Review

Clinicopathology of sialomucin: MUC1, particularly KL-6 mucin, in gastrointestinal, hepatic and pancreatic cancers

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Summary **MUC1, membrane-associated mucins, has various types based on different glycoforms in its extracellular domain and is widely expressed in gastrointestinal tissues. Many investigations have showed that aberrant expression of MUC1 in gastrointestinal cancer tissue has clinicopathological and biological importance in cancer disease. KL-6 mucin, one kind of MUC1, was also investigated and suggested to have a significant relationship with a worse tumor behavior especially cancer cell invasion and metastasis in various gastrointestinal cancers. On the other hand, clinicopathological availability of KL-6 mucin varied among each gastrointestinal cancer. In colorectal and gastric cancer, circumferential membrane and/or cytoplasmic localization of KL-6 mucin were frequently detected in the cancer tissue of patients with the presence of deeper invasion and lymph node metastasis of cancer cells. Therefore, the subcellular localization of KL-6 mucin in cancer tissues can be used for predicting a worse outcome for patients. In primary liver cancer, KL-6 mucin expression was detected in cholangiocarcinoma but not in hepatocellular carcinoma tissues. Therefore, it can be used as a good marker for discriminating cholangiocarcinoma from hepatocellular carcinoma. While various significant clinicopathological detections were clarified, the nature of KL-6 mucin is not yet clearly known. Alteration in expression or glycoform of KL-6 mucin is suggested to influence the invasive and adhesive ability of cancer cells. To clarify the characteristics and biological functions of KL-6 mucin in cancer disease, the clinical applications and study of this antigen is expected to be expanded.**

Keywords: Tumor marker, MUC1, KL-6, gastrointestinal cancer, hepatic cancer

1. Introduction: What is KL-6 mucin?

Invasion and metastasis have been the main malignant factors of cancer medicine in spite of the development of therapeutic technology including surgery. A number of studies have been performed to clarify the mechanism of these events from various perspectives and have produced innovations for cancer therapy such as the development of new anticancer drugs.

Carbohydrate moieties on cell surfaces change dramatically during oncogenesis (*1*). In particular, sialylation, the moiety of silalic acid, is considered to play an important role in tumor progression, and some studies suggest that aberrant expression of sialoglycoconjugates might relate to the process of metastasis such as the decline of adhesiveness (*2,3*). In Japan, various sialic acid-related antigens are clinically available as markers for screening patients with gastrointestinal cancers (Table 1). Histochemical

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Table 1. Clinically available gastrointestinal tumor markers related to sialic acid

Tumor marker	Characteristics	Ref.
CEA	180 kDa sialoglycoconjugates with 24~26 oligosaccharide chains. Serological level is elevated in patients with various gastrointestinal cancers.	*4,5*
Sialyl-Le^a	CA19-9, CA50, KMO1, and Span-1 antigens are detected by mAb recognizing sialyl-Le^a-related structure. Serological levels of these antigens are elevated in patients with various gastrointestinal cancers.	*6-9*
Sialyl-Le^x	SLX and NCC-ST-439 antigens are detected by mAb recognizing sialyl-Le^x-related structure. Serological levels of these antigens are elevated in patients with various gastrointestinal cancers.	*10,11*
Sialyl-Le^c	DUPAN-2 antigen is detected by mAb recognizing sialyl-Le^c-related structure. Serological level is elevated in patients with hepatobiliary and pancreatic cancers.	*9,12*
Sialyl Tn	STN and CA72-4 antigens are detected by mAb recognizing sialyl Tn-related structure. Serological levels of these antigens are elevated in patients with various gastrointestinal cancers	*13,14*
CA125	CA125 antigen is MUC16, transmembranous mucin carrying sialo-oligosaccharides. Serological level is elevated significantly in patients with ovarian and uterus cancer but also elevated in hepatobiliary and pancreatic cancers.	*15,16*

Abbreviations: CA, carbohydrate antigen; CEA, carcinoembryonic antigen; mAb, monoclonal antibody; sialyl-Le^a, sialyl-Lewis a; sialyl-Le^c, sialyl-Lewis c; sialyl-Le^x, sialyl-Lewis x.

studies with sialic acid-binding lectins and/or antibodies against sialylated carbohydrate antigens also showed that sialylation of glycoconjugates on the surface of tumor cells is thought to contribute to tumor progression and metastasis (*17-20*). Overexpression of sialoglycoconjugates, or some specific structures of sialo-oligosaccharides, has important functions in cancer cell metastasis such as attachment to endothelial cells at a metastatic site while its clinical application is still being investigated.

Mucins are large glycoproteins with high carbohydrate content and marked diversity both in the apoprotein and in the oligosaccharide moieties (*21*). MUC1 mucin, one kind of mucin glycoprotein, is abundantly expressed at the surface of epithelial cells in many tissues (*22,23*). Because the MUC1 molecule has sialic acid-containing oligosaccharides in a highly *O*-glycosylated tandem-repeat domain, the structure has a wide range and some kinds have a large molecular weight (*24,25*). In normal cells, MUC1 is known to interact with various molecules and seems to influence various physiological or biochemical events, for example, diminishing immune response (*26*). Development of various kinds of antibodies against MUC1 has been helpful for detecting MUC1 expression histologically or serologically. MUC1 expression was also observed in carcinomas that arise in various gastrointestinal organs, and its overexpression as well as overall sialoglycoconjugates was suggested to associate with invasive and metastatic potency of several adenocarcinomas (*27-31*). The core peptide of MUC1 had significant functions in tumor metastasis (*32*), and histochemical overexpression of the core peptide of MUC1 also indicated a worse prognosis for various cancer patients (*33*). However, the MUC1 molecule has many oligosaccharides in the extracellular domain as described previously, and these oligosaccharide moieties have a great deal of variety. Therefore, the qualitative change of oligosaccharides in MUC1 has great importance. Although the processing of the full length MUC1 core protein is similar in both normal and tumor cells, there is a remarkable diversity in oligosaccharide moieties between normal and cancer cells (*34,35*). Thus, it has been considered to be important to detect the specifically structured MUC1 in cancer cells and to clarify its role and clinical significance.

