

Overexpression of $\beta 3/\gamma 2$ chains of laminin-5 and MMP7 in biliary cancer

Toshikuni Oka, Hiroyuki Yamamoto, Shigeru Sasaki, Masanori Ii, Keiichi Hizaki, Hiroaki Taniguchi, Yasushi Adachi, Kohzoh Imai, Yasuhisa Shinomura

Toshikuni Oka, Hiroyuki Yamamoto, Shigeru Sasaki, Masanori Ii, Keiichi Hizaki, Hiroaki Taniguchi, Yasushi Adachi, Yasuhisa Shinomura, First Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo 060-8543, Japan

Kohzoh Imai, Sapporo Medical University, Sapporo 060-8556, Japan

Author contributions: Oka T and Yamamoto H designed the research; Oka T, Yamamoto H, Sasaki S, Ii M, Hizaki K, Taniguchi H and Adachi Y performed the research; Oka T, Yamamoto H, Imai K and Shinomura Y analyzed the data; Oka T, Yamamoto H and Shinomura Y wrote the paper.

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Correspondence to: Hiroyuki Yamamoto, MD, FJSIM, PhD, First Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo 060-8543, Japan. h-yama@sapmed.ac.jp

Telephone: +81-11-6112111 Fax: +81-11-6112282

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Abstract

AIM: To clarify the clinicopathological significance of laminin-5 $\gamma 2$ (LN $\gamma 2$) and $\beta 3$ (LN $\beta 3$) chains and MMP7 expression in biliary tract cancer.

METHODS: We analyzed the association between immunohistochemically detected LN $\gamma 2$, LN $\beta 3$, and MMP7 expression in biliary tract cancer and clinicopathological characteristics. Activity of MMP7 was analyzed by casein zymography. An *in vitro* invasion assay after treatment with MMP7-specific siRNA was performed.

RESULTS: LN $\gamma 2$ expression was predominantly observed in carcinoma cells at the invasive front. LN $\gamma 2$ expression was seen in 57% of patients with biliary tract cancer, and was associated with depth of invasion, histologic type, and advanced stage. The expression pattern of LN $\beta 3$ was classified into two types: invasive front dominant type (38%) and diffuse type (28%).

The invasive front dominant type was associated with histologic type and advanced stage. MMP7 positivity was correlated with LN $\gamma 2$ or LN $\beta 3$ expression but not with clinicopathological characteristics. Active MMP7 detected by casein zymography was correlated with depth of invasion and advanced stage. Downregulation of MMP7 expression by siRNA resulted in a significant decrease in biliary tract cancer cell invasion *in vitro*.

CONCLUSION: Our results suggest that LN $\gamma 2$ and LN $\beta 3$, in conjunction with MMP7, play a key role in the progression of biliary tract cancer.

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Key words: Biliary tract cancer; Laminin-5; Laminin $\gamma 2$; Laminin $\beta 3$; MMP7

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INTRODUCTION

Despite recent advances in diagnosis and treatment, the prognosis of patients with biliary tract cancer is still poor. Surgical resection is possible in only a small proportion of patients^[1,2]. Consequently, elucidating the biological characteristics of these carcinomas has become necessary to improve the prognosis of patients and to devise better treatment strategies.

Laminins are components of the extracellular matrix (ECM) that contribute to the architecture of the basal lamina surrounding the epithelial cells and mediate cell adhesion, growth, migration, proliferation, and differentiation. Laminins are heterotrimeric glycoproteins composed of three different polypeptide

chains (α , β and γ) arranged in a cruciform structure. A separate gene encodes each polypeptide chain and different combinations of these chains lead to the 15 different laminin isoforms^[3-5]. Laminin-5/laminin-332 (LN5), consists of $\alpha 3$, $\beta 3$, and $\gamma 2$ chains, which are encoded by three distinct genes (*LAMA3*, *LAMB3*, and *LAMC2*, respectively)^[6]. LN5 has been shown to promote the adhesion, migration, and scattering of a variety of cultured cells, mainly through integrin $\alpha 3\beta 1$, more strongly than other ECM proteins^[7]. Moreover, in hepatocellular carcinoma (HCC), LN5 reportedly plays an important role in epithelial mesenchymal transition through down-regulation of E-cadherin and translocation of β -catenin into the nuclei^[8].

Expression of the three subunits of LN5 is regulated differentially in cancer cell lines and in normal and malignant tissues including HCC^[9-11]. Indeed, LN $\gamma 2$ has been shown to be secreted as a single subunit in gastric cancer^[12]. Several lines of evidence suggest that the tumor-derived LN $\gamma 2$ contributes to invasion of tumor cells. LN $\gamma 2$ expression has been immunohistochemically detected in various types of carcinomas, such as HCC, colorectum, stomach, and esophagus^[9,12-16]. It is notable that LN $\gamma 2$ has been predominantly detected at the invasive front, where tumor cells with the most aggressive phenotype are localized^[17].

