SHORT REPORT

Aberrant maspin expression in gallbladder epithelium is associated with intestinal metaplasia in patients with cholelithiasis

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Objective: Aberrant expression of maspin protein related to DNA hypomethylation in the promoter region is frequently observed in gallbladder carcinomas, whereas the non-tumorous gallbladder epithelium is maspin negative. We investigated maspin expression in non-tumorous gallbladder epithelium in patients with cholelithiasis.

Methods: An immunohistochemical study of maspin expression was performed in 69 patients with cholelithiasis and 30 patients with gastric cancer without cholelithiasis.

Results: Immunoreactivity for maspin was observed in focal and patchy regions of the gallbladder epithelium. Positive immunoreactivity for maspin was significantly associated with the presence of intestinal metaplasia in patients with cholelithiasis ($p < 0.05$).

Conclusion: The high incidence of aberrant maspin expression in both intestinal metaplasia and carcinoma of the gallbladder supports the assumption that intestinal metaplasia of the gallbladder may predispose to gallbladder carcinoma.

Maspin is a protein of M_r 42 000, showing sequence homology to the serpin family protease inhibitors. In several tumour types, maspin acts as a tumour suppressor capable of inhibiting cell motility, invasion, and metastasis.¹ There is accumulated functional evidence that demonstrates that maspin blocks tumour metastasis, tumour cell motility and invasion, and apoptosis in vitro.¹ However, some in vivo analyses have shown that gain of maspin expression is associated with malignant behaviour.^{2,3} Thus, the role of maspin in tumour biology remains controversial.

We recently demonstrated that aberrant maspin expression is frequently present in intestinal metaplasia of gastric epithelium and carcinomas⁴ and also in undifferentiated thyroid carcinomas,⁵ whereas their normal tissue counterparts are negative. Futscher *et al*⁶ demonstrated that in normal tissues the maspin gene is strictly regulated in a cell type-specific manner by promoter DNA methylation. Interestingly, aberrant maspin expression has also been observed in preneoplastic and/or dysplastic lesions in lung.⁷ Aberrant maspin expression appears to be closely associated with morphological changes such as metaplasia, dysplasia, and dedifferentiation, probably as a result of disruption of epigenetic expression mechanisms.

We and other groups have reported a high incidence of aberrant maspin expression in pancreatobiliary tract carcinomas, including gallbladder carcinomas.^{3,8,9} We noticed that the background non-tumorous epithelium was also positive in restricted areas showing intestinal metaplasia. Several

factors are associated with the aetiology of gallbladder carcinomas, and gallstones should be considered as a risk factor. In the present study, therefore, we immunohistochemically investigated maspin expression in patients with cholelithiasis.

MATERIALS AND METHODS

Our subjects comprised 69 patients with cholelithiasis and 30 patients with gastric cancer without gallstones. Permission for the study was obtained from the institutional review board (Iwate Medical University School of Medicine, Morioka, Japan) and written consent was obtained from all patients prior to surgery. All the patients underwent cholecystectomy, and the surgical specimens of gallbladder were fixed in 10% buffered formalin solution and embedded in paraffin wax for immunohistochemistry. The longest section of each gallbladder was divided into three parts (proximal, middle, and distal) and three blocks of each part were made. Serial sections were stained with alcian blue (pH 2.5) and haematoxylin and eosin.

Immunohistochemistry for maspin was also performed on serial sections as described previously.^{4,5} After a microwave based antigen retrieval procedure, immunostaining with anti-human maspin antibody (clone G167-70, dilution 1:50; BD Pharmingen International, San Diego, CA, USA) was performed with a Histofine SAB-PO kit (Nichirei Co., Tokyo, Japan). The relative density of maspin positive cells was graded as described previously.⁸

RESULTS

Positive immunostaining for maspin was focal and patchy in each gallbladder. No case exhibited diffusely positive staining. Maspin protein was expressed in the cytoplasm (fig 1), and nuclear staining, which was extremely rare, was encountered in two patients with cholelithiasis (fig 1). Maspin positive regions were observed in 16 patients (14 patients with and 2 without cholelithiasis) (table 1). In total, 29 and 2 regions in patients with and without cholelithiasis, respectively, exhibited positive immunoreactivity for maspin (table 1). Although a tendency for higher maspin positivity was observed in patients with cholelithiasis compared with those without, the differences between the groups was not significant (table 1). Intestinal metaplasia was also more frequent in patients with cholelithiasis than in those without the disease, but again the difference was not statistically significant (table 1). Interestingly, strong immunoreactivity was seen only in regions exhibiting intestinal metaplasia (9 of 14 maspin positive patients with cholelithiasis) (fig 1). Faint signals were observed in epithelia with non-intestinal

Abbreviation: TFF, trefoil factor family

Figure 1 Histology and immunohistochemistry for maspin in gallbladder epithelia. (A and B) High power view of gallbladder epithelium with intestinal metaplasia in a patient with cholelithiasis. Marked infiltration of inflammatory cells into the mucosa and goblet cell metaplasia are apparent (A), and epithelial cells show dense immunoreactivity for maspin. (C) High power view of gallbladder epithelium without intestinal metaplasia in a patient with cholelithiasis. (D) The cell indicated by an arrow shows both nuclear and cytoplasmic staining for maspin. (A) Haematoxylin and eosin; (B–D) immunohistochemistry for maspin. Scale bars: (A–C) 100 µm; (D) 10 µm.

metaplasia (5 of 14 maspin positive patients with cholelithiasis and all of the maspin positive patients without cholelithiasis) (fig 1). Positive maspin staining was significantly associated with the presence of intestinal metaplasia in patients with cholelithiasis ($p<0.05$, table 2).