KL-6 mucin is a type of MUC1 mucin, recognized by a murine monoclonal antibody (mAb). KL-6 antibody, was obtained by Kohno *et al*. from a hybridoma established from BALB/c mouse splenocytes immunized with a human pulmonary adenocarcinoma cell line, VMRC-LCR (*36,37*). Biochemical analyses displayed that the molecular weight of KL-6 mucin was over 200 kDa because of a large amount of carbohydrate content (*38*). Histochemical expression of KL-6 has been observed not only in adenocarcinoma of the lung but also in various cancer cell lines, secretory epithelial tissues lining the respiratory, reproductive, gastrointestinal tracts, and bile duct, and carcinoma tissues (*36,37*). A well-investigated expression of KL-6 mucin in normal tissues is its presence on the surface of type II pneumocytes, and circulating KL-6 mucin in serum is likely derived from this expression (*39*). Past studies clarified that the serum KL-6 mucin level was significantly elevated in patients with interstitial pneumonitis compared with other pulmonary diseases, and that this elevation clinically correlated to interstitial pneumonitis activity (*39-42*). Thus KL-6 has been shown to be an effective serum marker for diagnosing behavior of interstitial lung disease and is currently used in clinical practice.

Although overexpression of MUC1 was showed in

many studies as previously described, the significance of KL-6 mucin in cancer diseases was also investigated. Kohno, the developer of KL-6 mAb, noted that the serum level of KL-6 mucin was elevated in pulmonary, breast, and pancreatic adenocarcinoma patients (*36*). Elevation of KL-6 mucin in serum was significantly associated with the behavior of breast cancer (*43*) or lung cancer (*44*). Moreover, the latest studies analyzed tissue expression of KL-6 mucin in various gastrointestinal cancers and suggested a relationship between its overexpression and a worse tumor behavior. The effectiveness of detecting MUC1 by KL-6 mAb is that the epitope of KL-6 mAb is a sialo-oligosaccharide-related structure. Since sialo-oligosaccharide moieties are exposed on mucin molecules, KL-6 antibody could effectively recognize the mucin without epitope masking as Cao *et al*. indicated with several antibodies against peptide epitopes of MUC1 (*45*). Although sialoglycoconjugates or MUC1 have been well-investigated and suggested to have significance in tumor behavior, research using KL-6 mAb have developed new findings in this field. In this article, we review the histochemical expression of KL-6 mucin in gastrointestinal, hepatic and pancreatic cancers while focusing on its clinicopathological significance. Expression profiles of KL-6 mucin in these cancer tissues are summarized in Table 2.

2. Ampullary cancer

Clinicopathological significance of sialoglycoconjugates has been studied in ampullary cancer, but the accumulated evidence is still inadequate because of the rarity of the lesions. Histochemical studies using sialic acid-binding lectins showed that expression of α2,3-linked sialoglycoconjugates had clinicopathological significance and lymph node metastasis (*46*). Some previous studies have indicated that ampullary cancer has a heterogeneous expression pattern of mucin glycoproteins including MUC1 (*47*,*48*). Paulsen *et al*. showed that the expression of MUC1 protein was not detected in ampulla of Vater and duodenum tissues while MUC1 mRNA was positive (*49*). However, since anti-MUC1 mAb against the specific core peptide sequence of MUC1 was used in that study, the highly glycosylated MUC1 might be undetectable. A detailed

Table 2. Expression profile of KL-6 mucin in gastrointestinal, hepatic and pancreatic tissues

Organ and tissue	KL-6 mucin expression
Stomach	
Normal epithelium	Positive in fundus gland.
Cancer	Negative/positive in apical surface/positive in circumferential membrane and cytoplasm. Circumferential membrane and cytoplasmic expression was related to malignant behavior.
Ampulla of Vater	
Normal epithelium	Negative.
Cancer	Negative/Positive. Positive expression was related to malignant behavior.
Colon	
Normal epithelium	Negative.
Cancer	Negative/positive in apical surface/positive in circumferential membrane and cytoplasm. Circumferential membrane and cytoplasmic expression was related to malignant behavior.
Liver	
Normal parenchyma	Negative.
Normal bile duct	Positive on apical surface of bile duct cells.
HCC	Negative.
CC	Positive. All analyzed specimens showed circumferential membrane and cytoplasmic expression. In cHCC-CC tissues, only CC components showed circumferential membrane and cytoplasmic expression.
Metastatic cancer	Positive in circumferential membrane and cytoplasm. This profile was matched with that in each primary CRC tissue.
Pancreas	
Normal duct	Positive.
Ductal cancer	Positive. All analyzed specimens were positive.
IPMT	Negative/Positive. The relationship between the expression profile and clinicopathological characteristics is still unclear.

