

KL-6 mucin expression in carcinoma of the ampulla of Vater: Association with cancer progression

Wei Tang, Yoshinori Inagaki, Norihiro Kokudo, Qian Guo, Yasuji Seyama, Munehiro Nakata, Hiroshi Imamura, Keiji Sano, Yasuhiko Sugawara, Masatoshi Makuuchi

Wei Tang, Norihiro Kokudo, Qian Guo, Yasuji Seyama, Hiroshi Imamura, Keiji Sano, Yasuhiko Sugawara, Masatoshi Makuuchi, Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, the University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
Yoshinori Inagaki, Department of Applied Biochemistry, Tokai University, Hiratsuka, Kanagawa 259-1292, Japan
Munehiro Nakata, Department of Applied Biochemistry and Institute of Glycotechnology, Tokai University, Hiratsuka, Kanagawa 259-1292, Japan

Supported by Grants-in-aid From the Ministry of Education, Science, Sports, and Culture of Japan and a grant for Hi-Tech Research from Tokai University

Correspondence to: Dr. Wei Tang, Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, the University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. tang-sur@h.u-tokyo.ac.jp

Telephone: +81-3-5800-8654 Fax: +81-3-5684-3989

Received: 2005-03-01 Accepted: 2005-04-09

Abstract

AIM: To assess histochemical expression of KL-6 and its clinicopathological significance in carcinoma of the ampulla of Vater.

METHODS: Ampullary carcinoma tissues were collected from 38 patients who underwent pancreatoduodenectomy or local resection. Tissues were subjected to immunohistochemical analysis using KL-6 antibody.

RESULTS: Positive staining of ampullary carcinoma cells was observed in 26 (68.4%) cases. Staining was not found in the surrounding non-cancer regions of the ampullary tissues. Remarkable KL-6 expression was observed in invasive carcinoma cells in pancreatic and duodenal tissues and in metastatic carcinoma cells in lymph nodes. Positive KL-6 expression was related to lymph node metastasis ($P = 0.020$), pancreatic invasion ($P = 0.016$), duodenal invasion ($P = 0.034$), and advanced stage of TNM clinical classification ($P = 0.010$). Survival analysis showed that positive expression of KL-6 was related to a poorer prognosis ($P = 0.029$).

CONCLUSION: The aberrant expression of KL-6 mucin is significantly related to unfavorable behaviors of carcinoma of the ampulla of Vater.

© 2005 The WJG Press and Elsevier Inc. All rights reserved.

Key words: KL-6 mucin; Carcinoma of the ampulla of Vater;

Invasion; Metastasis; Prognosis

Tang W, Inagaki Y, Kokudo N, Guo Q, Seyama Y, Nakata M, Imamura H, Sano K, Sugawara Y, Makuuchi M. KL-6 mucin expression in carcinoma of the ampulla of Vater: Association with cancer progression. *World J Gastroenterol* 2005; 11(35): 5450-5454

<http://www.wjgnet.com/1007-9327/11/5450.asp>

INTRODUCTION

Carcinoma of the ampulla of Vater is a malignancy with a low incidence^[1]. Although the prognosis for patients with this cancer is higher than that for patients with cancer arising within the pancreas or common bile duct, the 5-year survival rate after resection is less than 50%^[2,3]. Therefore, the mechanism for development of this cancer must be understood to improve the patient's prognosis.

Cell surface sialylation changes dramatically during oncogenesis^[4-6]. In several tumors, histochemical studies with sialic acid-binding lectins and antibodies against sialylated carbohydrate antigens showed that aberrant expression of sialylated glycoconjugates may play an important role in tumor progression^[7-10]. However, the natures of these glycoconjugates and their clinical application are not yet known.

KL-6 is a type of MUC1 mucin, recognized by a murine mAb, KL-6 antibody, as obtained by Kohno *et al.*, from a hybridoma established from the splenocytes of a BALB/c mouse immunized with a human pulmonary adenocarcinoma cell line, VMRC-LCR^[11,12]. The epitope recognized by the KL-6 antibody exists in sialylated carbohydrate moieties of the mucin molecule, and this sialylation is essential to the recognition by the KL-6 antibody^[12].

