Human Pathology (2015) **46**, 1633–1639

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Human PATHOLOGY

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Original contribution

Immunohistochemical characterization of the regenerative compartment in biliary atresia: a comparison between Kasai procedure and transplant cases☆

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Received 10 April 2015; revised 22 June 2015; accepted 1 July 2015

Keywords:
Biliary atresia;
Ductular reaction;
Kasai procedure;
Liver transplantation;
Hepatic progenitor cell

Summary Biliary atresia (BA) accounts for most cases of pathologic infantile jaundice, which may lead to cirrhosis and eventually necessitate liver transplantation (LT). A cardinal histologic feature of BA is ductular reaction (DR), reflecting activation of the regenerative compartment in liver. We examined the immunohistochemical attributes of the progenitor cell population and its immediate descendants in BA patients undergoing Kasai procedure (KP) or LT. The BA cases were divided into those undergoing KP (n = 24) and those undergoing LT (n = 64). Immunohistochemistry for CD56, CK7, and CK19 was performed. Patients with BA (both KP and LT groups) had more DR than controls (scores 2.4, 2.2, and 0.1, respectively; $P < .001$), but the degree of DR did not differ between BA patients undergoing KP compared to LT. There was significantly more CD56 staining in DR in LT (2.5) versus KP samples (1.3; $P < .001$), with a trend toward the same pattern in hepatocyte progenitor cells in these samples (0.6 versus 0.2; $P = .05$). In intermediate hepatocytes, CK7 staining was higher in LT versus KP samples (1.7 versus 0.3; $P < .001$). No differences in CK19 staining were noted in the cell types in different BA groups. Immunohistochemistry suggests that the regenerative compartment is expanded in patients undergoing LT for BA, compared to patients with an earlier stage of disease undergoing KP. These observations support the notion that there is more active regeneration in livers with advanced-stage BA and highlight the immunophenotypic heterogeneity of progenitor cells in different phases of the disease.

☆ Disclosures: The authors have no conflicts of interest or funding to disclose.

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http://dx.doi.org/10.1016/j.humpath.2015.07.003
0046-8177/

1. Introduction

Biliary atresia (BA) is an infantile disorder characterized by the complete obstruction or malformation of a portion or the entirety of the extrahepatic biliary ducts [1]. Early diagnosis is critical to patient management, as surgical intervention with a portoenterostomy (Kasai procedure [KP]) can slow the progression of disease in many and provide long-term survival for approximately one-fourth of patients [2]. However, despite timely performance of a KP, most patients with BA experience progressive biliary cirrhosis, ultimately requiring transplantation [3]. In fact, BA is the most common indication for liver transplantation (LT) in children.

One cardinal histologic feature of BA is ductular reaction (DR), a phenomenon reflecting activation of the regenerative compartment in the liver, accompanied by progressive sclerosing cholangitis, severe inflammation, fibrosis, and epithelial injuries [4]. Biopsies obtained at later stages of BA may eventually reveal ductopenia. In humans, increasing evidence supports the existence and activation of hepatic progenitor cells (HPCs) in a variety of liver diseases [5–9]. It is now believed that the role of HPCs in liver repair and regeneration mirrors their dual role in embryonic liver development. Biliary differentiation of HPCs manifests as reactive ductules near the biliary plate, whereas hepatocytic differentiation involves transition through an intermediate hepatocyte (IH) morphology [7], which consists of polygonal cells of an intermediate size, larger than HPCs but smaller than hepatocytes.

Many subtypes of keratin intracellular intermediate filaments have been characterized [10]. Particular subtypes are selectively expressed in different types of epithelia during the course of cell determination, differentiation, and organogenesis. Immunohistochemical analysis of keratin subtypes can help to identify the various components of the intrahepatic biliary system in normal liver. CK7 and CK19, for example, are strongly expressed by interlobular bile ducts, intraportal and intralobular bile ductules, as well as the biliary epithelial cells that partly line the canals of Hering [11]. Bipotential HPCs also express CK7 and CK19. The cytoplasm of IHs retains at least weak to moderate staining intensity for CK7 but lacks CK19. Mature normal hepatocytes express neither CK7 nor CK19 [7].

CD56 (neural cell adhesion molecule [N-CAM]) is commonly used as a marker of natural killer cells and cells with (neuro)endocrine differentiation. It is not normally expressed in mature biliary epithelium but is strongly expressed in reactive/proliferative biliary epithelial cells in obstructive biliary tract disease [12]. Cuboidal epithelial cells in the canals of Hering (particularly ductal elements), which are the potential niche for hepatic stem cells, also express this adhesion moiety. CD56 is thought to play an important role in morphogenesis, remodeling, and migration in several organs through cell-cell and cell-matrix interactions [13].

We examined the activity of the regenerative compartment in liver in BA by assessing the degree of DR in and by applying a panel of immunohistochemical markers, including CK7, CK19, and CD56, to early stage BA at the time of KP and late-stage BA at the time of LT. The potential utility of these analyses in prognostic assessment of BA patients was also explored.

2. Materials and methods

2.1. Liver specimens

The archives of the Department of Pathology, Kaohsiung Chang Gung Memorial Hospital, were retrospectively reviewed to identify all cases of extrahepatic BA from 1990 to 2007, with approval from institutional review boards. All cases with paraffin blocks available were included in this study. Patients undergoing KP or orthotopic LT were included only if the diagnosis of extrahepatic BA was confirmed histologically. A total of 88 cases met these inclusion criteria and were separated by the operation performed. KP specimens were wedge liver biopsies obtained at the time of the operation (n = 24), and LT specimens were liver explants, with or without previous KP (n = 64; 61 of them had previous KP). Wedge biopsies of living donor liver allografts constituted the normal control group (n = 20).

2.2. Immunohistochemistry

Five-micrometer-thick tissue sections prepared from representative formalin-fixed, paraffin-embedded tissue blocks were deparaffinized, followed by incubation with the following panel of antibodies: CK7 (clone OV-TL, 1:500 dilution; Dako, Carpinteria, CA), CK19 (clone BA17, 1:200 dilution; Dako), and CD56 (clone Ab-2, 1:100 dilution; Neomarker, Fremont, CA). Heat-induced epitope retrieval was achieved for all target antigens by heating sections in 0.1 mol/L citrate buffer (pH 6.0) in a commercial microwave oven. A visible reaction product was obtained using a (strept)avidin-biotin immunoperoxidase method using 3,3′-diaminobenzadine-4 HCl as chromogen. Appropriate positive and negative controls were performed.

The degree of DR was semiquantitatively scored as 0 (negative), 1 (<30% circumference in portal tracts), and 2 (≥30% to <60% circumference in portal tracts), and 3 (≥60% circumference in portal tracts). As previously described, the HPCs were small cells characterized by an oval nucleus and a narrow rim of cytoplasm, strongly CK7 and/or CK19 immunoreactive. The HPC compartment was evaluated as the CK-7–positive cells within the bile ductules as well as solitary CK-7–positive HPCs or those in small clumps that are localized in the parenchyma or at the portal interface. Cholangiocytes lining the interlobular bile ducts were excluded from the counts [14]. *IHs* are defined as cells with sizes between those of hepatocytes and HPCs (<40 but