Abbreviations: CC, cholangiocarcinoma; HCC, hepatocellular carcinoma; IPMT, intraductal papillary-mucinous tumors.

profile of MUC1 expression in ampullary cancer tissues was analyzed in a few studies. Gürbüz *et al*. showed that 72.7% of ampullary cancer tissues were positive for MUC1 expression, and they used the anti-MUC1 mAb of which the epitope was sialo-oligosaccharide (*50*). Zhou *et al*. divided ampullary cancer into 3 groups (intestinal type, pancreaticobiliary type and other) on the basis of cancer origin and analyzed the expression of various cancer-related antigens (*51*). In their results, the expression of MUC1 was detected in all differentiation types but there was no significant difference between the intestinal type and pancreaticobiliary type, and there was no relationship to patients' survival. However, in another study, a different profile of MUC1 expression was shown between intestinal type and pancreaticobiliary type though the number of cases was small (*52*). Thus, the expression profile of MUC1 in ampullary cancer tissues is controversial, and clinical availability of MUC1 in ampullary cancer is considered to be low.

On the basis of these investigations, an immunohistochemical analysis of ampullary cancer was performed using KL-6 mAb (*53*). Positive staining was obtained in 68.4% of all cases in ampullary cancer tissues but not in non-cancerous tissues (Figure 1), and a remarkable expression of KL-6 was found in invasive carcinoma cells in pancreatic and duodenal tissues and in metastatic carcinoma cells in lymph nodes. This study revealed that positive KL-6 mucin expression was significantly related to lymph node metastasis, pancreatic invasion, duodenal invasion, and the advanced stages of TNM clinical classification of ampullary cancer. Prognosis of the patients showing positive KL-6 mucin expression (5-year survival rate; 30.8%) was significantly poorer than those without KL-6 mucin expression (5-year survival rate; 75.0%). Therefore, this study suggested that histochemical analyses of preoperatively biopsied tissues using KL-6 mAb might be helpful in the assessment of the development of lymph node metastasis, pancreatic invasion, and duodenal invasion, which would increase the physician's ability to determine operative procedures or predict prognosis for individual patients. Although the clinicopathological significance of MUC1 was not clearly suggested in previous studies, KL-6 mucin is worth investigating to clarify availability as a diagnostic marker for ampullary cancer.

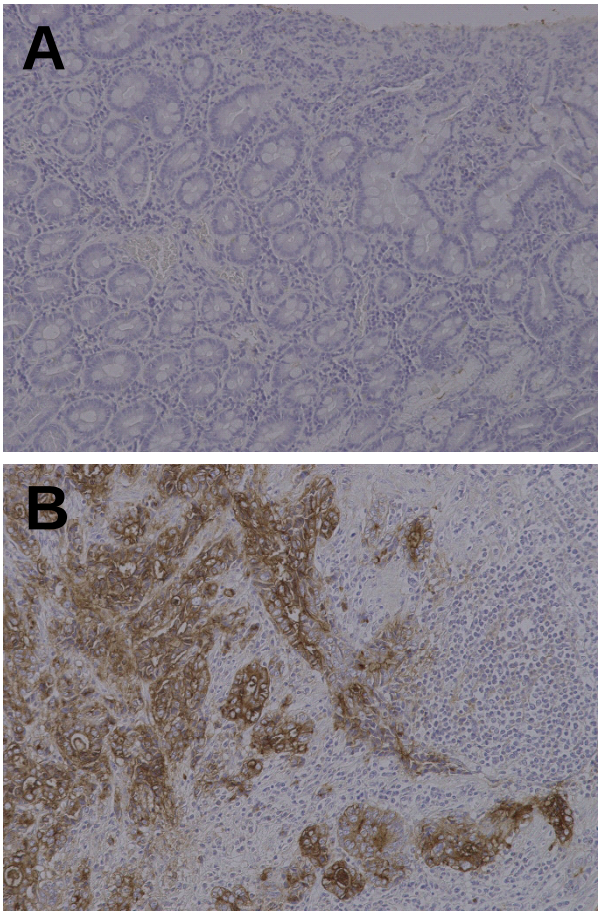

Figure 1. Histochemical expression of KL-6 mucin in noncancerous (A) and cancerous (B) tissues in ampulla of Vater. Original magnification ×200.

3. Primary colorectal cancer and its metastatic cancer

Concerning colorectal cancer (CRC), several sialoglycoconjugates have been used in clinical medicine as tumor markers, especially carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9 (*54-57*). Sialo-oligosaccharides and sialoglycoconjugates including CEA and CA19-9 have been well-investigated in CRC and those molecules are considered to have important functions in cell adhesion and cell migration. In clinicopathological studies, overexpression of molecules which contains sialo-oligosaccharides was frequently detected in cancer tissues or serum of CRC patients and suggested to have a significant relation to CRC behavior. The expression profile of overall sialoglycoconjugates has also been analyzed by lectin-immunohistochemistry, and α2,6-linked sialoglycoconjugates (recognized by SNA lectin) were significantly related to the presence of cancer cell invasion and lymph node metastasis (*58*). Antibodies against various kinds of sialo-oligosaccharides have been established and have been applied to detect the expression profile of sialoglycoconjugates in CRC. The well-investigated sialo-oligosaccharides are sialyl-Lewis x (sialyl-Le^x) antigen, sialyl-Lewis a (sialyl-Le^a) antigen and sialyl-Tn antigen. Nakagoe *et al*. demonstrated in their immunohistochemical study that overexpression of sialyl-Le^x antigen in CRC tissues was suggested as a predictor of cancer recurrence in patients with CRC without lymph node metastasis (*59*). In a serological study, elevated serum levels of sialyl-Le^x antigen and sialyl-Le^a antigen (identical with CA 19-9) as well as serum CEA levels suggested lymph node metastasis, distant metastasis and an advanced

stage of CRC (*60*). Overexpression of sialyl-Tn antigen in CRC tissues and CRC patients' serum also suggested prognostic factors in patients with advanced CRC (*61*). However, results that showed clinicopathological significance of these sialo-oligosaccharide antigens varied among studies. Thus, sensitivity and specificity of these antigens as the prognostic marker for CRC patients are considered to be insufficient though these antigens have important functions in cancer progression, especially in the process of metastasis.