Histochemical expression of KL-6 has been observed in malignant tissues in several organs^[11,12]. However, little is known about the expression profile and clinical significance of KL-6 in carcinoma of the ampulla of Vater. With due consideration to the importance of sialylation of glycoconjugates in tumor behavior, and that the epitope of KL-6 antibody is a sialylated carbohydrate, investigation of the expression profile of KL-6 in carcinoma of the ampulla of Vater will yield significant pointers in clarification of the mechanisms involved in the development of this cancer. In this study, we performed histochemical analysis of KL-6 in ampullary carcinoma tissues and examined the relationship between KL-6 expression and clinicopathological parameters in carcinoma of the ampulla of Vater.

MATERIALS AND METHODS

Patients

Ampullary carcinoma tissues were collected from 38 patients (22 males and 16 females; median age of 68 years; range of 54-85 years) surgically treated at the Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, the University of Tokyo, between April 1976 and December 2002. All of the patients except one underwent pancreatoduodenectomy, whereas one patient underwent local resection. The size of the tumors varied from 1.0 to 7.5 cm (2.4 ± 1.3 cm, mean \pm SD). Histopathological examination was performed according to the guidelines of the Japanese Society of Biliary Surgery^[13]. The TNM staging system of the International Union Against Cancer was used for TNM classification^[14].

Immunohistochemistry

Five-micrometer-thick sections were cut from archival formalin-fixed paraffin-embedded tissue blocks, deparaffinized, and dehydrated through a graded series of ethanol. Endogenous peroxidase activity was quenched with 0.3% hydrogen peroxide/methanol. The sections were incubated at 4 °C overnight with KL-6 antibody (Eisai, Tokyo, Japan) diluted to 1:150. The sections were then incubated with biotinylated anti-mouse antibody for 30 min at room temperature followed by development with the biotin-streptavidin-peroxidase complex method, using a commercial kit as per the manufacturer's instructions (Vectastain ABC Elite kit; Vector, Burlingame, CA, USA). 3,3'-Diaminobenzidine was used as the chromogen, and hematoxylin was used as a counterstain. Each tissue sample was microscopically analyzed over its entire area to determine histopathological characteristics. The percentage of cancer cells that were stained of the total number of cancer cells was determined in 10 random microscopic fields of each tissue sample, or using the entire area, if the tissue sample comprised less than 10 fields (magnification $\times 200$). Cases in which more than 10% carcinoma cells were stained were defined as positive.

Statistical analysis

The χ^2 test was used to evaluate the relationship between KL-6 expression and clinicopathological parameters. Survival curves were calculated by the Kaplan-Meier method and

compared with results of the log rank test. Differences of $P < 0.05$ were considered significant. Statview 5.0J (Abacus Concepts, Berkeley, CA, USA) statistical software was used for data analysis.

RESULTS

Immunohistochemistry

Figure 1 shows immunohistochemical analysis of ampullary carcinoma tissues using KL-6 antibody. Positive staining was observed in 26 (68.4%) of 38 cases. The population of KL-6-positive tumor cells in the cancer regions varied among the samples (mean 49.2%, range 17.0-84.0%). The staining was localized in the tumor-cell cytoplasm and cell membrane in cancer regions (Figure 1A), whereas staining was not detected in the surrounding non-cancer regions of the ampullary tissues. In some cases, the stained materials were also detected along the inner surfaces of glands (Figure 1B). On the other hand, in 12 of 38 cases (31.6%), stained cells were scarce (Figure 1C).

Immunohistochemical analysis was focused on pancreatic and duodenal tissues showing pathological invasion of the ampullary carcinoma cells (Figure 2). Strongly stained carcinoma cells were frequently observed in the invaded areas of pancreatic tissues (Figure 2A), whereas only weak staining was sporadically observed in the surrounding normal pancreatic tissues (Figure 2B). Similarly, strongly stained carcinoma cells were frequently observed in the invaded areas of duodenal tissues (Figure 2C), whereas the surrounding normal duodenal epithelium was scarcely stained by KL-6 antibody (Figure 2D).

In all of the metastatic lymph node tissues tested, strong staining was found in carcinoma cells, whereas staining was not found in the surrounding normal tissues (Figure 3). Staining was not found in metastatic negative lymph node tissues (data not shown).

