Original Article

Decreased Expression of SOX9 in Intraductal Papillary Mucinous Neoplasms of the Bile Duct

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Abstract

Background/Aims: SOX9 is an important transcription factor required for development and has been implicated in several types of malignant tumor. Our recent study showed that SOX9 played an important role in multi-step carcinogenesis in cases of intraductal papillary mucinous tumor of the pancreas (IPMN-P). This study aimed to investigate the expression of SOX9 in cases of intraductal papillary mucinous tumor of the bile duct (IPMN-B).

Material and Methods: SOX9 expression was immunohistochemically evaluated in the tumor and corresponding normal bile-duct epithelium of seven IPMN-B patients.

Results: In all cases, SOX9 expression in the IPMN-B was low compared with the normal biliary epithelium.

Conclusion: This study demonstrated that SOX9 expression may indicate a link between IPMN-B and IPMN-P. SOX9 may also have potential as a therapeutic target and/or prognostic marker in IPMN-B.

Key Words: SOX9, IPMN-B, IPMN, counterpart

Introduction

Some papillary bile duct tumors produce a large amount of mucin and cause several clinical symptoms including obstructive jaundice, abdominal pain, and fever. Mucin impedes the bile flow and causes biliary dilatation. Mucin-producing bile duct tumor is a rare disorder of the biliary tract, and several medical terms have been used to describe this tumor. Given its low incidence, the clinical features, diagnostic problems, and treatment strategies for this disorder are largely unclear [1-3]. "Intraductal papillary mucinous tumor of the bile duct" (IPMN-B) has been the most common term used to describe this condition in recent publications [4, 5]. IPMN-B is recognized as the counterpart of intraductal papillary mucinous tumor of the pancreas (IPMN-P) [6-8]. IPMN-B closely resembles IPMN-P in pathological characteristics and postoperative survival status [9, 10].

Recent reports have implicated Sex-determining Region Y (SRY) box 9 (SOX9), an important transcription factor required for development, in several types of malignant tumor [11-13]. In addition, SOX9 is an essential regulator for biliary development [14]. Our recent study showed that SOX9 plays an important role in the multi-step carcinogenesis of IPMN-P [15]. Herein, we described the SOX9 expression in IPMN-B and reveal the role of SOX9 in the progression of IPMN-B.

Materials and methods

Patients

From 1993 to 2011, seven patients with IPMN-B underwent surgery in our department. The profiles of our patients are summarized in Table 1. There were 4 females and 3 males, with a mean age of 68 years (range 61-76) at presentation. Five patients typically presented with recurrent cholangitis or jaundice while two patients were asymptomatic. A biliary-associated disease was found in three patients: hepatolithiasis in two and choledochoduodenal fistula in one. Major laboratory tests (total bilirubin, alkaline phosphatase, transaminases) were elevated in all five symptomatic patients but not in the two others. Serum carcinoembryonic antigen was within normal limits in all seven patients. Another tumor marker, CA19-9, was elevated in three patients. All laboratory tests, including tumor markers, were within normal limits in the two asymptomatic patients. Major diagnostic procedures including computed tomography (CT), ultrasonography (US), and endoscopic retrograde cholangiography (ERC) were performed in all seven patients. In all patients, ERC showed filling defects in the biliary tract the production of abundant mucin cause the orifice to be wide-open. ERC showed generalized dilatation of the intra- and extra-hepatic bile ducts in three patients, left hepatic duct and extra-hepatic bile ducts in two patients, right hepatic duct and extra-hepatic bile ducts in one patient, and segmental dilatation of the left hepatic ducts in one patient. Intraductal masses were revealed on enhanced CT in all patients. All patients underwent curative hepatectomy. Histological

grade findings showed four invasive carcinomas, one noninvasive carcinoma, and two

adenomas. Two patients died of recurrence. Two patients died of other disease. The other

three patients are alive and disease-free.

Immunohistochemical analysis

All the surgical specimens were fixed in 10% formalin. The specimens were then sectioned,

and serial sections were carefully cut from paraffin blocks and stained with hematoxylin

and eosin. Immunohistochemical examinations were performed as follows. Sections were

deparaffinized by ethanol concentrations and washed in phosphate-buffered saline (PBS).