Multiple studies on MUC1 expression in CRC have also been performed and investigated for clinical significance and relationships to other molecules (*62*,*63*). Histochemical studies focusing on the tandem-repeat domain of MUC1 suggested that CRC cells expressing high levels of MUC1 have increased invasive and metastatic potential (*26*). An increased percentage of MUC1 staining was frequently detected in advanced cancer patients and related to poorer survival of CRC patients (*64*). Histochemical studies analyzing the distribution of MUC1 and β-catenin clarified that MUC1 expression was observed at the tumor center and at the invasion front in over 50% of CRC tissues, and coexpression with β-catenin at the invasion front was also detected (*65*). However, some reports indicated that there was no significant relationship between MUC1 expression and worse tumor behavior. The histochemical analysis of MUC1 and MUC2 in CRC of African-American and Caucasian patients showed that the expression of MUC1 was detected more frequently in advanced cancer patients but was not significantly related to various clinicopathological features (*66*). Although it is clear that MUC1 is important for cancer progression, especially cancer cell invasion and metastasis of CRC, current evidence is insufficient to indicate the appearance of MUC1 as an independent clinicopathological marker. However, it is suggested that some specific kinds of MUC1 especially hyperglycosylated MUC1 is aberrantly expressed in CRC tissues but not in normal colorectal tissues. Thus, detecting some specific kinds of MUC1 can be used as a clinicopathological marker.

A recent immunohistochemical study of MUC1 in CRC was also carried out using KL-6 mAb (*67*). Because KL-6 mucin is thought to be sialylated or hyperglycosylated MUC1, it was expected that it would detect the different expression profile of MUC1 from the previous immunohistochemical studies of MUC1. As a result, positive staining was detected in CRC tissues and not in surrounding normal tissues. But this overall expression level had no clinicopathological significance, and this result was similar to previous histochemical studies of MUC1 in CRC. This study also focused on the subcellular localization of KL-6 mucin in CRC cells and classified the analyzed CRC patients into 3 groups: no expression (6/82, 7.3%), expression at apical surface of membrane (29/82, 35.4%), and expression at circumferential membrane and/or cytoplasm (47/82, 57.3%) as shown in Figure 2. Circumferential membrane and/or cytoplasmic localization of KL-6 mucin was correlated with the worse behavior of CRC, such as lymphatic vessel invasion, venous invasion, lymph node metastasis, and the advanced TNM stage. Five-year survival rate of patients who showed circumferential membrane and/or cytoplasmic localization of KL-6 mucin was 63.8%, and this was significantly lower than patients who

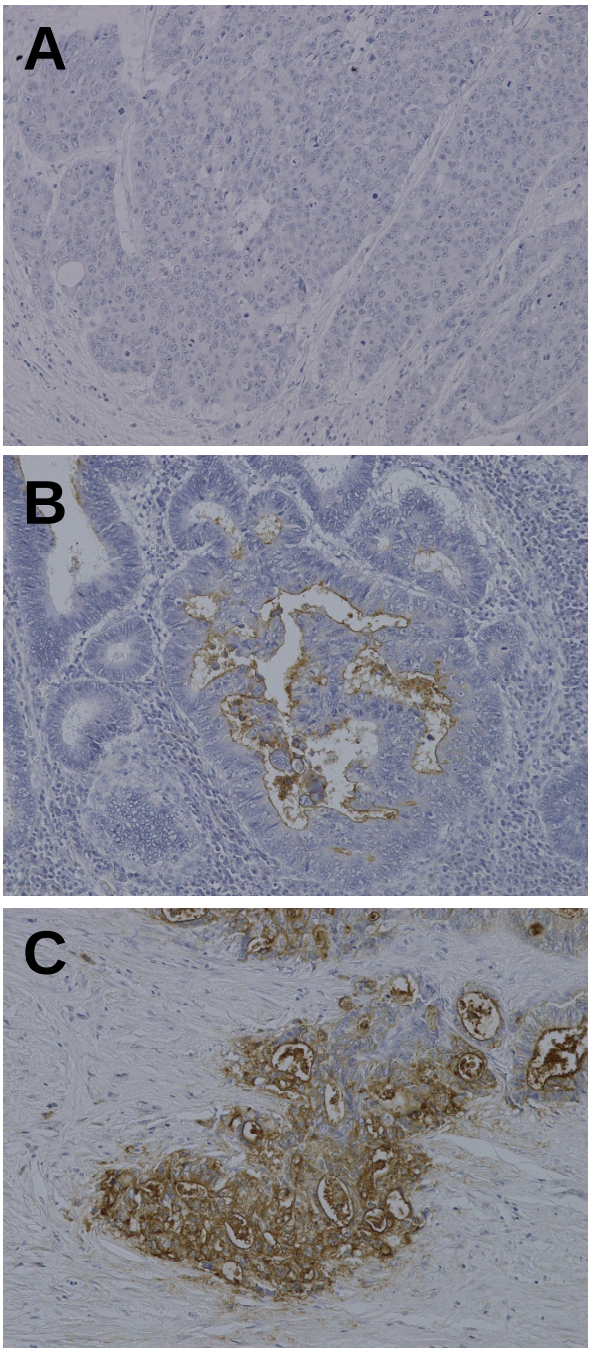

Figure 2. Subcellular localization of KL-6 mucin in CRC tissues. Expression profile of KL-6 mucin was categorized into 3 patterns; no expression (**A**), positive expression on apical surface of membrane (**B**) and positive expression in circumferential membrane and/or cytoplasm of cancer cells (**C**). Original magnification ×200.