Clinicopathological and survival characteristics

The relationship between KL-6 expression and clinicopathological characteristics of tumors was examined. As shown in Table 1, positive KL-6 expression was significantly frequent in cases of lymph node metastasis than those without it ($P = 0.020$), in cases of pancreatic invasion than those without it ($P = 0.016$), in cases of duodenal invasion than

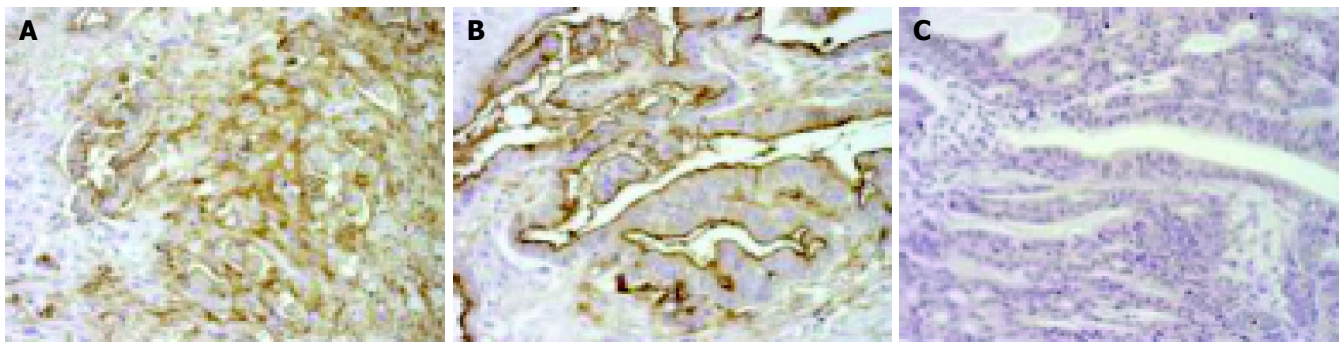


Figure 1 Immunohistochemical stain of the ampullary carcinoma tissues using KL-6 antibody. **A:** A typical example showing moderately positive staining; **B:** a typical example showing the moderately positive staining of inner surface of glands; **C:** a typical example in which stained cells are scarcely observed. Original magnification $\times 200$.

