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Different expression patterns of mucin core proteins and cytokeratins during intrahepatic cholangiocarcinogenesis from biliary intraepithelial neoplasia and intraductal papillary neoplasm of the bile duct—an immunohistochemical study of 110 cases of hepatolithiasis

Yoh Zen[1,2], Motoko Sasaki[1], Takahiko Fujii[1], Tse-Ching Chen[3], Miin-Fu Chen[4], Ta-Sen Yeh[4], Yi-Yin Jan[4], Shiu-Feng Huang[4], Yuji Nimura[5], Yasuni Nakanuma[1,*]

[1]*Department of Human Pathology, Kanazawa University Graduate School of Medicine, Kanazawa 920-8640, Japan*
[2]*Division of Pathology, Kanazawa University Hospital, Kanazawa, Japan*
[3]*Department of Pathology, Chang Gung Memorial Hospital, Lin Kou, Taiwan ROC*
[4]*Department of Surgery, Chang Gung Memorial Hospital, Lin Kou, Taiwan ROC*
[5]*Division of Surgical Oncology, Department of Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan*

See Editorial, pages 249–250

***Background/Aims*: Two types of neoplastic lesions preceding invasive intrahepatic cholangiocarcinoma (ICC) are identified: a flat-type neoplastic lesion called 'biliary intraepithelial neoplasia (BilIN)' and an intraductal papillary neoplasm of the bile duct (IPN-B). Multi-step carcinogenesis has been suggested in both lesions, although phenotypic changes during this process remain unclarified.**

***Methods*: We immunohistochemically examined expression patterns of MUC1, MUC2, MUC5AC, cytokeratin 7 (CK7), and CK20 in BilIN, IPN-B, and ICC in 110 cases of hepatolithiasis.**

***Results*: Thirty-seven cases of ICC in hepatolithiasis were divided into 18 tubular adenocarcinomas with BilIN, 10 tubular adenocarcinomas with IPN-B and nine colloid carcinomas with IPN-B. Carcinogenesis via BilIN was characterized by MUC2−/CK7+/CK20− with increasing MUC1 expression. IPN-B was characterized by the intestinal phenotype (MUC2+/CK20+), and carcinogenesis leading to tubular adenocarcinoma was associated with increasing MUC1 expression and that to colloid carcinoma with MUC1-negativity. Pathological stages of tubular adenocarcinoma of ICC with BilIN or IPN-B were more advanced than those of colloid carcinoma with IPN-B.**

***Conclusions*: Immunophenotypes of MUCs and cytokeratins might characterize three cholangiocarcinogenetic pathways in hepatolithiasis. Increased expression of MUC1 in BilIN and also IPN-B is associated with tubular adenocarcinoma, while colloid carcinoma in IPN-B is characterized by MUC1-negativity and less advanced pathologic stages.**

***Keywords*: Intrahepatic cholangiocarcinoma; Dysplasia; Colloid carcinoma; Biliary papillomatosis; PanIN**

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* Corresponding author. Tel.: +81 76 265 2195; fax: +81 76 234 4229.
E-mail address: pbcpsc@kenroku.kanazawa-u.ac.jp (Y. Nakanuma).

Abbreviations: BilIN, biliary intraepithelial neoplasia; CK, cytokeratin; ICC, intrahepatic cholangiocarcinoma; IPMN-P, intraductal papillary mucinous neoplasm of the pancreas; IPN-B, intraductal papillary neoplasm of the bile duct; MUC, mucin core protein; PanIN, pancreatic intraepithelial neoplasia.

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1. Introduction

Intrahepatic cholangiocarcinoma (ICC) is the second most common malignant tumor of the liver [1,2]. Once ICC shows invasive growth, it aggressively invades the surrounding tissue and is commonly associated with distant metastasis, finally resulting in a poor prognosis [2–4]. Two distinct neoplastic lesions preceding invasive ICC have been identified so far [5]. The first neoplastic lesion is a flat or micropapillary growth of atypical biliary epithelium, which has been called 'biliary dysplasia' [4]. 'Biliary intraepithelial neoplasia (BilIN)' was used for these lesions as one of the precursor lesions of ICC in World Health Organization's classification of tumors [6]. The other is an intraductal papillary neoplasm of the bile duct (IPN-B) with malignant potential, which is histologically characterized by the prominent papillary growth of atypical biliary epithelium with distinct fibrovascular cores and frequent mucin over-production [7,8].

BilIN and IPN-B share pathologic characteristics [4–8]: they usually occur in the large-sized intrahepatic bile ducts and are rarely present in the septal or interlobular bile ducts. In addition, both BilIN and IPN-B more frequently arise during chronic inflammatory biliary diseases, such as hepatolithiasis, primary sclerosing cholangitis, and infestation by liver flukes [6–9]. Among them, hepatolithiasis is unique in that both premalignant lesions (BilIN or IPN-B) can occur, whereas primary sclerosing cholangitis and parasitic infections are usually associated with only BilIN and not IPN-B [7]. On the other hand, BilIN and IPN-B have different pathologic characteristics. BilIN can be identified only microscopically (a microscopic lesion), whereas IPN-B is identifiable on radiologic images or through macroscopic examination [6,7]. When accompanied by invasive lesions, BilIN is known to progresses to conventional ICC (tubular adenocarcinoma), whereas IPN-B is associated with colloid carcinoma (mucinous carcinoma) in addition to conventional ICC [7,8,10].