Degradation of ECM components is mostly controlled by proteolytic enzymes called matrix metalloproteinases (MMPs)^[18]. Specific cleavage of LN5 ($\gamma 2$ subunit at residue 587) by MMP2 has been shown to induce migration of breast epithelial cells^[19]. This altered form of LN5 was found in tumors and in tissues undergoing remodeling, but not in quiescent tissues. LN5 is also converted into a migration-promoting substrate by MT1-MMP^[20]. MMP7, also known as matrilysin, is a "minimal domain MMP" that exhibits broad proteolytic activity against components of the ECM and non-ECM^[18]. MMP7 is often overexpressed at the invasive front in various types of human cancer and is associated with cancer progression^[21,22]. Both LN $\gamma 2$ and MMP7 are targets of the Wnt/ β -catenin pathway^[23,24].

Although there are only a few reports regarding LN $\beta 3$ expression in human cancer, coexpression of LN $\gamma 2$ and LN $\beta 3$ has been reported in HCC, squamous cell carcinoma of the tongue and colorectal carcinoma and basal cell carcinoma of the skin^[9,25,26]. It has recently been reported that human LN5 is a ligand for MMP7 and that a specific cleavage occurs in its $\beta 3$ chain^[27]. These results are interesting because MMP7 is overexpressed in HCC and colorectal carcinoma^[18,28]. However, expression of LN5 and MMP7 in biliary tract cancer has not been clearly addressed.

To clarify the possible involvement of LN5 and MMP7 in the progression of biliary tract cancer, we immunohistochemically analyzed these expressions in 61 primary biliary tract cancer. Activity of MMP7 was analyzed by casein zymography. An *in vitro* invasion assay of biliary tract cancer cell lines after treatment with MMP7-specific siRNA was performed.

MATERIALS AND METHODS

Cell lines and tissue samples

Human bile duct cancer cell lines (TFK-1, HuH-28, and MEC) were obtained from Cell Resource Center for Biomedical Research, Tohoku University. Bile duct cancer cell line TKKK and gallbladder carcinoma cell lines TGBC1TKB, TGBC2TKB, and TGBC14TKB were purchased from Riken Cell Bank (Tsukuba, Japan). Cells were cultured in RPMI1640 or DMEM supplemented with 10% fetal bovine serum. Formalin-fixed, paraffin-embedded sections of 61 biliary tract carcinomas (30 extrahepatic bile duct carcinomas, 18 gallbladder carcinomas, 13 carcinomas of the ampulla of Vater) were used for immunohistochemically. Sections containing the most invasive part of each tumor were used. Fresh specimens of extrahepatic bile duct carcinoma ($n = 10$), gallbladder carcinoma ($n = 7$), and carcinoma of the ampulla of Vater ($n = 3$) were obtained from patients who had undergone surgical treatment. Specimens were immediately frozen in liquid nitrogen at the time of surgery and stored at -80°C . Each tissue specimen was used for casein zymography. Histopathological features of the specimens were classified according to the pathological tumor-node-metastasis (TNM) classification system of the International Union Against Cancer. Informed consent was obtained from each subject and the institutional review committee approved the experiments.

Semi-quantitative reverse transcriptase-PCR (RT-PCR) and real-time RT-PCR

Semi-quantitative RT-PCR was carried out as described previously^[29]. Total RNA was extracted from cell lines using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. cDNA was synthesized from 1 μg of total RNA using SuperScript III reverse transcriptase (Invitrogen) with random hexamers. PCR was performed using primers specific for the *LAMA3*, *LAMB2* and *LAMC2* gene and the *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* gene. *GAPDH* served as an internal control of the reaction. Standard curves for semiquantitative RT-PCR were drawn as described previously^[30]. All reactions were carried out at least in duplicate and controlled without reverse transcriptase. PCR products were electrophoresed in 2% agarose gels. Real-time RT-PCR was performed using the TaqMan real-time PCR system as described previously^[31].

Immunohistochemistry

Immunohistochemistry was carried out as described previously^[32]. The antibodies used were as follows: anti-LN $\alpha 3$ rabbit polyclonal antibody (1/100 dilution, Santa Cruz, CA, USA), anti-laminin5 ($\gamma 2$ chain) mouse monoclonal antibody (1/50 dilution, Chemicon, Temecula, CA, USA), anti-LN $\beta 3$ rabbit polyclonal antibody (1/100 dilution, Santa Cruz), and anti-MMP7 mouse monoclonal antibody (1/50 dilution, Daiichi Fine Chemical, Takaoka, Japan). Normal mouse or rabbit

immunoglobulins were substituted for each primary antibody as negative controls. Cytoplasmic expression was defined as positive when immunoreactivity was observed in more than 10% of carcinoma cells. We defined the cells at the deepest invading part of the tumor as the invasive front.

Casein zymography

Casein zymography was performed as previously described with some modifications^[28]. Tissue extracts were electrophoresed on 8% polyacrylamide gel containing 1 mg/mL casein. After electrophoresis, gels were washed in 2.5% Triton-X 100 and incubated for 48 h at 37°C in 50 mmol/L Tris-HCl (pH 7.4), 10 mmol/L CaCl₂, 1 mmol/L ZnCl₂, and 0.02% NaN₃, followed by staining with 0.1% Coomassie brilliant blue.

siRNA transfection

siRNA transfection was performed as described previously^[33]. Levels of MMP7 inhibition were analyzed by RT-PCR and Western blotting. siRNA-transfected cells were used for the *in vitro* invasion assay.

In vitro invasion assay

Assays were performed by the modified Boyden Chamber method as described previously^[34]. Assays were also performed with 250 ng/mL of TIMP1, an MMP inhibitor. The results were presented as means ± SD for each sample.