showed no expression or apical membrane localization. This study suggested that subcellular localization of KL-6 mucin might have a significant role in cancer progression of CRC, especially metastasis to other tissues, and might be a useful histochemical marker for diagnosing tumor behavior of CRC and patients' prognosis. Although various kinds of sialo-oligosaccharides and sialoglycoconjugates were well-investigated these biological functions for CRC progression, the aberrant expression of KL-6 mucin, one of sialylated or hyperglycosylated MUC1, in the circumferential membrane and/or cytoplasm may be an important indicator for liver metastasis of colorectal carcinoma.

Moreover, expression of KL-6 mucin was also analyzed in metastatic liver cancer tissues (*68*). The results indicated that all examined cases were positive for circumferential membrane and/or cytoplasmic localization of KL-6 mucin, and suggested that metastatic lesions of CRC still retain primary pathological characteristics (Figure 3). On the other hand, no staining for KL-6 mucin was observed in any studied cases of hepatocellular carcinoma (HCC) tissues and the surrounding normal liver tissues. Therefore, histochemical evaluation of KL-6 mucin expression may also be helpful in distinguishing the pseudoglandular type of HCC from metastatic liver cancer. However, further examination of a larger population including both primary colorectal carcinoma and corresponding metastatic lesions should be performed to understand the clinical significance of KL-6 mucin with regard to the metastatic potency of individual tumors.

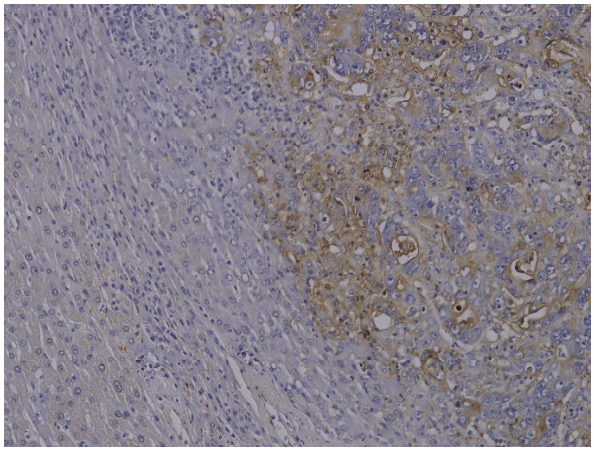

Figure 3. Subcellular localization of KL-6 mucin in metastatic liver cancer tissues. Histochemical staining was observed in circumferential membrane and/or cytoplasm of metastatic cancer cells (right side of picture). Hepatic parenchymal cells surrounding cancer tissue showed negative expression of KL-6 mucin (left side of picture). Original magnification ×200.

4. Primary liver cancer

Primary liver cancer can be classified as HCC and intrahepatic cholangiocarcinoma (CC). Previous studies showed that HCC and CC have different etiologic, epidemiologic, and clinical characteristics (*69,70*). The prognosis of CC patients is much worse than that of HCC patients and the latest reports indicated that the overall 5-year survival rate varied from 17 to 40% (*71*). Thus, diagnosis of CC in the earlier stages to distinguish it from HCC is important to improve the patients' prognosis. Furthermore, there is combined HCC and CC (cHCC-CC) although the number of these cancer patients is suggested to be approximately 5% of all liver cancers (*72*). Several reports showed that the prognosis of cHCC-CC patients was significantly poor as well as CC compared with HCC (*73-75*). Clinicopathological characteristics of cHCC-CC are suggested to be similar to patients with CC but this is controversial (*75-77*). The distribution of the CC and HCC components in cHCC-CC tissue, therefore, should be determined for assessment of the clinicopathological characteristics to select the best treatment of cHCC-CC patients.

Various studies have been performed to clarify the specific characteristics of CC for the purpose of discriminating it from HCC. In particular, hepatocyte paraffin 1 (Hep par 1) and cytokeratin 7 (CK7) are well-used antigens to discriminate hepatocytes and cholangiocytes, and it was suggested that these antigens were effective (*78-81*). But there is still a problem that the sensitivity and the specificity to distinguish between CC and HCC are insufficient. Investigations of sialoglycoconjugate expression, particularly CEA expression in CC, were also performed (*70,81*). The positive rate of CEA in CC (22%) was low compared with metastatic adenocarcinoma (62%) although HCC was not positive (*81*). Another study analyzed the histochemical expression of α2,6-linked sialoglycoconjugates and showed that its expression profile was changed between normal liver and HCC tissues but did not mention its clinical significance (*82*). Although this altered expression of silagoglycoconjugates might have some importance in the progression of CC, no clinicopathological significance has been clarified in these sialo-glycoconjugates. Thus, there were few effective molecules reported that can clearly discriminate CC from HCC.

