma and intrahepatic cholangiocarcinoma reported in the English literature

Case no.	Author ref (no.)	Age/Sex	Site	Size (cm)	EBER-1	Clinical Outcome	Follow-up
1	Hsu (2)	47/F	Left lobe	10	+	Lymph node, lung, rib, and spleen metastasis 8 mo after surgery, died of disease	4 yrs
2	Vortmeyer (3)	71/F	Porta hepatis	5	+	Regional lymph node metastasis and local recurrence in 2 yrs, alive with disease	2 yrs
3	Huang (4)	61/F	Caudate lobe	3.5	+	Alive without disease	24 mo
4	Chen (5)	67/F	Right lobe	5	+	Lymph node metastasis, died of post-operative pancreatitis	Unk
5	Chen (5)	41/M	Left lobe	2.5	-	Alive without disease	8 mo
6	Chen (6)	56/M	Right lobe	3	-	Regional lymph node metastasis 5 mo after surgery, died of disease	21 mo
7	Jeng (7)	67/F	Left lobe	3	+	Alive without disease	7 mo
8	Jeng (7)	42/M	Right lobe	3	+	Alive without disease	7 yrs
9	Jeng (7)	50/M	Right lobe	4	+	Alive without disease	16 mo
10	Jeng (7)	50/F	Right lobe	4	+	Alive without disease	2 mo
11	Kim (8)	64/M	Right lobe	2	-	unk	unk
12	Ortiz (9)	19/F	Left lobe	5.5	+	Local recurrence in 2 yrs, died of disease	44 mo
13	Henderson (this report)	63/F	Left lobe	4	+	Alive without disease	6 mo

Abbreviations: unk: unknown; F: female; M: male; yrs: years; mo: months

tastatic undifferentiated nasopharyngeal carcinoma, lymphoepithelioma-like hepatocellular carcinoma, and metastatic LELC from other body sites.

In this case, a thorough clinical and radiological

patient evaluation showed no extrahepatic primary tumors, supporting the hepatic origin of this tumor. Furthermore, the tumor negativity for HepPar1, and the normal serum AFP level, mitigated against the diagnosis of lymphoepithelioma-like hepatocellular carcinoma.

To the best of our knowledge, twelve cases of primary mixed ICC with LELC involving the liver have been reported. A summary of the reported cases of hepatobiliary LELCs is listed in **Table 1** [2-9]. In the reported cases, the patients presented with a variety of symptoms such as abdominal fullness, presence of an epigastric mass, or vague epigastric pain. Most of the hepatobiliary masses were found incidentally by sonography. The patients included eight women and five men. Their ages ranged from 19 to 71 years (mean 54; median 56). Grossly, the tumors were described as being well-demarcated, gray-white and firm [2, 4-9]. Six of the 13 cases reported that the tumors were non-encapsulated [2, 4-5, 8-9]. Tumor size ranged from 2 to 10 cm. In four patients the tumor was metastatic to regional lymph nodes at presentation or became metastatic during follow-up [2-3, 5-6]. Two of the patients with node metastases (50%) experienced recurrent liver disease within two years and both patients died of their disease.

As in our study, the reported cases showed histologically two architectural patterns: 1) The ill-defined, syncytial pattern infiltrated by small lymphocytes and 2) The ductular pattern. Cytologically the tumors revealed features similar to those described in our case, such as irregular vesicular nuclei with prominent nucleoli and indistinct cellular borders. The feature common to all of the reported cases was a background of dense lymphocytic infiltrate. Lymphoid follicles may be present. The desmoplastic stromal response is usually found within the ICC component of the tumor. Occasionally, a granulomatous reaction with multinucleated giant cells has also been described [5].

Similar to the previously reported tumors [3-5, 7], the immunohistochemical study in this case demonstrated strong positivity for CK7 and AE1/AE3 in the tumor cells that were negative for CK20. Focal mCEA positivity was present in our case. The tumor cells in four of the previously reported cases were negative for CEA. One unique case of hepatocellular carcinoma (HCC) with lymphoepithelioma-like features was reported [6]. The HCC diagnosis was supported by positive immunoreactivity for HePar 1, an immuno-histochemical marker specific for hepatocellular differentiation. Furthermore, that case was negative for EBV.

Nine of the 13 reported tumors (69%), including

the present case, were positive for EBV by EBER-1 in situ hybridization. It has been proposed that EBV may play a role in the tumorigenesis of ICC with LELC. EBV infected ductal epithelium may undergo transformation into cholangiocarcinoma and the viral genome may be sustained in the undifferentiated carcinoma component [1]. Clonal proliferation has been demonstrated in LELC of the liver [2-3], raising the possibility that EBV infection may have occurred before malignant transformation [1,9]. Interestingly two of the 3 patients with negative EBV had chronic viral hepatitis C and one had chronic viral hepatitis B. These patients had liver cirrhosis in the background. The development of LELC in these patients was attributed to the hepatitis virus C and/or B. In our patient the hepatitis viral profile was negative.

The mechanism of viral carcinogenesis for biliary epithelial cells and for hepatocytes, and the molecular mechanism associated with the nasopharyngeal carcinoma-like morphology warrants further study. Detection of EBV may be significant in the diagnosis and prognosis of these tumor types.

The prognosis of ICC with LELC is difficult to assess due to a limited number of cases reported to date and to the short and inconsistent follow-up periods. Seven out of thirteen (54%) patients are alive without disease. Lymph node metastasis and recurrent tumor appeared to be associated with a poor outcome. Careful long-term follow-up of additional cases is warranted to determine the disease course.

There is no consensus on standardized treatment strategy for primary mixed ICC/LELC. Considering the reported cases, the patients underwent surgical resection of their tumors. Post-operative chemotherapy and radiation were given to two of the patients [3, 6] one of whom was alive with local recurrence and no distant metastases 2 years after treatment, while the other succumbed to her malignancy 21 months after receiving chemoradiation.

In summary, this report describes an unusual and rare case of primary mixed ICC with LELC that revealed the presence of EBV encoded RNA-1 in the tumor cells. When faced with such a difficult diagnostic challenge, thorough clinical and radiologic testing is required to rule out the possibility of a metastatic LELC to a liver harboring a primary intra-hepatic cholangiocarcinoma.

A regular post-operative follow-up of such patients will be important for early detection of recurrent/metastatic disease and to evaluate the efficacy of the therapeutic modalities offered. Furthermore, studies are needed to determine the exact role of EBV in the genesis of this morphologically distinctive neoplasm.

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