Statistical analysis

Expression was assessed for associations with clinicopathological parameters using the chi-square two-tailed test or Fisher's exact test. A *P* value < 0.05 was considered statistically significant. A *P* value between 0.05 and 0.10 was considered as a trend toward an association.

RESULTS

Expression of LN α 3, LN β 3 and LN γ 2, and MMP7 in cell lines

Expression levels of LN α 3, LN β 3 and LN γ 2, and MMP7 in cancer cell lines were analyzed using semi-quantitative RT-PCR (Figure 1). LN α 3 mRNA was detected in all seven cell lines but at very low levels in TGBC-14TKB. LN β 3 mRNA was detected in six cell lines but at very low levels in MEC and TKKK. LN γ 2 mRNA was detected in all seven cell lines but at very low levels in MEC. TFK-1, HuH-28, TGBC-1TKB, and TGBC-2TKB expressed considerable amounts of all 3 chains of LN5. MMP7 mRNA was detected in all seven cell lines. There were no significant correlations between these expression patterns and characteristics of the cell lines. Similar data were observed by real-time RT-PCR (data not shown).

Overexpression of LN γ 2, LN β 3, and MMP7 in biliary tract cancer tissues

Expression of LN α 3 was detected in normal basement

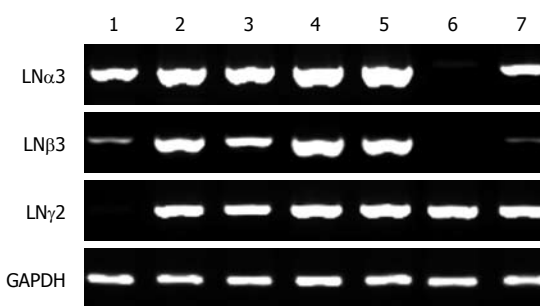


Figure 1 RT-PCR analysis of the LN α 3, LN β 3, LN γ 2 genes in biliary tract cancer cell lines. 1: MEC; 2: TFK-1; 3: HuH28; 4: TGBC1TKB; 5: TGBC2TKB; 6: TGBC14TKB; 7: TKKK.

membranes but not in carcinoma cells (data not shown). Figure 2 shows representative results of immunohistochemistry for LN γ 2 in biliary tract cancer. In carcinoma tissues, the cytoplasm of carcinoma cells was stained for LN γ 2 at levels much stronger than those in normal basement membranes. The cytoplasmic immunoreactivity was often more intense at the invasive front. Cancer cells budding or dissociating from the tumor nests showed intense cytoplasmic staining. Sections with immunostaining signals in over 10% of carcinoma cells, which were observed in 35 (57%) of 61 cases, were judged to be positive for LN γ 2. LN γ 2 positivity was 67% in extrahepatic bile duct cancer, 50% in gallbladder cancer, and 46% in carcinoma of the ampulla of Vater. Figure 3 shows representative results of immunohistochemistry for LN β 3 in biliary tract cancer. In carcinoma tissues, the cytoplasm of carcinoma cells was stained for LN β 3 at levels much stronger than those in normal basement membranes. The expression pattern of LN β 3 was classified into 2 patterns: invasive front dominant pattern and diffuse pattern. The invasive front dominant pattern and diffuse pattern were observed in 23 (38%) and 17 (28%) of 61 cases, respectively. Positivity for invasive front dominant pattern and diffuse pattern was 47% and 17% in extrahepatic bile duct cancer, 28% and 39% in gallbladder cancer, and 31% and 38% in carcinoma of the ampulla of Vater. Figure 4 shows representative results of immunohistochemistry for MMP7 in biliary tract cancer tissues. MMP7 immunoreactivity was intense at the invasive front in several cases. In general, MMP7 immunoreactivity was diffuse rather than invasive front dominant like LN γ 2. Sections with immunostaining signals in over 10% of carcinoma cells, which were observed in 42 (69%) of 61 cases, were judged to be positive for MMP7. MMP7 positivity was 80% in extrahepatic bile duct cancer, 50% in gallbladder cancer, and 69% in carcinoma of the ampulla of Vater.

Association of LN γ 2, LN β 3, and MMP7 expression with clinicopathological characteristics

The relationship between LN γ 2 positivity and clinicopathological characteristics is summarized in Table 1. LN γ 2 positivity was significantly correlated with histologic type (less differentiated type), depth of invasion