Investigations targeting the expression of mucin glycoprotein in CC have also been performed. Sasaki *et al*. studied the expression of various kinds of mucin glycoproteins in CC and cHCC-CC tissues and clarified that MUC1 glycoprotein was extensively expressed in CC tissues and was also detected in CC regions of cHCC-CC tissues (*83*). Matsumura *et al*. reported that clinicopathological significance of cytoplasmic expression of MUC1 core protein in CC tissues was related to lymph node metastasis and poor survival of patients (*84*). In immunohistochemical analyses using several different antibodies that recognize MUC1 core peptide sequence or highly sialylated MUC1

glycoprotein, the results indicated that positive staining of MUC1 was significantly related to a worse prognosis for CC patients regardless of the glycosylation degree of MUC1 (*85*). Thus, these investigations suggested that the overexpression of MUC1 glycoprotein in CC tissues might be related to some unfavorable clinicopathological features such as lymph node metastasis and be able to use this expression as a prognostic marker for CC patients. On the other hand, several studies showed the clinicopathological importance of MUC1 in HCC tissues. Yamamoto *et al*. showed that the expression of MUC1 core protein at the luminal surface membrane of tumor cells was detected frequently in HCC tissues while the cytoplasmic expression had no significance (*86*). Yuan *et al*. indicated that the expression of MUC1 glycoprotein had no significant difference between HCC and CC, but the strong expression of MUC1 was significantly related to lymph node metastasis and tumor recurrence (*87*). According to these studies, the expression profile of MUC1 has clinicopathological significance to detect unfavorable behavior of primary liver cancers but is not useable as a marker to discriminate CC from HCC.

Tang *et al*. performed immunohistochemical analyses of KL-6 mucin in HCC and CC tissues (*88*). This report showed that KL-6 staining was positive in all of the CC tissues examined, while it was not positive in any of the HCC tissues or normal hepatic parenchyma examined (Figures 4A and B). Interestingly, a similar selective pattern of KL-6 staining was also found in cHCC-CC tissues, and the cholangiocellular areas could be clearly detected by using KL-6 (Figure 5). Thus, KL-6 mucin was suggested to be an effective marker for separating CC from HCC in resected or biopsied liver tissues. Moreover, the same study showed that 79.5% of HCC specimens and 66.7% of cHCC-CC specimens were positive for Hep1 expression in the HCC tissues and areas, respectively, while none of the CC tissues and CC areas of cHCC-CC specimens were positive. On the other hand, staining for CK7 was observed in 95.2% of CC specimens and 35.9% of HCC specimens, although it was faint in some of the HCC specimens. Also, 58.3 and 25.0% of the cHCC-CC specimens were positive for CK7 in the CC and HCC areas, respectively. Conclusively, the report suggested that KL-6 mucin might be more effective for differentiating CC from HCC than the combination of Hep1 and CK7. In addition, KL-6 mucin was positive in the cholangiocellular tissues but not in the hepatocellular tissues of cHCC-CC, so this antibody may be useful to detect the cholangiocellular component of cHCC-CC and provide pathological information for selecting clinical strategy.

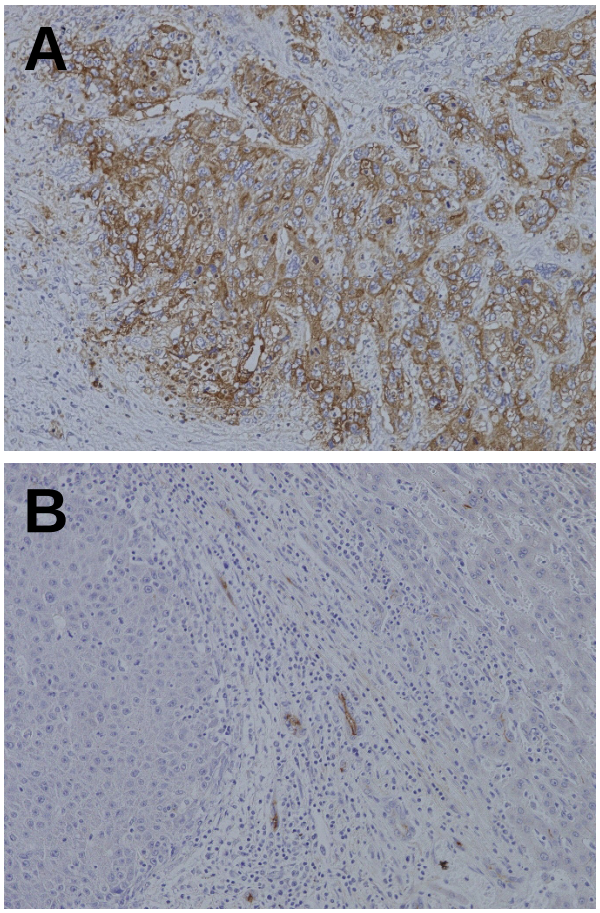

Figure 4. Histochemical expression of KL-6 mucin in primary liver cancer tissues. Positive expression was observed in CC tissues (**A**) but not in HCC tissues (left side of **B**). Surrounding noncancerous hepatic cells displayed negative expression of KL-6 mucin except for luminal surface of bile duct (right side of **B**). Original magnification ×200.

5. Gastric cancer

Expression of overall sialoglycoconjugates in gastric cancer tissues was investigated by immunohistochemistry. Overexpression of α2,3-linked sialoglycoconjugates that was detected only in cancerous tissues but not in normal gastric mucosa had a significant relationship to the presence of cancer cell invasion and lymph node metastasis (*20*). This overexpression was nominated as an independent prognostic factor alongside the deeper invasion of cancer cells and the presence of venous invasion. This result showed different evidence from CRC that many studies indicated significant expression of α2,6-linked sialoglycoconjugates as described before. α2,6-linked sialoglycoconjugates were also detected in gastric cancer tissues as well as normal mucosa but not related to clinicopathological parameters. Histological differentiation of gastric and colorectal mucosa is considered to cause the clinicopathological difference between α2,3- and α2,6-linked, but it is still under investigation. On the other hand, several researchers indicated that sialyl-Le^x and sialyl-Le^a antigens were frequently detected in patients with lymphatic invasion and lymph node metastasis, and particularly related to the incidence of liver metastasis (*89-92*). The significant relation between overexpression of sialyl-Le^x antigen and a worse tumor