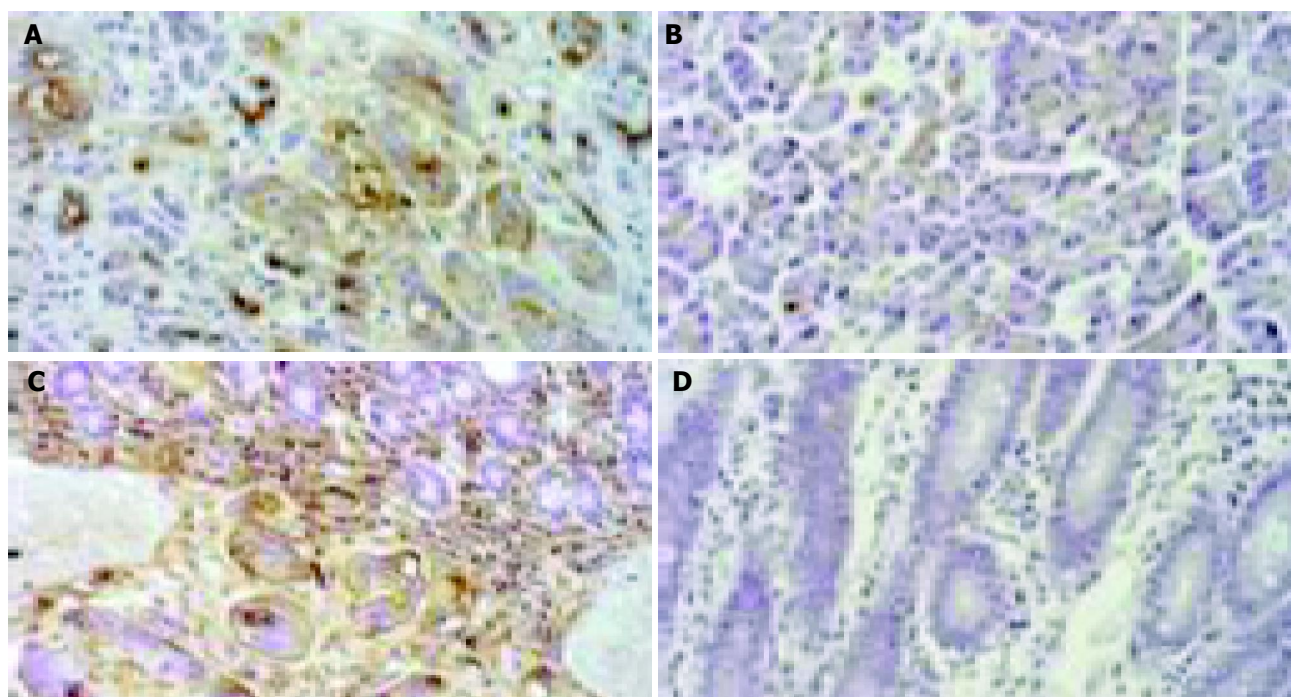


Figure 2 Immunohistochemical stain of KL-6 in invasive area of tumor. **A:** Area of pancreatic tissue invaded by carcinoma; **B:** normal pancreatic tissue; **C:** area of duodenal tissue invaded by carcinoma; **D:** normal duodenal tissue. Original magnification $\times 200$.

Table 1 Relationship between KL-6 expression and clinicopathological parameters in patients with carcinoma of the ampulla of Vater

Parameters	<i>n</i>	Positive KL-6 expression (<i>n</i> = 26; %)	<i>P</i>
Age (yr)			
≥ 65	24	14 (58.3)	0.080
< 65	14	12 (85.7)	
Sex			
Male	22	16 (72.7)	0.503
Female	16	10 (62.5)	
Histological grade			
Well differentiated	31	22 (71.0)	0.390
Moderately differentiated	4	3 (75.0)	
Poorly differentiated	3	1 (33.3)	
Lymphatic vessel invasion			
Present	23	18 (78.3)	0.106
Absent	15	8 (53.3)	
Lymph node metastasis			
Present	23	19 (82.6)	0.020
Absent	15	7 (46.7)	
Venous invasion			
Present	17	14 (82.4)	0.096
Absent	21	12 (57.1)	
Pancreatic invasion			
Present	26	21 (80.8)	0.016
Absent	12	5 (41.7)	
Duodenal invasion			
Present	30	23 (76.7)	0.034
Absent	8	3 (37.5)	
TNM clinical classification			
III+IV	18	16 (88.9)	0.010
I+II	20	10 (50.0)	



Figure 3 A typical example showing moderately positive staining of KL-6 in carcinoma cells in a metastatic lymph node tissue. Original magnification $\times 100$.

those without it ($P = 0.034$), and in cases classified into III or IV by TNM clinical classification than those classified into I or II ($P = 0.010$). On the other hand, KL-6 expression was not associated with age, sex, histological grade, lymphatic vessel invasion, and venous invasion (Table 1).

The relationship between KL-6 expression and patient survival was then analyzed. As shown in Figure 4, patients showing positive KL-6 expression ($n = 26$) displayed a significantly poorer prognosis than those showing negative KL-6 expression ($n = 12$): 5-year survival rates were 30.8 and 75.0%, respectively, as determined by the Kaplan-Meier method ($P = 0.029$ by the log rank test).

DISCUSSION

Mucins are large extracellular glycoproteins with high carbohydrate content and marked diversity both in the apoprotein and in the oligosaccharide moieties^[15]. It has been

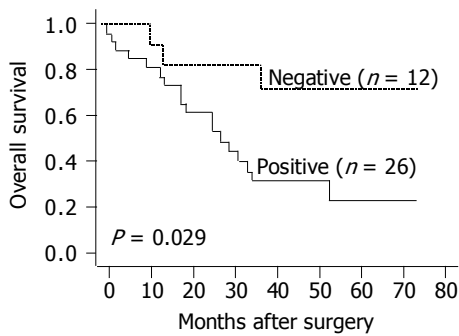


Figure 4 Kaplan-Meier curves for overall survival rates of patients with carcinoma of the ampulla of Vater. Patients with positive (solid line, $n = 26$) and negative (dotted line, $n = 12$) KL-6 expression were followed-up for over 70 mo.