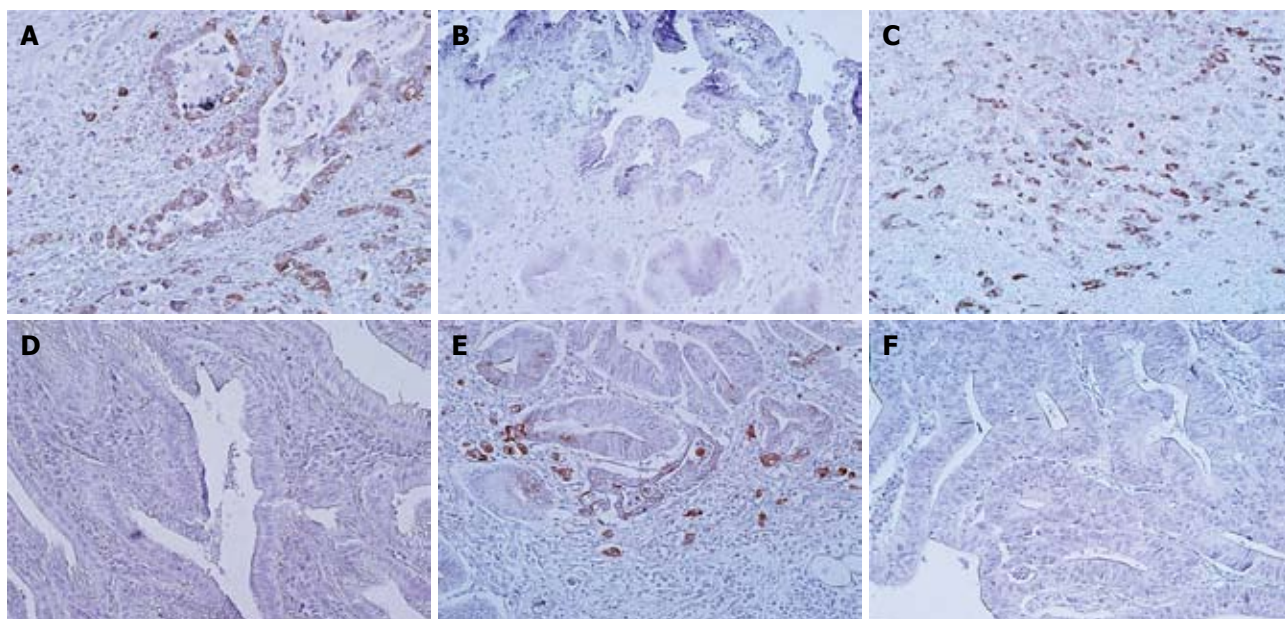


Figure 2 LN γ 2 expression in biliary tract cancer tissues. A, B: Extrahepatic bile duct cancer tissues; A: Moderately differentiated tubular adenocarcinoma positive for staining. Note that LN γ 2 is strongly positive in tumor cells at the invasive front; B: Papillary adenocarcinoma negative for staining; C, D: Gallbladder cancer tissues; C: Moderately differentiated tubular adenocarcinoma positive for staining; D: Well differentiated tubular adenocarcinoma negative for staining; E, F: Carcinoma tissues of the ampulla of Vater; E: Moderately differentiated tubular adenocarcinoma positive for staining. Note that LN γ 2 is strongly positive in tumor cells at the invasive front; F: Well differentiated tubular adenocarcinoma negative for staining.