Multi-step carcinogenesis has been suggested in ICCs arising from BilIN and IPN-B [4,8]. However, little is known about the phenotypic or genetic changes during their carcinogenetic pathways, because only a few studies using both BilIN and IPN-B have been conducted. In this study, we immunohistologically examined the phenotypic changes of mucin core proteins (MUCs) and cytokeratins (CKs) during cholangiocarcinogenesis from BilIN and IPN-B in 110 hepatolithiasis cases.

2. Materials and methods

2.1. Case selection and classification of biliary lesions

A total of 110 hepatolithiasis cases were obtained from the liver disease file of Department of Human Pathology, Kanazawa University Graduate School of Medicine, and that of Department of Pathology, Chang Gung Memorial Hospital (Table 1). All were surgically-resected cases (65 cases, left lobectomy; 39 cases, right lobectomy; and six cases, segmentectomy.

Table 1
Cases analyzed in this study and their clinical features

	Number	Average age (range)	Male: female
Non-neoplastic epithelium	10	49.1 (40–60)	6:4
BilIN (total)	38	56.8 (36–83)	17:21
BIIIN-1	12	50.9 (41–68)	2:10
BilIN-2/3	26	59.5 (36–83)	15:11
ICC with BilIN	18	61.0 (43–83)	8:10
IPN-B (total)	25	55.7 (35–66)	9:16
IPN-B1	12	58.8 (50–66)	5:7
IPN-B2	13	50.1 (35–65)	4:9
ICC with IPN-B	19	59.8 (46–78)	6:13

BilIN, biliary intraepithelial neoplasia; BilIN-1, low-grade BilIN; BilIN-2/3, high-grade BilIN; IPN-B, intraductal papillary neoplasm of the bile duct; IPN-B, intraductal papillary neoplasm of the bile duct; IPN-B1, low-grade IPN-B corresponding to benign and borderline lesions; IPN-B2, high-grade IPN-B corresponding to carcinoma in situ; ICC, intrahepatic cholangiocarcinoma.

All biliary lesions examined were found in the intrahepatic large bile ducts, which correspond to the first to third branches of the right and left hepatic ducts [11]. The biliary epithelial lesions in hepatolithiasis were classifiable into five categories (Table 1); non-neoplastic biliary epithelium, BilIN, ICC with BilIN, IPN-B, and ICC with IPN-B. BilIN was histologically defined as a flat or micropapillary proliferation of atypical biliary epithelium showing multilayering, piled-up nuclei, an increased nucleocytoplasmic ratio, a partial loss of nuclear polarity, and nuclear hyperchromasia and pleomorphism. In contrast, IPN-B was microscopically defined as a prominent papillary proliferation of atypical biliary epithelium with distinct fibrovascular cores, showing nuclear stratification, piled-up nuclei, and nuclear enlargement. In this study, one lesion representative of these categories was chosen from each case of hepatolithiasis: non-neoplastic biliary epithelium (regenerative and hyperplastic changes) was chosen in 10 cases of hepatolithiasis, BilINs in 38 cases, ICC with BilIN in 18 cases, IPN-Bs in 25 cases, and ICC with IPN-B in 19 cases.

Next, BilINs and IPN-Bs were graded. Based on previously reported criteria, BilINs were classified into three grades: BilIN-1 (corresponding to low-grade dysplasia), BilIN-2 (high-grade dysplasia), and BilIN-3 (carcinoma in situ) [12]. Briefly, BilIN-1 showed mild cellular/nuclear atypia such as nuclear membrane irregularity or nuclear enlargement with only a minimal disturbance of cellular polarity. BilIN-2 had evident cellular/nuclear atypia, but not enough to suggest overt carcinoma, with a focal disturbance of cellular polarity. BilIN-3 showed a diffuse disturbance of cellular polarity with or without distinct cellular/nuclear atypia corresponding to an overt carcinoma. In this study, BilIN-2 (chosen from 17 cases of hepatolithiasis) and BilIN-3 (from nine cases) were grouped together as high-grade BilIN (BilIN-2/3). Eighteen cases of ICC with BilIN were all histologically tubular adenocarcinoma (eight cases, well differentiated; six cases, moderately differentiated; and four cases, poorly differentiated).

Because there are no well-established criteria for grading IPN-B, we graded IPN-B in consideration of World Health Organization's criteria for intraductal papillary mucinous neoplasm of the pancreas (IPMN-P) [13]. IPN-Bs were classified into two subgroups: IPN-B1 (corresponding to benign and borderline lesions of IPMN-P) (chosen from 12 cases of hepatolithiasis) and IPN-B2 (corresponding to carcinoma in situ) (from 13 cases). One IPN-B2 histologically consisted of oncocytic tumor cells (oncocytic type). Out of 19 ICCs with IPN-B, nine were histologically colloid carcinomas, whereas nine were tubular adenocarcinomas (eight cases, well differentiated; and one case, poorly differentiated). The remaining case was oncocytic papillary adenocarcinoma with invasion.

BilIN-1, BilIN-2/3 and ICC with BilIN were regarded as BilIN lineage, while IPN-B1, IPN-B2 and ICC with IPN-B were regarded as IPN-B lineage.