Then, the sections were treated with hot water at 95°C for antigen retrieval (Target

Retrieval Solutions pH 9.0 (Dako, Japan)) for 20 minutes. After washing, the samples were

treated with 0.03% hydrogen peroxide in methyl alcohol. Thereafter, they were treated with

0.25% casein in PBS, containing stabilizing protein and 0.015 mol/L sodium azide (Protein

Block, Serum-Free, Dako, Japan) at room temperature for 20 min and covered with a

mouse monoclonal antibody to SOX9 (Abcam Discover More, Tokyo, Japan) at a dilution

of 1:500 in PBS at room temperature for 30 minutes. After being rinsed with PBS, the

sections were treated with a rabbit polyclonal antibody against mouse IgG, IgA, and IgM at

room temperature for 30 minutes and washed with PBS. The peroxidase/antiperoxidase

complex was allowed to react with the rabbit antibody, and the sections were stained with 3,

3-diaminobenzidinetetrahydrochloride (DAB) containing 0.03% hydrogen peroxide

(Envison kit / HRP (DAB), Dako, Japan). The sections were counterstained with Mayer's

hematoxylin.

Expression of SOX9 was evaluated by the percentage of positive cells of each

tumor specimen and that of the corresponding normal epithelium of the bile ducts. The

percentages were analyzed by counting 200 cells at ×200 magnification in four random

views.

Statistical analysis

Continuous data are expressed as the median (range). Categorical data and continuous data

were compared using the Mann Whitney-U test. We assigned statistical significance at <

0.05. The calculations were performed with the help of Excel statistics version 2009 (Social

Survey Research Information Co., Ltd, Japan).

Results

SOX9 positivity was detected in nuclei and was observed throughout the normal biliary

epithelium. The results of the immunohistochemical analyses of SOX9 expression are

presented in Table 2. Almost all normal biliary duct epithelial cells were SOX9 positive

(Fig. 1). The SOX9-positive rate ranged from 75% to 83% in normal biliary epithelia. In

comparison, all cases showed low SOX9 expression in IPMN-B (Fig. 2). The range of the

SOX9-positive rate in IPMN-B cells was from 7% to 45% (Table 2).

Discussion

The expression of SOX9 was decreased in IPMN-B compared with the corresponding normal bile ducts in this immunohistochemical study. Our recent study demonstrated that the expression of SOX9 in pancreatic cancer was extremely low, and that in IPMN-P, SOX9 expression progressively and gradually decreased according to IPMN-P progression, including adenoma, noninvasive carcinoma, and invasive carcinoma [15]. Recent studies have revealed that the IPMN-B is a counterpart of IPMN-P in terms of its histopathological features, production of a large amount of mucin, and clinical findings [4-10]. IPMN-B comprises a histological spectrum that ranges from benign to malignant: adenoma, noninvasive, and invasive carcinoma [16, 17]. Interestingly, the SOX9 expression in the two cases of adenoma of IPMN-B was higher than that in the more malignant cases of carcinoma of IPMN-B in the present study. The down-regulation of SOX9 expression is an early event in the stepwise carcinogenesis of IPMN-B. This same phenomenon was found in the stepwise carcinogenesis of IPMN-P [15].

SOX9 was originally known as a key regulator of development processes, including chondrogenesis, neurogenesis, and male sex determination [18, 19]. Subsequently, SOX9 was shown to be a multifaceted transcription factor indispensable for the development of several organs, including the intestines, the pancreas, and the biliary tract [20]. Recent studies have shown the important role of SOX9 in carcinogenesis for several organs [21-]. Sun et al. [21] reported that upregulation of SOX9 is related to gastric cancer

development. Lu et al. [22] reported that SOX9 overexpression in colorectal cancer as an indicator of an unfavorable outcome. On the other hand, SOX9 expression in melanoma is downregulated; SOX9 overexpression in melanoma cell lines inhibited tumorigenicity in a human *ex vivo* model of melanoma [23]. In biliary tract cancers, a recent study demonstrated that lack of nuclear SOX9 expression was associated with a higher tumor stage [24]. Moreover, SOX9 is strongly expressed both in intrahepatic bile ducts and gallbladder epithelium [24]. The present study showed that SOX9-positive cells were expressed in almost all normal bile duct cells. It is clearly neither an oncogenic gene or anti-oncogenic gene but rather one that plays different roles in different cancers. Interestingly, Passeron et al. [23] reported that treatment of melanoma cell lines with prostaglandin D2 increased SOX9 expression and restored sensitivity to retinoic acid. These findings suggested that SOX9 has a potential to inhibit the growth of IPMN-B at an early, low-malignancy stage.

In summary, our results of SOX9 expression may indicate a link between IPMN-B and IPMN-P and confirm the recently proposed notion that these two mucin-producing cancers are counterparts. SOX9 may also have potential as a therapeutic target and/or prognostic marker in IPMN-B.