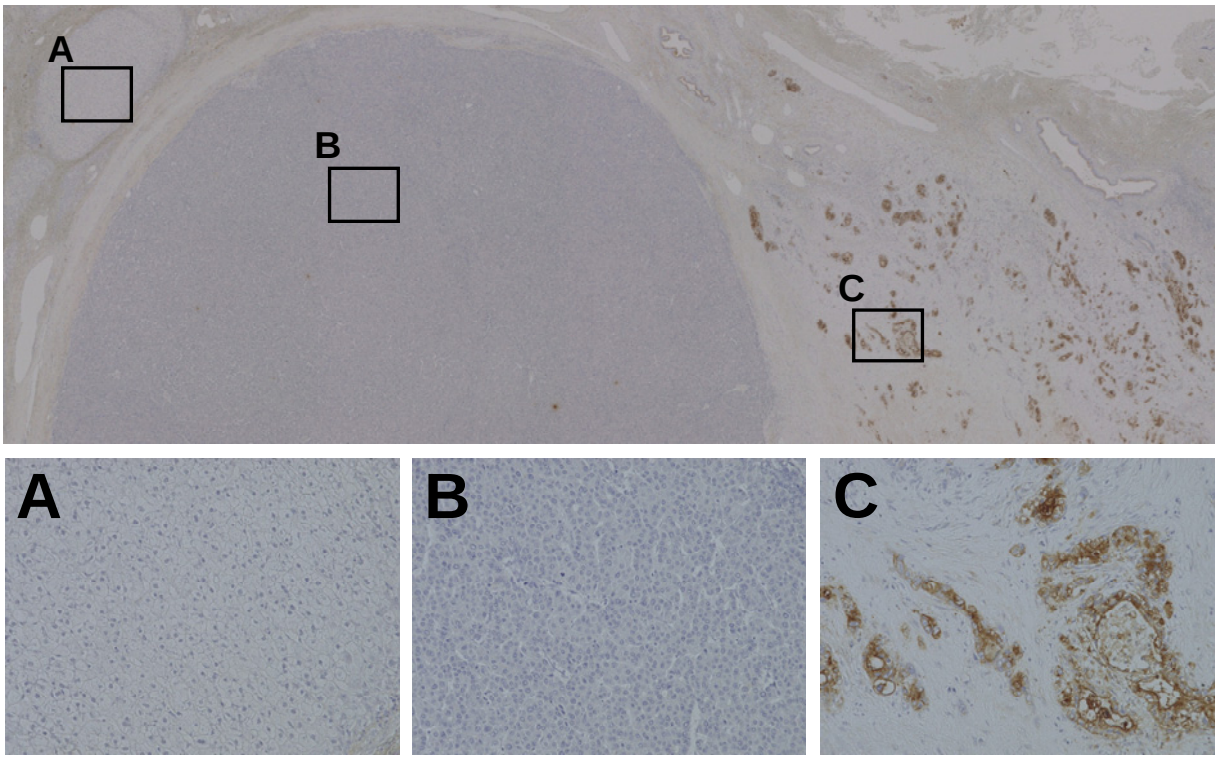

Figure 5. Histochemical expression of KL-6 mucin in cHCC-CC tissues. Positive expression in circumferential membrane and/or cytoplasm was observed in cholangiocellular areas (**C**) but not in hepatocellular areas including noncancerous liver parenchyma (**A**) and HCC (**B**). Original magnification; extensive area, ×4; close-up areas (**A**-**C**), ×200.

outcome was also observed in patients with stage 0 to II gastric cancer (*93*). Thus, overexpression of these sialo-oligosaccharides in gastric cancer cells can be predictable for a worse result for patients with overall gastric cancer. As described before, these specific sialo-oligosaccharides have various functions in cancer cell metastasis, especially attachment to endothelial cells at metastatic sites. Overexpression of these sialo-oligosaccharides might perform the same role in cancer cell metastasis in gastric cancer as CRC. But Ikeda *et al*. reported that expression of sialyl-related antigens including sialyl-Le^x and sialyl-Le^a antigens was detected heterogeneously in primary and metastatic lesions (*94*). Further studies are needed to clarify the biological effect of sialo-oligosaccharides in gastric cancer cells.

The expression profile of MUC1 has been well-investigated in gastric cancer as well as CRC. Many immunohistochemical studies analyzed the expression profile of MUC1 along with other mucins such as MUC2, MUC3 and MUC5AC, and compared the clinicopathological significance. MUC1 was frequently expressed in the antrum and superficial foveolar epithelium in normal tissue, whereas various expression profiles of MUC1 were observed in gastric cancer tissues. Most of the studies regarding MUC1 showed overexpression of MUC1 was an unfavorable marker in gastric cancer. Aberrant expression of MUC1 was frequently observed in Lauren's intestinal type of gastric cancer (*95,96*) or in glandular-forming types of gastric cancer (*97*). Clinicopathological analyses were performed and showed that expression of MUC1 was significantly related to deeper invasion of cancer cells, the presence of lymphatic invasion and lymph node metastasis (*98-100*). This MUC1 expression is also suggested to be an independent prognostic factor for gastric cancer patients, but it is controversial. The latest study analyzed the expression of KL-6 mucin in gastric cancer tissues and observed its localization in the apical surface and/or cytoplasm of cancer cells like CRC cells (unpublished data), but its clinicopathological importance is still unclear. Because MUC1 is an insufficient prognostic factor independently, the results of expression profiles of plural mucins were combined and its clinicopathological significance was analyzed. Each kind of mucin has a distinct expression profile and the combination therefore resulted in a unique parameter. Utsunomiya *et al*. showed that MUC1 expression was related to a worse outcome while MUC2 expression was correlated with a favorable outcome and suggested the combined effectiveness of measurement of these mucins as a prognostic predictor (*101*). Wang *et al*. indicated that patients with a MUC1-positive and MUC5AC-negative profile showed the worst prognosis (*102*). Furthermore, the combination of MUC1 and some other functional proteins such as E-cadherin and β-catenin were also analyzed. As a result, patients with positive expression of MUC1 and abnormal E-cadherin had a significantly poorer prognosis (*103*). Because gastric cancer has various types of tissue differentiation