noted that ampullary carcinoma have a heterogeneous mucin expression pattern^[16,17] and that overexpression of MUC1 was associated with invasive and metastatic potency of several adenocarcinoma^[18-20]. However, the studies on MUC1 expression were mostly done with different antibodies, which recognize different carbohydrate epitopes or the core peptide. This study addresses clinicopathological significance of histochemical expression of KL-6, MUC1 mucin-bearing sialylated carbohydrate epitope recognized by KL-6 antibody, in carcinoma of the ampulla of Vater. Sialylation of tumor cell surface glycoconjugate is thought to contribute to tumor progression and metastasis^[7-10]. Furthermore, since sialylated oligosaccharide moieties are exposed on the mucin molecules, KL-6 antibody could effectively recognize the mucin without epitope masking as Cao and Karsten indicated with several antibodies against peptide epitopes of MUC1^[21]. Therefore, immunohistochemical detection of KL-6 mucin seems to be a reasonable strategy.

The present data shows that aberrant expression of KL-6 mucin is related to unfavorable behaviors of the carcinoma, such as lymph node metastasis, pancreatic invasion, duodenal invasion, and the advanced stage of TNM clinical classification (Table 1), and poorer prognosis ($P = 0.029$, Figure 4). Furthermore, remarkable expression of KL-6 was found in invasive carcinoma cells in pancreatic and duodenal tissues and in metastatic carcinoma cells in lymph nodes (Figures 2 and 3). These results suggest that KL-6 mucin might play an important role in unfavorable tumor behaviors, such as invasions and metastasis of carcinoma of the ampulla of Vater.

Several tumor-associated carbohydrate antigens have been identified on mucins^[22,23]. Aberrant forms of mucins expressed in cancer cells have been considered to arise as a consequence of the deregulation of expression of enzymes that modify them^[15]. The epitope recognized by KL-6 antibody is sialylated carbohydrates included in MUC1 molecule^[12], although the detailed structure of the epitope remains to be determined. Among the many types of carbohydrates, sialic acid is vital for cancer growth, since enhanced sialylation is thought to play a role in tumor progression and metastasis^[5,24]. Carbohydrate moieties of glycoconjugates are constructed by complex interactions involving a series of glycosyltransferases^[25,26]. In our previous study, the aberrant sialylation of glycoconjugates in carcinoma

of the ampulla of Vater has been found in histochemical analyses using sialic acid-binding lectins such as *Maackia amurensis* leukoagglutinin and *Sambucus nigra* agglutinin^[27]. Therefore, it is postulated that the aberrant expression of KL-6 stems from the aberrant expression of the glycosyltransferase (s) such as sialyltransferase, which participates in the construction of the epitope for KL-6 antibody. Relationship of KL-6 expression to invasions and metastasis has also been suggested in colorectal carcinoma^[28,29].

Surgery is still the only option that provides a cure for patients with carcinoma of the ampulla of Vater. Patient outcome after surgery for carcinoma of the ampulla of Vater is better than that for pancreatic cancer or bile duct cancer. However, patients with lymph node metastasis or invasion of carcinoma to adjacent organs including the pancreas or duodenum display a poorer prognosis^[30-32]. The present study revealed that positive KL-6 expression was significantly related to lymph node metastasis, pancreatic invasion, and duodenal invasion of ampullary carcinoma. These clinicopathological parameters are recognized as prognostic factors of ampullary carcinoma^[30,31]. In particular, the presence of lymph node metastasis, pancreatic invasion, and duodenal invasion was found in 73% (19/26), 81% (21/26), and 88% (23/26), respectively, of KL-6-positive cases. Further, strong staining of KL-6 was frequently found in invasive carcinoma cells in pancreatic and neonatal tissues and in metastatic carcinoma cells in lymph nodes. Therefore, histochemical analyses of preoperatively biopsied tissues using anti-KL-6 antibody might be helpful for the assessment of the development of lymph node metastasis, pancreatic invasion, and duodenal invasion, which would increase a physician's ability to determine operative procedures or predict prognosis for individual patients. Examination with a larger population and a biochemical approach is needed to understand clinical significance of KL-6 expression and biochemical role of carbohydrate moiety of KL-6 mucin in carcinoma of ampulla of Vater.