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FIGURE LEGENDS

Fig. 1 SOX9 expression and immunohistochemical stain of biliary epithelium. Almost all normal biliary duct epithelial cells were SOX9 positive.

Fig. 2 SOX9 expression was low in IPMN-B compared with the normal biliary ductal epithelium.

Fig. 1
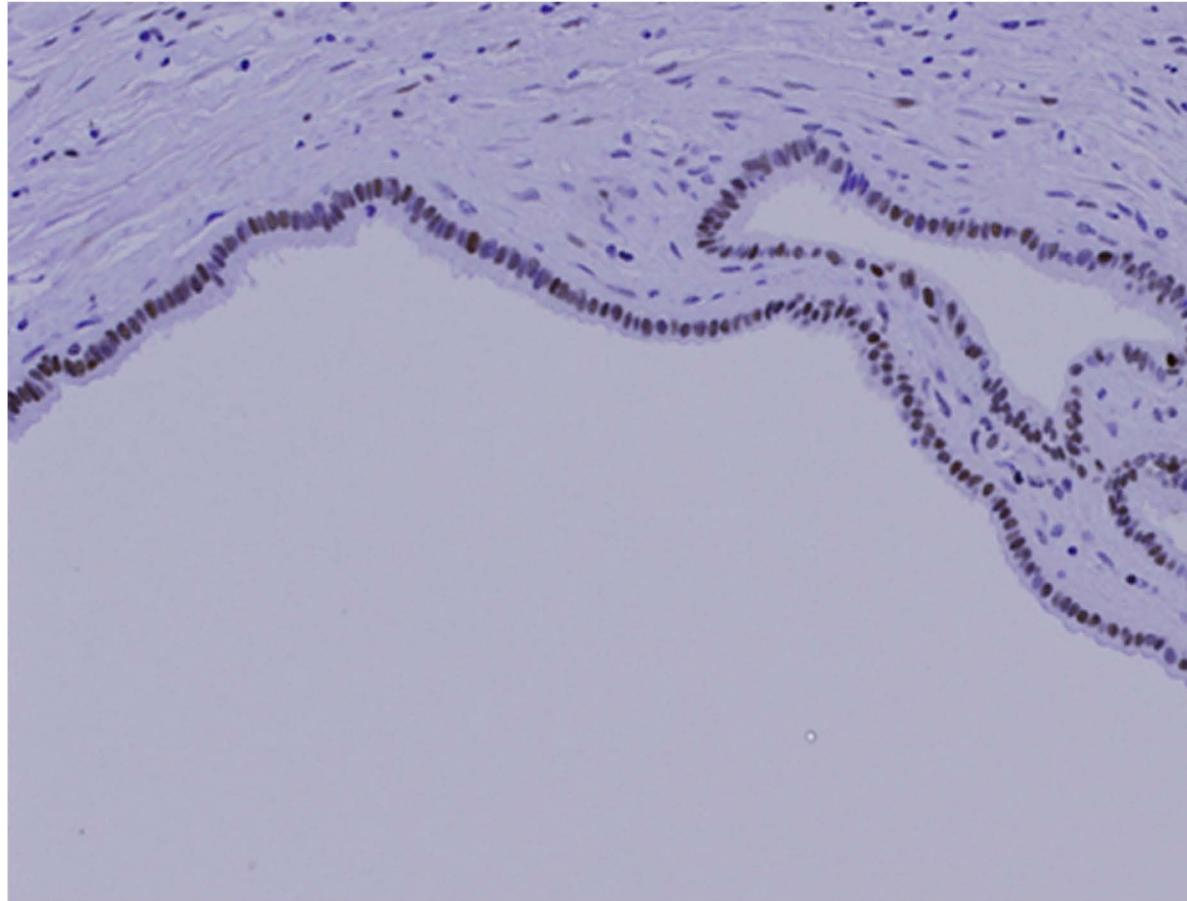
Fig. 2
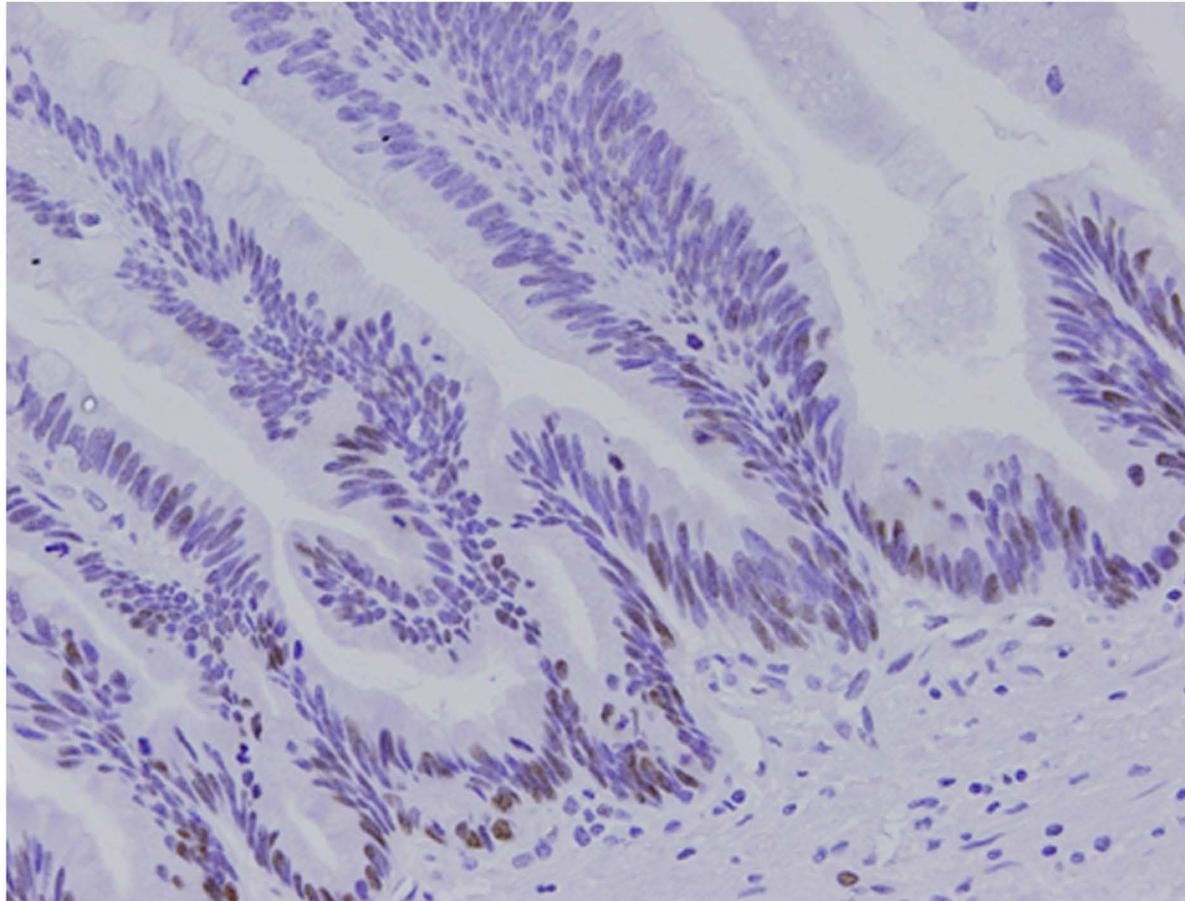
Table 1 Clinicopathologic findings of seven patients with IPMN-B

