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Original contribution

Characterization of intraductal papillary neoplasm of bile duct with respect to histopathologic similarities to pancreatic intraductal papillary mucinous neoplasm☆,☆☆

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Summary Intraductal papillary neoplasm of bile duct (IPNB) is a papillary tumor covered by well-differentiated neoplastic epithelium with fine fibrovascular cores in the dilated bile ducts. It reportedly shows similarities to intraductal papillary mucinous neoplasm of pancreas (IPMN), to various degrees. Herein, IPNB was pathologically analyzed by classifying 52 cases into 4 groups based on the histopathologic similarities to IPMN: group A (identical to IPMN, 19 cases), group B (similar to but slightly different from IPMN, 18 cases), group C (vaguely similar to IPMN, 5 cases), and group D (different from IPMN, 10 cases). In group A, intrahepatic and perihilar regions were mainly affected, most cases were of low/intermediate or high grade without invasion, and gastric type was the most common phenotype, followed by oncocytic and intestinal types. In groups C and D, perihilar and distal bile ducts were affected, almost all cases were of high grade with invasion, and most of them were of intestinal and pancreatobiliary phenotypes. Most group B cases were of intestinal phenotype, and all were of high grade with or without invasion. In conclusion, these 4 groups of IPNB showed unique pathologic features and behaviors. Group A cases were less aggressive and shared many features with IPMN, whereas group C and D cases were more aggressive and mainly found in perihilar and distal bile ducts. Group B resembling IPMN was intermediate between them. This classification may be useful in clinical practice and holds promise for a novel approach to analyze IPNB tumorigenesis.

1. Introduction

In the biliary tree, grossly visible papillary neoplasms including papillary carcinoma and papillomatosis occasionally occur and reportedly represent 4% to 10% of all biliary

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epithelial neoplasms [1-7]. Although the term *intraductal papillary neoplasm of bile duct* (IPNB) has recently been proposed for such biliary neoplasms in the *World Health Organization (WHO) Classification of Tumors of the Digestive System* [2,4], many pathologic terms have been coined, such as papillary carcinoma, biliary papillomatosis, mucin-secreting biliary tumor, and intraductal papillary mucinous tumor [1-7], implying that the histology, biological behavior, and even the mechanisms of tumorigenesis of these papillary neoplasms are heterogeneous.

Intraductal papillary mucinous neoplasm of pancreas (IPMN) is also characterized by the papillary proliferation of atypical mucinous epithelium in the pancreatic ductal system, and the affected pancreatic ducts are often cystically or cylindrically dilated [1,3,7]. IPMN is a spectrum of diseases ranging from low or intermediate (low/int)–grade and high-grade intraepithelial neoplasia to IPMN with an associated invasive carcinoma [8]. IPNB is reported to resemble IPMN and can be regarded as an example of a biliary disease with a pancreatic counterpart [9,10]. However, IPNB is also known to differ from IPMN in some aspects. For example, the incidence of mucus hypersecretion is relatively low in IPNB, and the proportion of cases of malignant or high-grade intraepithelial neoplasia is high in IPNB in comparison with that in IPMN [11,12].

In this study, we histopathologically and immunohistochemically examined IPNB with an emphasis on its similarities to IPMN. We found that IPNB can be classified into several types, which show different distributions in terms of their main location along the biliary tree and different phenotypes and grades of intraepithelial neoplasia. The histopathologic similarities and dissimilarities to IPMN may reflect the different mechanisms of tumorigenesis of IPNB, and this approach of comparing these 2 entities may lead to a novel clinicopathological analysis and evaluation of IPNB.

2. Materials and methods

2.1. Anatomy of the bile duct and definition of IPNB

The bile ducts are divided into the intrahepatic, perihilar, and distal bile ducts [13,14]. Peribiliary glands are distributed around the intrahepatic large bile ducts and perihilar and distal bile ducts. IPNB was diagnosed in accordance with the 2010 WHO classification [1,4]. That is, IPNB was characterized by dilated bile ducts filled with a grossly visible noninvasive papillary biliary neoplasm covering delicate fibrovascular stalks. The covering neoplastic epithelial cells were well differentiated, although small amounts of tubular component and mucinous carcinoma were occasionally identifiable.

2.2. Case selection and tissue preparation

A total of 326 consecutive surgically resected cases with a diagnosis of bile duct tumor at Shizuoka Cancer Center, Shizuoka, Japan (2002-2014), were surveyed with a reference to "grossly visible papillary or polypoid neoplastic lesions in the bile duct lumen." This hospital opened in 2002, and these 326 cases constitute all of the surgically resected bile duct tumors experienced at this hospital up until 2014. A total of 75 cases of bile duct tumors fulfilled the aforementioned gross criteria. Among them, a total of 52 cases fulfilled the aforementioned histopathologic criteria of IPNB, and these cases were examined. The surgical operations for the main tumor were as follows: left lobectomy or segmentectomy (15 cases), right lobectomy or segmentectomy (8 cases), pancreaticoduodenectomy (19 cases), and combined left or right lobectomy or segmentectomy with simultaneous pancreaticoduodenectomy (10 cases). The main tumors with adjacent proximal and distal bile ducts were examined in all cases in this study. Postoperative survival (overall survival) was examined in all cases. The study protocol was approved by the institutional review board of Shizuoka Cancer Center.

The number of tissue blocks that included the main IPNB tumor and were sampled in individual cases for pathologic diagnosis ranged from 10 to 50 (mean, 28; median, 22). These blocks were fixed in 10% neutral buffered formalin and embedded in paraffin. Data regarding the resected specimen, including the tumor size and status of the resected margin, were available.

More than 10 serial thin sections, 4 μm in thickness, were prepared from each paraffin block. After deparaffinization, thin sections were stained with hematoxylin and eosin, Azan-Mallory, and periodic acid–Schiff after diastase digestion for histologic observation. The remaining sections were used for immunohistochemistry as follows.

2.3. Immunohistochemistry

Immunohistochemical staining of CDX2, MUC1, MUC2, MUC5AC, MUC6, CK7, CK20, CD10, cyclin D1, Smad4, p21, p53, p16, EZH2, γH2AX, and Ki-67 was performed using formalin-fixed, paraffin-embedded tissue sections of surgically resected specimens. CDX2, MUC1, MUC2, MUC5AC, MUC6, CK7, CK20, and CD10 were used to facilitate the phenotyping of IPNB [3,8]. Cyclin D1, Smad4, p21, p53, and Ki-67 were used for assessing cell cycle regulation and cell proliferative activities [15], whereas p16, p21, and EZH2 were used for cellular senescence and the escape from cellular senescence [16], and γH2AX was used for DNA damage [17]. Immunohistochemistry was performed with an EnVision+ system (Dako, Glostrup, Denmark). The primary antibodies and their sources, optimal dilution, and antigen retrieval methods are shown in Table 1. After blocking of the endogenous peroxidase, the sections were incubated overnight at 4°C with the primary antibody to p21 and for 30 minutes with each of the remaining primary antibodies. Color development was performed using 3,3′-diaminobenzidine tetrahydrochloride.