and the expression profile of MUC1 is not homogenous among these types, MUC1 alone is insufficient for the precise discrimination of patients with a worse tumor behavior. Combined analysis of KL-6 mucin and other functional proteins might lead to a new discovery for this field.

6. Pancreatic cancer

An elevated level of several sialic acid-containing antigens such as CA19-9, DU-PAN-2 and Span-1 has been used as a diagnostic marker of pancreatic cancer. These tumor markers have high sensitivity to detect patients with exocrine pancreatic cancer but are considered to be insufficient for discrimination of a small-sized early cancer (*104,105*). Although surgical techniques and systematic chemotherapy have been developed, patients with pancreatic ductal cancer still have a poor prognosis because of its highly invasive properties and nonspecific symptoms. Therefore, a diagnostic marker of exocrine pancreatic tumors is required to detect the disease in the early stages with higher sensitivity.

In normal pancreatic tissue, MUC1 molecules with various glycoforms were detected in the apical surface of centroacinar cells, intercalated ducts, and intralobular ducts but not in the main pancreatic ducts, acini and islets (*106*). While many studies performed to detect the expression profile of mucins in pancreatic tissues, MUC1 expression has been investigated in pancreatic ductal cancer tissues. The results were similar among those histochemical studies that a high rate of pancreatic ductal cancer tissues showed positive expression of MUC1. Although it might be one reason for the high rate of MUC1 expression that most pancreatic ductal cancers are already at an advanced stage, availability of MUC1 expression for diagnosing the clinicopathological status of pancreatic ductal cancer patients including the prediction of patients' prognosis is still vague. Availability of MUC1 as a marker for discriminating pancreatic ductal cancer from other pancreatic diseases has been tried to be developed. Various studies have analyzed the differences of MUC1 expression between pancreatic ductal cancer and intraductal papillary-mucinous tumors (IPMT) of various pathological types which display better or worse tumor behavior. Expression of MUC1 was detected not only in pancreatic ductal cancer but also in the carcinoma type of IPMT while it was not detected in the adenoma type and borderline type of IPMT (*107,108*). Therefore, MUC1 can be used effectively to diagnose IPMT with malignant characteristics. Immunohistochemical analysis of KL-6 mucin was also performed and all specimens of pancreatic ductal cancer were positive for KL-6 mucin (unpublished data). Although expression of KL-6 mucin in IPMT also varied like some other MUC1s detected by ordinary mAb, further analyses must be performed in order to clarify its clinicopathological significance. Moreover, some studies described that combined analysis of several mucins such as MUC1, MUC2 and MUC5AC are available for screening pancreatic ductal cancer in file-needle aspiration specimens (*109,110*). This combination was also analyzed using various histological types of IPMT as well as pancreatic ductal cancer (*111*). IPMT-dark cell type tumor and IPMT-clear cell type tumor, which have a favorable outcome, showed a negative pattern for MUC1 while the IPMT-compact cell type tumor frequently showed a positive pattern for MUC1. The expression pattern of those mucins varied among the types of IPMT and might be caused by the different biological behavior of each IPMT. According to these studies, expression of MUC1 is thought to be related to the attainment of invasive ability of pancreatic cancer cells. In the study of Adsay *et al*., the rate of patients with positive MUC1 expression gradually increased according to the invasive status of pancreatic tumors (*112*). But, in contrast, Gold *et al*. showed in immunohistochemical analysis using PAM4, an anti-MUC1 mAb, that PAM4-reactive MUC1 was detected frequently not only in invasive pancreatic adenocarcinomas but also in the early stage of pancreatic intraepithelial neoplasia (*113*). The induction of MUC1 expression itself was suggested to be initiated in the early stage of pancreatic tumorigenesis, therefore some other components of MUC1 such as sialo-oligosaccharide content might be significantly related to the invasive status of pancreatic cancer cells (*114*).

7. Conclusions

MUC1 has been investigated for a long period of time and its importance for cancer progression has been clarified. However, MUC1 was also shown to have various functions and complex characteristics in its molecular structure because many kinds of anti-MUC1 mAb have been developed. MUC1 is not only one glycoprotein but it shows various specific styles affected by altered biological systems, especially in malignant cells. KL-6 mucin is one such kind of MUC1 molecule although the detailed characteristics are still unknown. While KL-6 mAb has already been applied to the diagnosis of interstitial pneumonitis, the latest immunohistochemical analysis has clarified KL-6 mucin's clinicopathological significance in gastrointestinal and hepatic cancer tissues. The expression profile and clinicopathological significance of KL-6 mucin were different among each organ or disease as described in this review, the biological role of KL-6 mucin might have a different importance in each location and state. To accumulate knowledge of the molecular biology regarding MUC1 or KL-6 mucin, its mechanism on cancer progression and novel method for its medical applications must be further studied.

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