No.	Age	Sex	Location	Operation	Histological Grade	Outcome
1	72	M	Left hepatic duct	Left trisectionectomy	Invasive carcinoma	25 months
				Caudate lobectomy		Death of
				Resection of the extrahepatic bile duct		other disease
2	62	F	Left hepatic duct	Left hemihepatectomy	Noninvasive carcinoma	97 months
				Caudate lobectomy		Death of
						recurrence
3	70	M	Hilar duct	Right hemihepatectomy	Invasive carcinoma	25 months
				Caudate lobectomy		Death of
				Resection of the extrahepatic bile duct		recurrence
4	62	F	Common bile duct	Anterior segmentectomy	Invasive carcinoma	79 months
				Resection of the extrahepatic bile duct		Death of
						Other disease
5	61	F	Left hepatic duct	Left hemihepatectomy	Adenoma	142 months
						Alive and
						disease-free
6	76	F	Left hepatic duct	Left hemihepatectomy	Adenoma	27 months
				Caudate lobectomy		Alive and
						disease-free
7	76	M	Right hepatic duct	Extended right hemihepatectomy	Invasive carcinoma	50 months
				Caudate lobectomy		Alive and
				Resection of the extrahepatic bile duct		disease-free

Table 2 SOX9 expression in seven patients with IPMN-B

No.	SOX9 positive rate	
	Normal epithelial cells	tumor cells
1	75%	31%
2	77%	22%
3	79%	19%
4	77%	33%
5	76%	36%
6	83%	45%
7	77%	7%