Human Pathology (2011) 42, 1454–1458

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Human PATHOLOGY

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Original contribution

Intestinal phenotypes in pediatric gallbladder epithelium ☆

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Received 4 November 2010; revised 8 December 2010; accepted 10 December 2010

Keywords:
Intestinal metaplasia;
Biliary epithelium;
Dysplasia;
MUC2;
Goblet cell

Summary The aim of this study was to characterize the physiologic expression of "intestinal" features in gallbladders of infants and children. The study group consisted of 56 pediatric (age, 2 weeks to 7 years) and 15 adult (15-25 years) patients who underwent incidental cholecystectomy during surgery for other lesions. All gallbladders examined were histologically unremarkable without inflammation, gallstones, or neoplasia. The presence of goblet cells and the expression of cytokeratin 7, cytokeratin 20, mucin core protein 2, and caudal-related homeobox protein 2 were examined. Intestinal features were frequently detected in the pediatric gallbladders: goblet cells in 34 cases (61%), cytokeratin 20 expression in 25 (45%), mucin core protein 2 expression in 32 (57%), and caudal-related homeobox protein 2 expression in 16 (29%). In contrast, none of these features was identified in adult gallbladders. The expression of mucin core protein 2 was mostly restricted to goblet cells in pediatric gallbladders, whereas cytokeratin 20 and caudal-related homeobox protein 2 were expressed in both goblet and nongoblet cells. Cytokeratin 7 was diffusely and consistently expressed in both pediatric and adult gallbladder epithelium including goblet cells. Intestinal features became less frequent with age and were scarce in children aged 6 to 7 years. Thus, goblet cells were identified in 14 (93%) of 15 children aged <1 year, together with the common expression of cytokeratin 20 (73%), mucin core protein 2 (93%), and caudal-related homeobox protein 2 (53%). In conclusion, intestinal features are physiologically present in gallbladder epithelium of children, particularly those aged <6 years. Intestinal metaplasia, as associated with cholangiopathy or carcinogenesis in adult patients, may represent an immature phenotype of biliary epithelium.

1. Introduction

The biliary epithelium lining large bile ducts and the gallbladder consists of columnar cells with consistent expression of cytokeratin (CK) 7 and CK19. Intestinal markers such as CK20, mucin core protein 2 (MUC2), and caudal-related homeobox protein 2 (CDX2) are normally absent from the biliary epithelium of adults, although cholangiocytes have a capacity for intestinal metaplasia particularly during prolonged inflammation or neoplastic transformation, as evidenced by the presence of goblet cells or aberrant expression of intestinal-type molecules [1-4]. *Intestinal metaplasia* is defined as the replacement of one differentiated cell type by another differentiated intestinal cell type.

Previously, we have identified goblet cells in otherwise unremarkable pediatric gallbladders, suggesting the possibility that such "intestinal" features may be physiologically

☆ Financial support: nothing to declare.
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doi:10.1016/j.humpath.2010.12.012

present in pediatric biliary epithelium. We know of no attempts to examine this possibility systematically, although one earlier article described goblet cells in gallbladders of newborn infants [5]. Interestingly, it is documented that fetal gallbladder epithelium contains goblet cells, which appear at 4 months of gestation [6,7].

In this study, we attempted to characterize the extent and prevalence of intestinal features in otherwise normal gallbladder epithelium of infants and children.