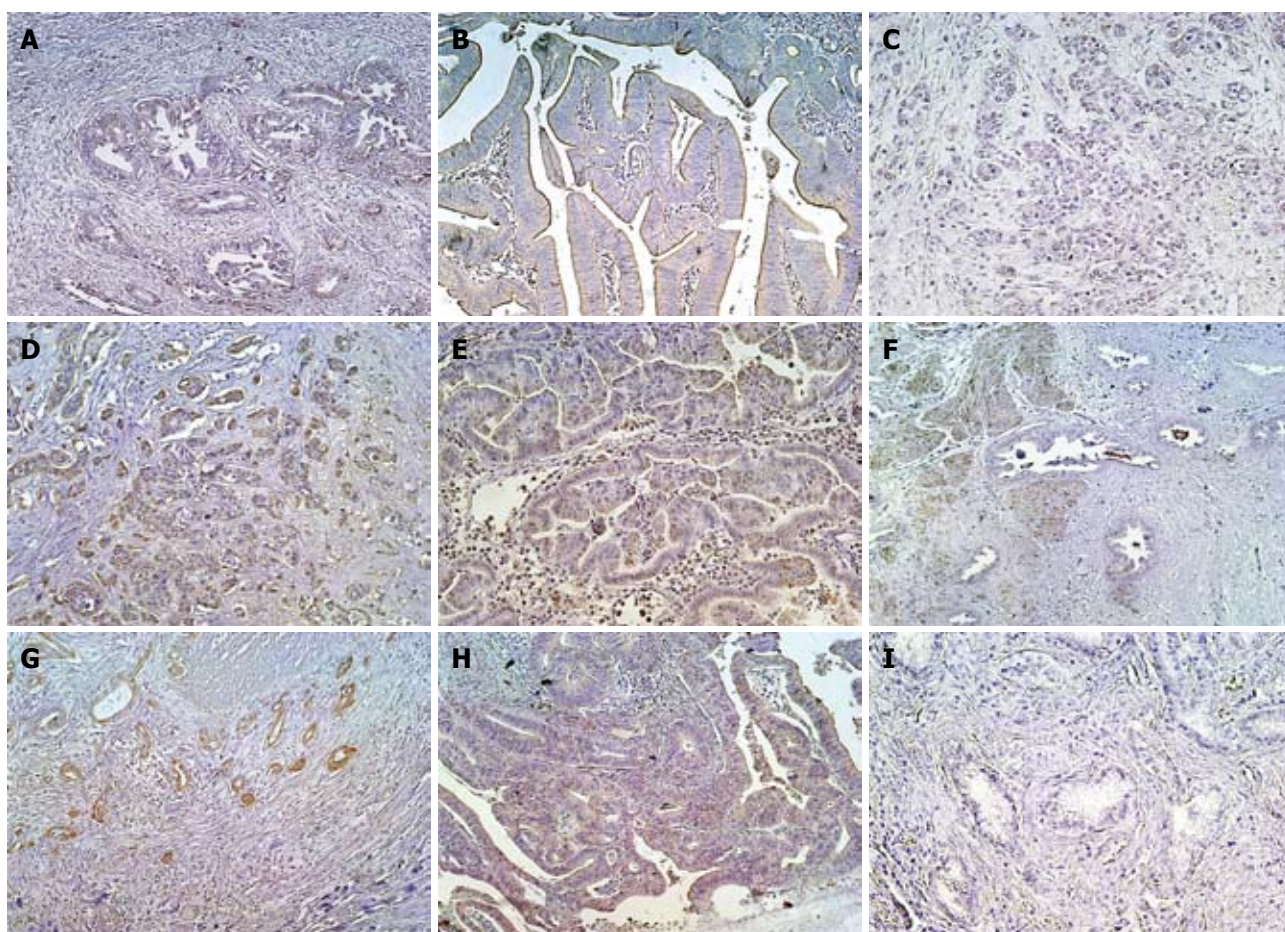


Figure 3 LN β 3 expression in biliary tract cancer tissues. A-C: Extrahepatic bile duct cancer tissues; A: Well differentiated tubular adenocarcinoma positive for invasive front dominant staining; B: Papillary adenocarcinoma positive for diffuse staining; C: Moderately differentiated tubular adenocarcinoma negative for staining; D-F: Gallbladder cancer tissues; D: Moderately differentiated tubular adenocarcinoma positive for invasive front dominant staining; E: Papillary adenocarcinoma positive for diffuse staining; G-I: Carcinoma tissues of the ampulla of Vater; G: Well differentiated tubular adenocarcinoma positive for invasive front dominant staining; H: Well differentiated tubular adenocarcinoma positive for diffuse staining; I: Well differentiated tubular adenocarcinoma negative for staining.

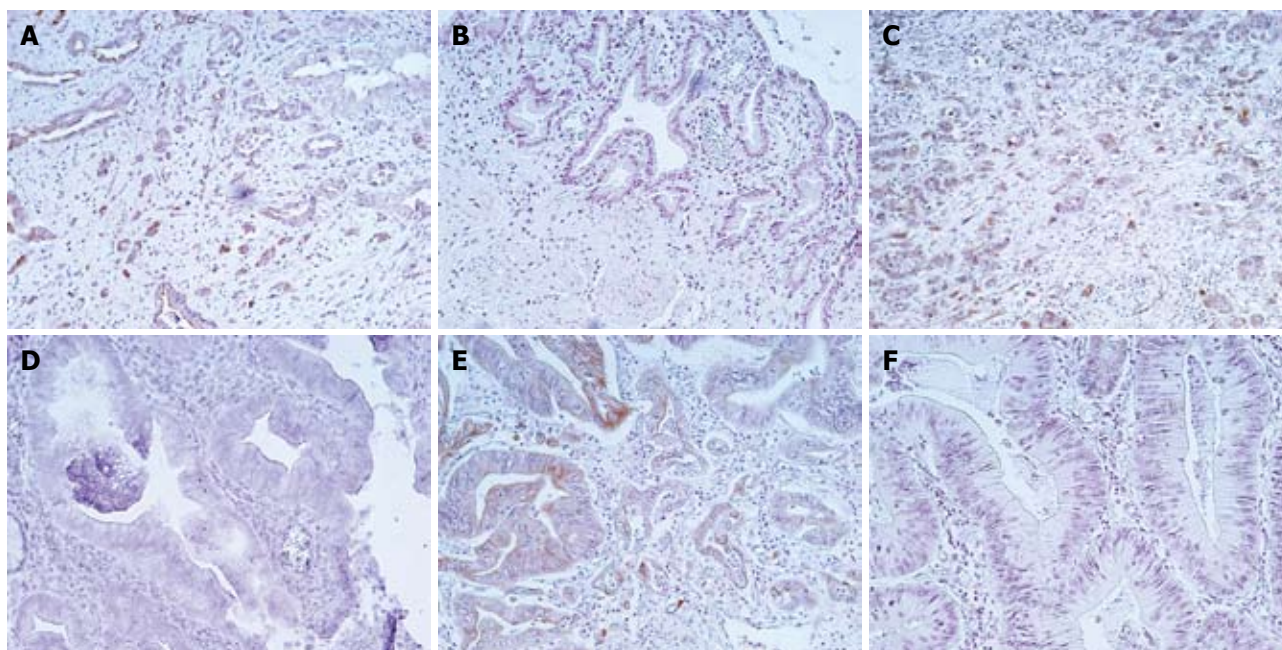


Figure 4 MMP7 expression in biliary tract cancer tissues. A, B: Extrahepatic bile duct cancer tissues; A: Moderately differentiated tubular adenocarcinoma positive for staining. Note that MMP7 is strongly positive in tumor cells at the invasive front; B: Papillary adenocarcinoma negative for staining; C, D: Gallbladder cancer tissues; C: Moderately differentiated tubular adenocarcinoma positive for staining; D: Well differentiated tubular adenocarcinoma negative for staining; E, F: Carcinoma tissues of the ampulla of Vater; E: Moderately differentiated tubular adenocarcinoma positive for staining. Note that MMP7 is strongly positive in tumor cells at the invasive front; F: Well differentiated tubular adenocarcinoma negative for staining.