REFERENCES

- 1 Conlon KC, Dougherty E, Klimstra DS, Coit DG, Turnbull AD, Brennan MF. The value of minimal access surgery in the staging of patients with potentially resectable peripancreatic malignancy. *Ann Surg* 1996; **223**: 134-140
- 2 Klempnauer J, Ridder GJ, Pichlmayr R. Prognostic factors after resection of ampullary carcinoma: multivariate survival analysis in comparison with ductal cancer of the pancreatic head. *Br J Surg* 1995; **82**: 1686-1691
- 3 Nakao A, Harada A, Nonami T, Kishimoto W, Takeda S, Ito K, Takagi H. Prognosis of cancer of the duodenal papilla of Vater in relation to clinicopathological tumor extension. *Hepatogastroenterology* 1994; **41**: 73-78
- 4 Fogel M, Altevogt P, Schirmacher V. Metastatic potential severely altered by changes in tumor cell adhesiveness and cell-surface sialylation. *J Exp Med* 1983; **157**: 371-376
- 5 Numahata K, Satoh M, Handa K, Saito S, Ohyama C, Ito A, Takahashi T, Hoshi S, Orikasa S, Hakomori S. Sialosyl-Le^x expression defines invasive and metastatic properties of bladder carcinoma. *Cancer* 2002; **94**: 673-685
- 6 Hakomori S. Tumor malignancy defined by aberrant glycosylation and sphingo(glyco)lipid metabolism. *Cancer Res* 1996; **56**: 5309-5318
- 7 Li XW, Ding YQ, Cai JJ, Yang SQ, An LB, Qiao DF. Studies on mechanism of Sialy Lewis-X antigen in liver metastases of human colorectal carcinoma. *World J Gastroenterol* 2001; **7**:

- 425-430
- 8 **Ikeda Y**, Mori M, Kajiyama K, Haraguchi Y, Sasaki O, Sugimachi K. Immunohistochemical expression of sialyl Tn, sialyl Lewis^x, sialyl Lewis^{a-b}, and sialyl Lewis^x in primary tumor and metastatic lymph nodes in human gastric cancer. *J Surg Oncol* 1996; **62**: 171-176
 - 9 **Tang W**, Mafune K, Nakata M, Konishi T, Kojima N, Mizuochi T, Makuuchi M. Association of histochemical expression of *Maackia amurensis* leucoagglutinin-positive glycoconjugates with behaviour of human gastric cancer. *Histopathology* 2003; **42**: 239-245
 - 10 **Vierbuchen MJ**, Fruechtnicht W, Brackrock S, Krause KT, Zienkiewicz TJ. Quantitative lectin histochemical and immunohistochemical studies on the occurrence of alpha(2,3)- and alpha(2,6)-linked sialic acid residues in colorectal carcinomas. Relation to clinicopathologic features. *Cancer* 1995; **76**: 727-735
 - 11 **Kohno N**. Serum marker KL-6/MUC1 for the diagnosis and management of interstitial pneumonitis. *J Med Invest* 1999; **46**: 151-158
 - 12 **Kohno N**, Akiyama M, Kyoizumi S, Hakoda M, Kobuke K, Yamakido M. Detection of soluble tumor-associated antigens in sera and effusions using novel monoclonal antibodies, KL-3 and KL-6, against lung adenocarcinoma. *Jpn J Clin Oncol* 1988; **18**: 203-216
 - 13 Japanese Society of Biliary Surgery. General rules for surgical and pathological studies on cancer of biliary tract, 5th ed. Tokyo: Kanehara 2003
 - 14 **Sobin LH**, Wittekind C. TNM classification of malignant tumors, 5th ed. New York: Wiley-Liss 1997
 - 15 **Hollingsworth MA**, Swanson BJ. Mucins in cancer: protection and control of the cell surface. *Nat Rev* 2004; **4**: 45-60
 - 16 **Adsay NV**, Mrati K, Basturk O, Iacobuzio-Donahue C, Levi E, Cheng JD, Sarkar FH, Hruban RH, Klimstra DS. Pathologically and biologically distinct types of epithelium in intraductal papillary mucinous neoplasms. *Am J Surg Pathol* 2004; **28**: 839-848
 - 17 **Gürbüz Y**, Klöppel G. Differentiation pathways in duodenal ampullary carcinomas: a comparative study on mucin and trefoil peptide expression, including gastric and colon carcinomas. *Virchows Arch* 2004; **444**: 536-541
 - 18 **Kashiwagi H**, Kijima H, Dowaki S, Ohtani Y, Tobita K, Tsukui M, Tanaka Y, Matsubayashi H, Tsuchida T, Yamazaki H, Nakamura M, Ueyama Y, Tanaka M, Tajima T, Makuuchi H. DF3 expression in human gallbladder carcinoma: significance for lymphatic invasion. *Int J Oncol* 2000; **16**: 455-459
 - 19 **Luttges J**, Feyerabend B, Buchelt T, Pacena M, Kloppel G. The mucin profile of noninvasive and invasive mucinous cystic neoplasms of the pancreas. *Am J Surg Pathol* 2002; **26**: 466-471
 - 20 **Nakamori S**, Ota DM, Cleary KR, Shirotani K, Irimura T. MUC1 mucin expression as a marker of progression and metastasis of human colorectal carcinoma. *Gastroenterology* 1994; **106**: 353-361
 - 21 **Cao Y**, Karsten U. Binding patterns of 51 monoclonal antibodies to peptide and carbohydrate epitopes of the epithelial mucin (MUC1) on tissue sections of adenolymphomas of the parotid (Warthin's tumours): role of epitope masking by glycans. *Histochem Cell Biol* 2001; **115**: 349-356
 - 22 **Kim YS**, Gum J, Brockhausen I. Mucin glycoproteins in neoplasia. *Glycoconj J* 1996; **13**: 693-707
 - 23 **Lloyd KO**. The chemistry and immunohemistry of blood group A, B, H, and Lewis antigens: past, present and future. *Glycoconj J* 2000; **17**: 531-541
 - 24 **Hakomori S**. Possible functions of tumor-associated carbohydrate antigens. *Curr Opin Immunol* 1991; **3**: 646-653
 - 25 **Brockhausen I**. Pathways of O-glycan biosynthesis in cancer cells. *Biochim Biophys Acta* 1999; **1473**: 67-95
 - 26 **Kornfeld R**, Kornfeld S. Assembly of asparagine-linked oligosaccharides. *Annu Rev Biochem* 1985; **54**: 631-664
 - 27 **Tang W**, Guo Q, Usuda M, Kokudo N, Seyama Y, Minagawa M, Sugawara Y, Nakata M, Kojima N, Makuuchi M. Histochemical expression of sialoglycoconjugates in carcinoma of the papilla of Vater. *Hepato-gastroenterology* 2005; **52**: 67-71
 - 28 **Hiraga Y**, Tanaka S, Haruma K, Yoshihara M, Sumii K, Kajiyama G, Shimamoto F, Kohno N. Immunoreactive MUC1 expression at the deepest invasive portion correlates with prognosis of colorectal cancer. *Oncology* 1998; **55**: 307-319
 - 29 **Tanimoto T**, Tanaka S, Haruma K, Yoshihara M, Sumii K, Kajiyama G, Shimamoto F, Kohno N. MUC1 expression in intramucosal colorectal neoplasms. Possible involvement in histogenesis and progression. *Oncology* 1999; **56**: 223-231
 - 30 **Roder JD**, Schneider PM, Stein HJ, Siewert JR. Number of lymph node metastases is significantly associated with survival in patients with radically resected carcinoma of the ampulla of Vater. *Br J Surg* 1995; **82**: 1693-1696
 - 31 **Yoshida T**, Matsumoto T, Shibata K, Yokoyama H, Morii Y, Sasaki A, Kitano S. Patterns of lymph node metastasis in carcinoma of the ampulla of Vater. *Hepato-gastroenterology* 2000; **47**: 880-883