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Original contribution

Diagnostic value of maspin in distinguishing adenocarcinoma from benign biliary epithelium on endoscopic bile duct biopsy☆,☆☆

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Summary Histopathologic distinction between benign and malignant epithelia on endoscopic bile duct biopsy can be extremely challenging due to small sample size, crush artifact, and a propensity for marked inflammatory and reactive changes after stent placement. Our previous studies have shown that the insulin-like growth factor II mRNA-binding protein 3, S100P, and the von Hippel–Lindau gene product (pVHL) can help the distinction. This study analyzed 134 endoscopic bile duct biopsy specimens (adenocarcinoma 45, atypical 31, and benign 58) by immunohistochemistry for the expression of maspin, a serine protease inhibitor. The results demonstrated that (1) maspin expression was more frequently detected in malignant than in benign biopsies; (2) malignant biopsies frequently showed diffuse, strong/intermediate, and combined nuclear/cytoplasmic staining patterns for maspin, which were much less commonly seen in benign biopsies; (3) the malignant staining patterns for maspin observed in atypical biopsies were consistent with follow-up data showing that 67% of these patients were subsequently diagnosed with adenocarcinoma; (4) a maspin+/S100P+/pVHL− staining profile was seen in 75% of malignant biopsies but in none of the benign cases. These observations demonstrate that maspin is a useful addition to the diagnostic immunohistochemical panel (S100P, pVHL, and insulin-like growth factor II mRNA-binding protein 3) to help distinguish malignant from benign epithelia on challenging bile duct biopsies.

1. Introduction

Cholangiocarcinoma, either intrahepatic or extrahepatic, is a highly aggressive malignancy with an average 5% to

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10% 5-year survival rate [1]. Histopathologic examination of an endoscopic bile duct biopsy is the criterion standard for diagnosing extrahepatic cholangiocarcinoma (adenocarcinoma of the bile duct). However, bile duct biopsy frequently consists of a small tissue sample often with crush artifact and/or marked inflammatory/reactive changes particularly when there is a history of stent placement for biliary stricture. Histopathologic distinction between benign and malignant bile duct epithelial lesions can be extremely challenging on those biopsies.

Maspin, mammary serine protease inhibitor, has been shown to participate in the regulation of cell adhesion and apoptosis and the inhibition of cell invasion, metastasis, and angiogenesis [2-4]. Maspin may function differently in different tumor types because both overexpression and down-regulation have been reported in cacners of various organs [5-15]. A few studies have also shown that maspin expression is a useful prognostic factor for cancers [7,8,16-18]. Recently, the biological functions of maspin have been linked to its subcellular localization [3,4]. There are only a few publications that have examined maspin expression in biliary cancers [10,19-23]. However, these studies primarily focused on the gallbladder with resection specimens.

We have previously shown that an immunohistochemical panel comprising insulin-like growth factor II mRNA-binding protein 3 (IMP3), S100P, and the von Hippel–Lindau gene product (pVHL) is valuable in helping the detection of adenocarcinoma of the bile duct on biopsy specimens, which frequently shows an IMP3+/S100P+/pVHL− staining profile. In contrast, benign/reactive biliary epithelium frequently exhibits an IMP3−/S100P−/pVHL+ staining pattern [24,25]. The same malignant staining profile was also demonstrated in pancreatic ductal carcinoma and gallbladder carcinoma [9,10,26]. In the present study, we examined the expression of maspin by immunohistochemistry on endoscopic bile duct biopsy specimens to determine its usefulness in the distinction between adenocarcinoma and benign biliary epithelium.

2. Materials and methods

2.1. Cases

A total of 134 endoscopic bile duct biopsy specimens were selected from authors' institutions, which were divided into 3 groups. Group 1 comprised 45 malignant cases where a definitive diagnosis of adenocarcinoma was rendered. Group 2 comprised 31 atypical cases where a diagnosis of adenocarcinoma or reactive/inflammatory atypia could not be definitively made. Group 3 comprised 58 benign cases where the diagnosis of adenocarcinoma was completely excluded. Hematoxylin and eosin–stained slides, pathology reports, endoscopic reports, operative notes, and other pertinent medical records for each case were reviewed to confirm the original diagnoses and to determine the follow-up results for those who underwent repeat biopsy and/or surgical resection. The ages of the patients with a malignant diagnosis ranged from 51 to 95 years (mean, 72.2 years; median, 73.0 years). Twenty-seven patients were male and 18 were female. The ages of the patients with an atypical diagnosis ranged from 40 to 96 years (mean, 68.4 years; median, 72.0 years). Thirteen patients were male and 18 were female. The ages of the patients with a benign diagnosis ranged from 23 to 94 years (mean, 60.0 years; median, 61.0 years). Thirty-four patients were male and 24 were female. The study was approved by the institutional review boards at authors' institutions.

2.2. Immunohistochemistry

Immunohistochemical staining for maspin, S100P, IMP3, and pVHL was performed following previously published protocols using a Dako (Dako North America, Carpinteria, CA) autostaining system with a standard EnVision-HRP detection kit [21]. For maspin immunostaining, specifically, deparaffinized tissue sections were incubated in 3% hydrogen peroxide for 5 minutes to quench endogenous tissue peroxidase, followed by antigen retrieval with EDTA (pH 8.0) for 15 minutes at 100°C. The sections were then incubated for 40 minutes at room temperature with a mouse monoclonal antibody specific for maspin (G167-70) obtained from Becton Dickinson Immunocytometry Systems (BD Biosciences, San Jose, CA), at a dilution of 1:200. Positive controls included a pancreatic ductal adenocarcinoma, a large cell neuroendocrine carcinoma of the lung, normal placental tissue and normal kidney tissue for maspin, IMP3, S100P, and pVHL, respectively. For the negative controls, the primary antibodies were replaced with nonhuman-reactive mouse or rabbit serum.

2.3. Evaluation of immunohistochemical stains

Although we arbitrarily set 1% as the cutoff for positive immunostains as we did in our previous studies [24,25], positive cases always showed greater than 10% of the cells of interest exhibiting immunoreactivity. Positive stains were graded as weak, intermediate, or strong for staining intensity (using positive controls as references) and as focal if 1% to 50% of the cells stained, or diffuse if greater than 50% of the cells stained. Nuclear or combined nuclear/cytoplasmic staining was considered positive for maspin and S100P, and the stain was considered negative for these 2 markers if only cytoplasmic positivity was detected. Cytoplasmic staining was considered positive for IMP3. Membranous and cytoplasmic staining was considered positive for pVHL. Immunohistochemical stains were independently evaluated by 2 observers. Cases with significantly discrepant interpretation were resolved by review with a third observer. Consensus data were used for statistical analysis.