(invasion into serosa), and advanced stage. The relationship between LN β 3 positivity and clinicopathological characteristics is summarized in Table 2. LN β 3 invasive front dominant pattern was significantly correlated with histologic type (less differentiated type) and advanced stage. There was a tendency that lymph node metastasis was more frequently observed in cases with LN β 3 invasive front dominant pattern than in other cases ($P = 0.063$). The relationship between MMP7 positivity and clinicopathological characteristics is summarized in Table 3. MMP7 positivity was not significantly correlated with clinicopathological characteristics. The expression of LN γ 2, LN β 3 invasive front dominant pattern, and MMP7 were correlated with each other (Tables 4-6).

MMP7 activity detected by zymography

Using casein zymography, the levels of secreted matrilysin were analyzed (Figure 5). Nontumorous tissues secreted neither latent (28 kDa) nor activated (19 kDa) MMP7 activity. Latent and activated forms of MMP7 were detected in 16 (80%) and 12 (60%) of 20 carcinoma tissues, respectively. The activity was eliminated by the addition of the metalloproteinase inhibitor EDTA (data not shown). The activated form but not the latent form was correlated with depth of invasion and advanced stage.

Suppression of cancer cell invasiveness by MMP7 siRNA treatment

In vitro invasion assays after treatment with specific siRNA for the MMP7 gene were carried out to assess the direct role of the expression of MMP7 in cancer

Table 1 Correlation between laminin γ 2 staining and clinicopathologic factors n (%)

	Case ($n = 61$)	Laminin γ 2		P value
		Positive ($n = 35$)	Negative ($n = 26$)	
Age (yr)				0.47
≤ 65	25 (41)	15 (60)	10 (40)	
> 65	36 (59)	20 (56)	16 (44)	
Gender				0.29
Male	41 (67)	25 (61)	16 (39)	
Female	20 (33)	10 (50)	10 (50)	
Location				0.34
Bile duct	30 (49)	20 (67)	10 (33)	
Gall bladder	18 (30)	9 (50)	9 (50)	
Ampulla of Vater	13 (21)	6 (46)	7 (54)	
Maximum diameter (mm)				0.29
< 30	41 (67)	25 (61)	16 (39)	
≥ 30	20 (33)	10 (50)	10 (50)	
Histologic type				0.03
Tub1 & pap	35 (57)	16 (46)	19 (54)	
Others	26 (43)	19 (73)	7 (27)	
Depth				0.02
Serosa negative	37 (61)	17 (46)	20 (54)	
Serosa positive	24 (39)	18 (75)	6 (25)	
Lymph node metastasis				0.37
Absent	29 (48)	16 (55)	13 (45)	
Present	27 (44)	17 (63)	10 (37)	
Stage				0.046
< IV	43 (70)	21 (49)	22 (51)	
≥ IV	17 (28)	13 (76)	4 (24)	

cell invasiveness. Transfection efficiency determined by fluorescein isothiocyanate-labeled oligonucleotide uptake was $84\% \pm 6\%$ in TFK-1 cells and $86\% \pm 5\%$ in TGBC-

Table 2 Correlation between parameters and the pattern of laminin $\beta 3$ expression n (%)

	Total ($n = 61$)	Laminin $\beta 3$			P value
		Invasive ($n = 23$)	Diffuse ($n = 17$)	Negative ($n = 21$)	
Age (yr)					
≤ 65	25 (41)	9 (36)	8 (32)	8 (32)	0.83
> 65	36 (59)	14 (39)	9 (25)	13 (36)	
Gender					
Male	41 (67)	17 (41)	8 (20)	16 (39)	0.11
Female	20 (33)	6 (30)	9 (45)	5 (25)	
Location					
Bile duct	30 (49)	14 (47)	5 (17)	11 (37)	0.40
Gall bladder	18 (30)	5 (28)	7 (39)	6 (33)	
Ampulla of Vater	13 (21)	4 (31)	5 (38)	4 (31)	
Maximum diameter (mm)					
< 30	40 (66)	18 (45)	10 (25)	12 (30)	0.27
≥ 30	21 (34)	5 (24)	7 (33)	9 (43)	
Histologic type					
Tub1 & pap	35 (57)	9 (26)	14 (40)	12 (34)	0.02
Others	26 (43)	14 (54)	3 (12)	9 (35)	
Depth					
Serosa negative	37 (61)	11 (30)	13 (35)	13 (35)	0.18
Serosa positive	24 (39)	12 (50)	4 (17)	8 (33)	
Lymph node metastasis					
Absent	29 (48)	8 (28)	11 (38)	10 (34)	0.13
Present	27 (44)	14 (52)	5 (19)	8 (30)	
Stage					
< IV	43 (70)	10 (23)	15 (35)	18 (42)	0.003
≥ IV	17 (28)	12 (71)	2 (12)	3 (18)	

Table 3 Correlation between MMP7 staining and clinicopathologic factors n (%)