DISCUSSION

Chronic irritation due to cholelithiasis may be associated with neoplastic transformation of gallbladder epithelium.^{10 11} Two types of metaplasia (antral and intestinal) are frequently observed with ageing in gallbladder epithelium.^{10 11} Several investigators have demonstrated that intestinal metaplasia is observed more frequently in patients with cholelithiasis than in those without, and furthermore, intestinal metaplasia is accompanied by dysplasia and carcinoma.^{10 11} Such histological evidence suggests a stepwise progression of gallbladder carcinoma through a path comprising intestinal metaplasia, dysplasia, and carcinoma.^{10 11} Although aberrant maspin

Table 2 Relationship between maspin immunoreactivity and presence of intestinal metaplasia in patients with and without cholelithiasis				
Intestinal metaplasia*	Cholelithiasis positive		Cholelithiasis negative	
	Intestinal metaplasia*		Intestinal metaplasia*	
	+	–	+	–
Positive	9 (64)	5 (9)	0 (0)	2 (7)
Negative	5 (36)	50 (91)	3 (100)	25 (93)
Total	14 (100)	55 (100)	3 (100)	27 (100)

* $p<0.05$ by Fisher's exact test.

expression did not significantly correlate with the presence of cholelithiasis, a tendency for higher maspin positivity was observed in patients with cholelithiasis compared with those without. Moreover, strong immunoreactivity for maspin protein was significantly correlated with the presence of intestinal metaplasia. Our previous (gallbladder carcinomas)⁸ and present (intestinal metaplasia) observations of aberrant maspin expression support the assumption that intestinal metaplasia of the gallbladder epithelium might increase susceptibility to the development of gallbladder carcinoma. In hepatolithiasis, it is well established that aberrant expression of certain proteins is associated with the presence of gastric and/or intestinal metaplasia. Ishikawa *et al*¹² demonstrated that aberrant expression of CDX2 was closely related to intestinal metaplasia and MUC2 overexpression in patients with mucinous intrahepatic cholangiocarcinoma (ICC) and intraductal papillary neoplasm of the liver. Furthermore, their group also demonstrated aberrant expression of trefoil factor family (TFF)-1, which has tumour suppressor properties, in biliary epithelial dysplasia and non-invasive ICC, associated with overexpression of gastric-type apomucin (MUC5AC).¹³ Interestingly, TFF-1 protein was negative in normal biliary epithelium and gain of TFF-1

Table 1 Patient characteristics, maspin immunostaining, and presence of intestinal metaplasia in patients with and without cholelithiasis		
	Cholelithiasis	
	+	–
Number of patients	69	30
Patient characteristics		
Mean age (range), years	52 (39 to 62)	64 (51 to 72)
Male:female	27:42	18:12
Maspin immunostaining		
Positive (%)	14 (20)	2 (7)
Immunoreactivity*	9/0/5	0/0/2
Negative (%)	55 (80)	28 (93)
Intestinal metaplasia		
Presence (%)	14 (20)	3 (10)
Absence (%)	55 (80)	27 (90)

*Strong/moderate/faint.

expression was observed in biliary dysplasia and non-invasive ICC. Moreover, loss of maspin expression was observed in invasive ICC. DNA methylation status in the TFF-1 promoter region was inversely correlated with protein expression at each stage.¹³ This switchback phenomenon in both epigenetic status and protein expression suggests the existence of epigenetic instability in the sequence that extends through normal epithelium, metaplasia, and cancer.

In gallbladder carcinogenesis, Wu *et al*¹⁴ recently demonstrated aberrant CDX2 expression in intestinal metaplasia and dysplasia of the gallbladder epithelium and in tubular-type gallbladder carcinoma. The possibility that CDX2 gene expression in gastric cancer is regulated by an epigenetic mechanism has been reported.¹⁵ These facts led us to speculate that epigenetic instability might contribute to metaplastic and/or neoplastic transformation of gallbladder epithelium. Although we attempted to investigate the DNA methylation status of the maspin promoter by use of a methylation specific PCR method combined with microdissection,⁵ we were unable to obtain sufficient DNA for bisulphite treatment because of the extremely small size of maspin positive areas.