2. Patients and methods

2.1. Patients

Fifty-six patients aged 2 weeks to 7 years with histologically unremarkable gallbladders were identified from histopathology files for the period January 1997 to August 2006. Four preterm infants born at 34 to 36 gestational weeks were included in this study. They had cholecystectomy at the age of 2, 5, 11, and 35 weeks. Forty-five patients underwent cholecystectomy as a part of liver transplantation for genetic or metabolic disorders (n = 30), acute/subacute liver failure (n = 12), Budd-Chiari syndrome (n = 1), cryptogenic cirrhosis (n = 1), or Wilson disease (n = 1) (Table 1). Eleven patients underwent a cholecystectomy at surgery for focal nodular hyperplasia (n = 2), mesenchymal hamartoma (n = 1), hepatic artery ligation for vascular malformation or tumor (n = 3), pancreatitis (n = 2), trauma (n = 1), and liver surgical biopsy (n = 2). Fifteen patients, aged 15 to 25 years, transplanted for acute liver failure were used as controls. Patients who had a large duct cholangiopathy such as extrahepatic biliary atresia, choledochal cyst, or sclerosing cholangitis or who had had chemotherapy before surgery (eg, for hepatoblastoma) were excluded. All gallbladders were, therefore, histologically normal without conspicuous inflammatory cell infiltration or gallstones.

Table 1 Original diseases of patients examined in this study

Liver transplantation	n
Acute/subacute liver failure	12
Alagille syndrome	3
α 1-Antitrypsin deficiency	3
Budd-Chiari syndrome	1
Crigler-Najjar syndrome	2
Cryptogenic cirrhosis	1
Factor VII deficiency	3
Glucose-6-phosphate dehydrogenase deficiency	1
Glycogen storage disorder, type IV	1
Mitochondrial disorder	2
Neonatal hemochromatosis	2
Ornithine transcarbamylase deficiency	1
Primary hyperoxaluria, type 1	1
Progressive familial intrahepatic cholestasis	10
Propionic acidaemia	1
Wilson disease	1
Partial liver resection with cholecystectomy	
Focal nodular hyperplasia	2
Mesenchymal hamartoma	1
Cholecystectomy	
Cholecystectomy during arterial ligation [a]	3
Cholecystectomy during nonhepatobiliary surgery [b]	3
Cholecystectomy during surgical liver biopsy [c]	2

[a] Arterial ligation for arteriovenous malformation (n = 1) and performed before chemotherapy for epithelioid hemangioendothelioma (n = 2).
[b] Pancreatitis (n = 2) and trauma (n = 1).
[c] Progressive familial intrahepatic cholestasis (n = 1) and autoimmune hepatitis (n = 1).

2.2. Pathologic examination

Gallbladder tissue samples were taken from the body and the neck to the cystic duct in each case. One original section stained with hematoxylin and eosin (H&E) was reviewed for each case. Sections 4-μm thick were cut from formalin-fixed and paraffin-embedded specimens and used for H&E staining and immunohistochemistry. The presence or absence of goblet cells was examined on H&E-stained sections. Immunostaining for CK7, CK20, MUC2, and CDX2 was performed with an autostainer (Leica BOND-MAX, Leica Microsystems, Milton Keynes, United Kingdom) as per the manufacturer's instructions. The primary antibodies used were mouse monoclonal antibodies against CK7 (clone OV-TL 12/30; 1:200; Dako Cytomation, Glostrup, Denmark), CK20 (clone Ks20.8; 1:100; Dako Cytomation), MUC2 (clone Ccp58; 1:100; Novocastra Laboratories, Newcastle, United Kingdom), and CDX2 (clone AMT28; 1:100; Novocastra Laboratories). Sections were pretreated with a heated plate and a pH 9.0 buffer. Specimens of livers (for CK7) and colons (for CK20, MUC2, and CDX2) were used as positive controls.

2.3. Statistical analysis

Statistical analyses were performed using the χ^2 test. $P < .05$ was considered significant.

3. Results

3.1. Presence of goblet cells

Goblet cells were noted in 34 (61%) of 56 pediatric gallbladders, compared with none in the adult group ($P < .001$). Goblet cells were scattered or focally accumulated (Fig. 1) and present in the surface epithelium but not in the mucinous glands of the neck. Goblet cells were similarly identified in sections from the body and the neck to the cystic duct. Nongoblet cells had a tall columnar epithelium, which did not differ morphologically between