	Case ($n = 61$)	MMP7		P value
		Positive ($n = 42$)	Negative ($n = 19$)	
Age (yr)				
≤ 65	25 (42)	17 (68)	8 (32)	0.66
> 65	36 (58)	25 (69)	11 (31)	
Gender				
Male	41 (67)	30 (73)	11 (27)	0.23
Female	20 (33)	12 (60)	8 (40)	
Location				
Bile duct	30 (49)	24 (80)	6 (20)	0.09
Gall bladder	18 (30)	9 (50)	9 (50)	
Ampulla of Vater	13 (21)	9 (69)	4 (31)	
Maximum diameter (mm)				
< 30	41 (67)	29 (71)	12 (29)	0.43
≥ 30	20 (33)	13 (65)	7 (35)	
Histologic type				
Tub1 & pap	35 (57)	23 (66)	12 (34)	0.37
Others	26 (43)	19 (73)	7 (27)	
Depth				
Serosa negative	37 (61)	25 (68)	12 (32)	0.58
Serosa positive	24 (39)	17 (71)	7 (29)	
Lymph node metastasis				
Absent	29 (48)	21 (72)	8 (28)	0.43
Present	27 (44)	18 (67)	9 (33)	
Stage				
< IV	43 (70)	31 (72)	12 (28)	0.24
≥ IV	17 (28)	10 (59)	7 (41)	

Table 4 Expression of laminin $\gamma 2$ and MMP7 in biliary tract carcinoma n (%)

MMP7 expression	Laminin $\gamma 2$		P value
	Positive ($n = 42$)	Negative ($n = 19$)	
Positive ($n = 34$)	28 (46)	6 (10)	0.01
Negative ($n = 27$)	14 (23)	13 (21)	

Table 5 Expression of laminin $\beta 3$ and MMP7 in biliary tract carcinoma n (%)

MMP7 expression	Laminin $\beta 3$			P value
	Invasive ($n = 23$)	Diffuse ($n = 17$)	Negative ($n = 21$)	
Positive ($n = 34$)	17 (28)	6 (10)	14 (23)	0.037
Negative ($n = 27$)	6 (10)	11 (18)	7 (11)	

Table 6 Expression of laminin $\beta 3$ and laminin $\gamma 2$ in biliary tract carcinoma n (%)

Laminin $\gamma 2$ expression	Laminin $\beta 3$			P value
	Invasive ($n = 23$)	Diffuse ($n = 17$)	Negative ($n = 21$)	
Positive ($n = 42$)	18 (30)	8 (13)	9 (15)	0.036
Negative ($n = 19$)	5 (8)	9 (15)	12 (20)	

2TKB cells (data not shown). Transfection with siRNA resulted in over 80% inhibition of mRNA and protein expression (data not shown). Transfection with *MMP7*-

specific siRNA decreased invasiveness of TFK-1 cells compared with control siRNA-transfected counterparts ($P < 0.01$, Figure 6). This difference was significantly diminished by the addition of TIMP1. Similar results were observed in *MMP7*-specific siRNA-transfected TGBC-2TKB cells (data not shown).

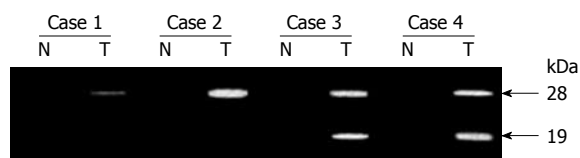


Figure 5 Casein zymography of surgical specimen pairs of biliary tract carcinoma and adjacent nontumor tissue. N and T: Matched samples from nontumor and tumor tissue, respectively.

DISCUSSION

In the current study, LN γ 2 positivity in carcinoma cells at the invasive front was immunohistochemically observed in 57% of patients with biliary tract cancer, and was associated with histologic type (less differentiated type), depth of invasion, and advanced stage. LN β 3 invasive front dominant pattern was immunohistochemically observed in 38% of patients with biliary tract cancer, and was associated with histologic type (less differentiated type) and advanced stage. These results suggest that LN γ 2 and LN β 3 expression in carcinoma cells at the invasive front contributes to the more aggressive phenotype of carcinoma cells, resulting in the progression of biliary tract cancer.

Preferential expression of LN γ 2 and LN β 3 in carcinoma cells at the invasive front and its correlation with tumor progression suggest that these molecules play a role in the acquisition of a migrating and invading epithelial cell phenotype that is a prerequisite for malignancy^[13]. Also in metastatic HCC, LN5 was mainly distributed along the tumoral advancing edge^[9]. The mechanism underlying the preferential distribution of LN γ 2 and LN β 3 at the invasive front in cancer is not known. It is known that activation of cancer-related genes in carcinoma cells affects their associated stromal cells. Certain stromal cell populations lying close to carcinoma cells may be induced to assist the invasion process by signals sent out by the cancer cells, stimulating the synthesis of gene products that facilitate cancer cell invasion and migration^[35]. Interactions of carcinoma cells with stromal cells or with the surrounding extracellular matrix at the invasive front may result in an accumulation of LN γ 2 and LN β 3 at the invasive front, where they may play a direct role in tumor invasion processes^[19].