Recently, the issue of whether the maspin gene has tumour suppressor or tumour promoting activity has been highlighted. Although tumour suppressive functions have been confirmed *in vitro*, several investigators have demonstrated that cancer patients with gain of maspin expression have a poor prognosis.²⁻³ Therefore, we propose an explanation for this apparently paradoxical expression of maspin protein. Maspin plays an important role in the establishment and/or maintenance of normal cells in a cell specific manner (breast and prostate).⁶ In tumours arising from these organs, maspin has tumour suppressive activity, such as inhibition of cell motility, invasion, and metastasis. Conversely, in gallbladder, pancreatic, and ovarian carcinomas, maspin shows aberrant expression.^{2-3, 8} In these tissues, maspin is usually repressed in a cell type restricted manner, and the maspin gene is expressed aberrantly when differentiated normal epithelial cells transform to metaplastic or dysplastic cells. Tumour cells derived from these tissues are disrupted in a cell type restricted manner. In addition, Sood *et al*² have reported that nuclear subcellular localisation of maspin protein was frequently observed in tumours with low malignant potential, such as ovarian tumours, and that invasive tumours also showed cytoplasmic staining for maspin.² The authors hypothesised that the maspin protein might act as β -catenin in the Wnt signalling pathway.

Definitive conclusions about the relationship between morphological transformation of cells and the subcellular localisation of maspin, and that between aberrant protein expression and epigenetic instability in areas of the gallbladder showing metaplastic formation, will require a more functional investigation of the maspin gene.

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TAKE HOME MESSAGES

- Aberrant expression of maspin protein is frequently observed in gallbladder carcinomas, but not in non-cancerous gallbladder epithelium.
- Using immunohistochemistry on samples from 69 patients with cholelithiasis and 30 patients with gastric cancer without cholelithiasis, immunoreactivity for maspin was observed in regions of the gallbladder epithelium.
- Positive immunoreactivity for maspin was significantly associated with the presence of intestinal metaplasia in patients with cholelithiasis ($p < 0.05$)
- This study supports the assumption that intestinal metaplasia of the gallbladder may predispose to gallbladder carcinoma.

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REFERENCES

- 1 Zou Z, Anisowicz A, Hendrix MJ, *et al*. Maspin, a serpin with tumor-suppressing activity in human mammary epithelial cells. *Science* 1994;**263**:526-9.
- 2 Sood AK, Fletcher MS, Gruman LM, *et al*. The paradoxical expression of maspin in ovarian carcinoma. *Clin Cancer Res* 2002;**8**:2924-32.
- 3 Maass N, Hojo T, Ueding M, *et al*. Expression of the tumor suppressor gene maspin in human pancreatic cancers. *Clin Cancer Res* 2001;**7**:812-17.
- 4 Akiyama Y, Maesawa C, Ogasawara S, *et al*. Cell-type-specific repression of the maspin gene is disrupted frequently by demethylation at the promoter region in gastric intestinal metaplasia and cancer cells. *Am J Pathol* 2003;**163**:1911-19.
- 5 Ogasawara S, Maesawa C, Yamamoto M, *et al*. Disruption of cell-type-specific methylation at the maspin gene promoter is frequently involved in undifferentiated thyroid cancers. *Oncogene* 2004;**23**:1117-24.
- 6 Futscher BW, Oshiro MM, Wozniak RJ, *et al*. Role for DNA methylation in the control of cell type specific maspin expression. *Nat Genet* 2002;**31**:175-9.
- 7 Smith SL, Watson SG, Ratschiller D, *et al*. Maspin – the most commonly-expressed gene of the 18q21.3 serpin cluster in lung cancer – is strongly expressed in preneoplastic bronchial lesions. *Oncogene* 2003;**22**:8677-87.
- 8 Fujisawa K, Maesawa C, Sato R, *et al*. Epigenetic status and aberrant expression of the maspin gene in human hepato-biliary tract carcinomas. *Lab Invest* 2004;**85**:214-24.
- 9 Fitzgerald M, Oshiro M, Holtan N, *et al*. Human pancreatic carcinoma cells activate maspin expression through loss of epigenetic control. *Neoplasia* 2003;**5**:427-36.
- 10 Kozuka S, Hachisuka K. Incidence by age and sex of intestinal metaplasia in the gallbladder. *Hum Pathol* 1984;**15**:779-84.
- 11 Duarte I, Llanos O, Domke H, *et al*. Metaplasia and precursor lesions of gallbladder carcinoma. Frequency, distribution, and probability of detection in routine histologic samples. *Cancer* 1993;**72**:1878-84.
- 12 Ishikawa A, Sasaki M, Ohira S, *et al*. Aberrant expression of CDX2 is closely related to the intestinal metaplasia and MUC2 expression in intraductal papillary neoplasm of the liver in hepatolithiasis. *Lab Invest* 2004;**84**:629-38.
- 13 Sasaki M, Tsuneyama K, Nakanuma Y. Aberrant expression of trefoil factor family 1 in biliary epithelium in hepatolithiasis and cholangiocarcinoma. *Lab Invest* 2003;**83**:1403-13.
- 14 Wu XS, Akiyama Y, Igari T, *et al*. Expression of homeodomain protein CDX2 in gallbladder carcinomas. *J Cancer Res Clin Oncol* 2005;**131**:271-8.
- 15 Yuasa Y, Nagasaki H, Akiyama Y, *et al*. Relationship between CDX2 gene methylation and dietary factors in gastric cancer patients. *Carcinogenesis* 2005;**26**:193-200.