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Original contribution

Proliferative index facilitates distinction between benign biliary lesions and intrahepatic cholangiocarcinoma☆,☆☆

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Summary Differentiation between benign and malignant lesions of the hepatic biliary tree may pose a diagnostic problem because well-differentiated intrahepatic cholangiocarcinoma may mimic biliary hamartoma, bile duct adenoma, or parenchymal extinction. We evaluated Ki-67 proliferative index and p53 status by immunohistochemical staining to aid in exclusion of cholangiocarcinoma. Fourteen biliary hamartomas, 21 bile duct adenomas, and 11 livers with parenchymal extinction were compared with 26 intrahepatic cholangiocarcinomas (16 well-differentiated and 10 moderately or poorly differentiated tumors). We found an increased proliferative index in intrahepatic cholangiocarcinomas compared with benign biliary lesions (average 23.0% in cholangiocarcinoma versus 1.4% in all benign biliary lesions, n = 26 versus n = 46, $P < .001$). No difference in average proliferative index was observed between well-differentiated and moderately/poorly differentiated cholangiocarcinomas (average 22.7% versus 23.3%, n = 16 versus n = 10, $P = .92$). Average proliferation indices of benign biliary lesions were uniformly low (biliary hamartoma, 1.2%; bile duct adenoma, 2%; parenchymal extinction, 0.5%). Most cholangiocarcinomas (23/26; 88.5%), but none of the benign lesions (0/46; 0%), had proliferative indices greater than 10%. Strong nuclear p53 immunohistochemical staining was only seen in cholangiocarcinomas (9/26; 34.6%) and not in benign biliary lesions (0/46; 0%), although many of the benign lesions showed weak to moderate staining. Immunohistochemical staining for Ki-67 facilitates distinction between benign and malignant lesions of the intrahepatic biliary tree, whereas p53 immunohistochemical staining is less helpful.

1. Introduction

Cholangiocarcinomas represent approximately 3% of all gastrointestinal tumors worldwide and 15% to 20% of primary liver malignancies [1-3]. Diagnosis of cholangiocarcinoma confers a dismal prognosis, even at an early stage, with 5-year survival of less than 5% [2,3]. The incidence of cholangiocarcinoma,

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particularly intrahepatic cholangiocarcinoma, has increased in the United States, as has its consequent mortality [3]. Benign biliary lesions may show overlapping morphologic features with small or early intrahepatic cholangiocarcinoma. Here we describe use of immunohistochemical staining for Ki-67 and, to a limited extent, p53 in distinguishing between intrahepatic cholangiocarcinoma and its benign mimics.

2. Materials and methods

2.1. Case selection

After approval by the University of California, San Francisco, Institutional Committee on Human Research, the liver specimens of 14 patients with biliary hamartomas (3 core biopsies, 2 wedge biopsies, 9 resections), 21 patients with bile duct adenomas (1 core biopsy, 19 resections, 1 autopsy), 11 patients with parenchymal extinction (5 core biopsies, 6 resections), and 26 patients with intrahepatic cholangiocarcinoma (3 core biopsies, 1 wedge biopsy, 22 resections) were retrieved from the files at the Department of Pathology, University of California, San Francisco (Table 1). All histologic slides of all patients were reviewed by R. M. G. and C. G. T. The diagnoses were confirmed according to most recent World Health Organization criteria. Demographic and follow-up data were extracted from the clinical records when possible.

2.2. Immunohistochemistry

All formalin-fixed, paraffin-embedded tissue samples were routinely processed, and serial sections from representative paraffin blocks were used for hematoxylin-eosin staining and immunohistochemistry. Immunohistochemical analysis was performed using previously described techniques [4]. Briefly, 4-μm paraffin-embedded sections were heat-treated; deparaffinized; heated in citrate buffer; blocked for endogenous peroxidase, avidin, and biotin; and incubated with antibodies for either Ki-67 (Dako, Carpinteria, CA; clone MIB-1, 1:50) or p53 (Vector, Burlingame, CA; clone DO7, 1:100). Sections were subsequently washed and developed with the Vector Labs ABC kit. For all antibodies, the number of immunopositive and immunonegative cells was manually counted in a 1000-cell count (focusing on regions with the most positive cells), when available. All lesional cells were counted in cases with less than 1000 available lesional cells. For Ki-67, any degree of nuclear staining was scored as positive. For benign biliary proliferations and cholangiocarcinomas, the number of cases in which greater than 5% and greater than 10% of cells stained positive were tabulated for comparison (Table 2). For p53, nuclear staining was scored as positive, and the intensity of staining was further graded as weak (1+), moderate (2+), or strong (3+). For benign biliary proliferations and cholangiocarcinomas, the number of cases with greater than 1% strong nuclear staining is tabulated for comparison (Table 3). Appropriate positive controls for Ki-67 and p53 were performed. Slides were scored by C. G. T. and reviewed by R. M. G. to reach consensus.

2.3. Statistical analysis

Statistical differences between groups were analyzed using Student *t* test and analysis of variance.

3. Results

3.1. Study populations

The study population demographics and pathologic features of the cases are summarized in Table 1. There was no significant difference in age of patients diagnosed as having benign biliary lesions compared with cholangiocarcinoma ($P = .30$; Table 1). The mean age was 59 years (range, 41-80 years) for biliary hamartoma, 55 years (range, 28-75 years) for bile duct adenoma, 55 years (range, 48-65 years) for parenchymal extinction, and 62 years (range, 38-85 years) for cholangiocarcinoma. Forty-three percent of the biliary

Table 1 Demographics of studied cases

	No. of cases	Sex (% male)	Average age (y)	*vs* WD CC (*P*)	Size (range)[a]	*vs* WD CC (*P*)
Biliary hamartoma	14	42.9	58.9	.90	0.2 (0.1-0.4)	<.001
Bile duct adenoma	21	52.4	55.2	.31	0.5 (0.1-1.8)	<.001
Parenchymal extinction	11	63.6	54.7	.18	0.2 (0.15-1.8)	<.001
All benign lesions	46	52.2	56.2	.30	0.4 (0.1-1.8)	<.001
WD cholangiocarcinoma	16	37.5	59.4	n/a	4.3 (0.9-9)	n/a
MD/PD cholangiocarcinoma	10	50	66.5	.13	6.7 (0.9-9.8)	.08
All cholangiocarcinomas	26	42.3	62.2	.61	5.3 (0.9-9.8)	.45

Abbreviations: n/a, not applicable; WD, well-differentiated; CC, cholangiocarcinoma; MD/PD, moderately/poorly differentiated cholangiocarcinomas.

[a] Sizes (cm) were available for 14, 19, 5, 13, and 9 of the biliary hamartomas, bile duct adenomas, parenchymal extinctions, WD cholangiocarcinomas, and MD/PD, respectively.