Although there are only a few reports regarding LN β 3 expression in human cancer, coexpression of LN γ 2 and LN β 3 has been reported in HCC, squamous cell carcinoma of the tongue, colorectal carcinoma and basal cell carcinoma of the skin^[9,25,26]. Sordat *et al.*^[14] reported that the heterodimer of the LN γ 2 and LN β 3 chains is accumulated in the cytoplasm of dissociating (or budding) tumor cells from neoplastic tubules of colon carcinomas. Since LN γ 2 and LN β 3 were not always coexpressed in biliary tract cancer, further analysis is necessary to elucidate the mechanism of overexpression and localization of LN γ 2 and LN β 3 in biliary tract cancer. LN5 reportedly plays an important role in epithelial mesenchymal transition through down-regulation of E-cadherin and translocation of β -catenin

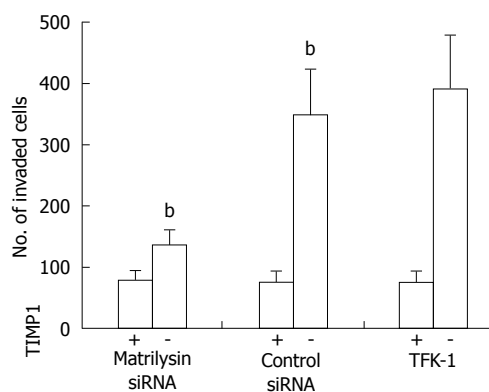


Figure 6 *In vitro* invasion assay with or without TIMP1 (250 ng/mL) in TFK-1 and siRNA transfectants. Each column indicates the means of three experiments; ^b $P < 0.01$.

into the nuclei^[8]. It will be interesting to address this issue in biliary tract cancer in the near future.

In contrast to preferential expression of MMP7 in carcinoma cells at the invasive front in various carcinomas, the expression pattern of MMP7 was diffuse in biliary tract cancer. The mechanism underlying the differential distribution of MMP7 in carcinomas needs to be further analyzed. MMP7 positivity was not significantly correlated with clinicopathological characteristics. However, the expression of LN γ 2, LN β 3 invasive front dominant pattern, and MMP7 were correlated with each other. It has been suggested that the controlled up-regulation of gene products is one of the characteristics of invading cancer cells and that these gene products have functions crucial for the invasive phenotype of cancer cells^[13]. It is notable that limited proteolysis of LN β 3 by MMP7 increases the cell motility activity of LN5 in colon carcinoma cells^[27].

The activated form but not the latent form of MMP7 was correlated with depth of invasion and advanced stage, suggesting that active MMP7 plays an important role in the progression of biliary tract cancer. The implication of up-regulation of MMP7 expression in tumor progression was further substantiated by the *in vitro* invasion analysis. We revealed that down-regulation of MMP7 expression by siRNA resulted in a significant decrease in biliary tract cancer cell invasion *in vitro*, suggesting that up-regulation of MMP7 contributes to the more invasive phenotype of biliary tract cancer cells. Taken together, our results suggest that LN γ 2 and LN β 3, in conjunction with MMP7, play a key role in the progression of biliary tract cancer.

COMMENTS

Background

Biliary tract cancers are relatively rare human malignancies involving the gallbladder and/or the bile ducts, but the prognosis is poor. Understanding the molecular biological features of biliary tract cancer progression is necessary for improving the prognosis. The potential role of laminin-5 (LN5) and MMP7 in human cancer is receiving increasing attention.

Research frontiers

Altered expression patterns of LN5, especially the LN γ 2 chain, and MMP7 have been correlated with tumor behavior, such as invasiveness, vascularization, metastatic potential, and patients' poor prognosis. However, expression of LN5

and MMP7 in biliary tract cancer has not been clearly addressed. In this study, the authors demonstrate that LN γ 2, LN β 3, and active MMP7 play a key role in the progression of biliary tract cancer.

Innovations and breakthroughs

This is the first study to report that invasive front dominant expression of LN γ 2 and LN β 3, and active MMP7 play a key role in the progression of biliary tract cancer. Furthermore, our *in vitro* studies suggest that MMP7 plays an important role in biliary tract cancer cell invasion.

Applications

Detection of LN γ 2, LN β 3, and active MMP7 could be molecular markers for tumor aggressiveness in biliary tract cancer. Understanding how LN γ 2, LN β 3, and active MMP7 are induced and how their expression is blocked may represent a future strategy for therapeutic intervention in the treatment of patients with biliary tract cancer.

Terminology

Laminin: A heterotrimeric glycoprotein composed of three different polypeptide chains (α , β and γ), is a component of the extracellular matrix (ECM) that contributes to the architecture of the basal lamina surrounding the epithelial cells and mediates cell adhesion, growth, migration, proliferation, and differentiation. LN-5/LN-332: LN5, consists of α 3, β 3 and γ 2 chains, and is involved in cell adhesion, migration, and scattering. Altered expression of LN-5 plays an important role in cancer. MMP7: Degradation of ECM components is mostly controlled by proteolytic enzymes called MMP. MMP7, also known as matrilysin, is a minimal domain MMP that exhibits broad proteolytic activity against components of the ECM and non-ECM.

Peer review

This paper reports the expression of LN-5 chains and MMP-7 in biliary cancer. The authors showed that LN γ 2 and LN β 3, in conjunction with MMP7, play a key role in the progression of biliary cancer. The study sounds interesting and confirms the role of LN-5 and MMP7 in human cancer.

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