Expression of gastric mucin *MUC5AC* and gastric transcription factor *SOX2* in ampulla of vater adenocarcinoma: Comparison between expression patterns and histologic subtypes

YUICHI SANADA, KAZUHIRO YOSHIDA, KAZUO KONISHI, MAMORU OEDA, MASAHIRO OHARA and YASUHIRO TSUTANI

Department of Surgical Oncology, Research Institution for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan

Received October 10, 2005; Accepted November 28, 2005

Abstract. The purpose of this study was to examine the expression pattern of *MUC5AC* and *SOX2* in ampulla of vater adenocarcinoma and evaluate the association between expression of these gastric epithelial markers and the histologic phenotype of ampulla of vater carcinoma. Six surgically resected samples of ampulla of vater adenocarcinoma, including four intestinal type carcinomas and two pancreatobiliary type carcinomas, were studied. We performed immunohistochemistry with a monoclonal antibody against *MUC5AC* and a polyclonal anti-*SOX2* antibody. In two of the four intestinal type carcinomas, MUC5AC and SOX2 were focally expressed in the superficial neoplastic mucosa. However, in the centre of the tumour and in other invasive lesions, including vascular invasive lesions and metastatic lymph nodes, neither *MUC5AC* nor *SOX2* was expressed. In contrast, in both pancreatobiliary type carcinomas, expression of *MUC5AC* and *SOX2* was maintained or increased in invasive lesions. Our immunohistochemistry data suggest that *MUC5AC* and *SOX2* are associated with the pancreatobiliary phenotype of ampulla of vater carcinoma and involved in later events in carcinogenesis, such as invasion and metastasis.

Introduction

The ampulla of vater is located at the confluence of the main biliary and pancreatic ducts and the duodenum. Consequently, it is composed of three distinct epithelial elements: duodenal mucosa, pancreatic ductal epithelium, and biliary ductal epithelium. Because the ampulla is made up of multiple epithelial elements, ampullary carcinomas demonstrate a wide spectrum of histologic features and biological behavior (1).

According to the World Health Organization classification of tumours, carcinomas of the vater's ampulla are classified under carcinomas of the extrahepatic bile duct system (2). Albores-Saavedra *et al* defined pancreatobiliary and intestinal as the main types of ampullary carcinomas (3). Based on the classification of Albores-Saavedra *et al*, several molecular studies have reported histogenesis and tumourigenesis in ampullary carcinoma (1,4-6). Hansel *et al* reported that caudal type homeodomain transcription factor (*CDX1/2*) is an independent marker of intestinal type ampullary carcinoma (4), and Zhou *et al* reported that gastric mucin *MUC5AC* is associated with pancreatobiliary type ampullary carcinoma (1).

MUC5AC and *CDX1/2* have been used as markers to identify diffuse and intestinal type gastric carcinomas (7). Prasad *et al* reported that expression of several gastric transcription factors, including *GATA4/5/6* and *SOX2,* is up-regulated in pancreatic intraepithelial neoplasia (PanIN) (8). Kim *et al* showed frequent expression of MUC5AC in pancreatic adenocarcinoma (9).

We hypothesised that gastric morphogenesis is involved in the histogenesis of pancreatobiliary type ampullary carcinoma. In the present study, we performed immunohistochemical analyses of the gastric mucin *MUC5AC* and gastric epithelial transcription factor *SOX2* in six resected samples of ampullary adenocarcinoma. We compared the patterns of expression of these markers with histologic subtypes and several components, including mucosal epithelium, neoplastic ducts, vascular invasive lesions, and metastatic lymph nodes.

Materials and methods

Cases. We obtained six specimens of primary ampulla of vater adenocarcinoma that were surgically resected in the Department of Surgical Oncology, Research Institution for Radiation Biology and Medicine at Hiroshima University between 1994 and 2003. All tumours included in the study were limited to the ampulla or located mainly in the ampulla

Correspondence to: Dr Kazuhiro Yoshida, Department of Surgical Oncology, Research Institution for Radiation Biology and Medicine, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734-8551, Japan
E-mail: kyoshida@hiroshima-u.ac.jp

Key words: MUC5AC, SOX2, ampulla of vater carcinoma, carcinogenesis

Table I. Histopathologic evaluation of six resected cases with ampulla vater adenocarcinoma.

Case no.	Age/sex	Macro	Size	Type	Diff	T-grade	N-grade	Stage	Ly orV
1	75/F	Mass	5.2	Intestinal	Pap	3	1	IIB	(+)
2	61/M	Mass	5.3	Intestinal	Well	1	0	IA	(-)
3	65/M	Ulcer	6.0	Pancreat	Mod	4	1	III	(+)
4	75/F	Mass	4.5	Intestinal	Well	1	0	IA	(-)
5	70/F	Mass	3.0	Pancreat	Well	3	1	IIB	(+)
6	81/F	Mass	5.0	Intestinal	Well	3	0	IIA	(+)

M, male; F, female; macro, macroscopic classification; mass, mass-forming type; ulcer, ulcerative or infiltrating type; type, histologic classification; intestinal, intestinal type; pancreat, pancreatobiliary type; diff, differentiation; well, well-differentiated adenocarcinoma; pap, papillary adenocarcinoma; mod, moderately-differentiated adenocarcinoma; T, depth of the tumour; 1, limited to the mucosa; 3, Invasion beyond the muscularis propria; 4, invasion to the adjacent organs; N, lymph node metastasis; 0, absence; 1, presence; stage, WHO classification; Ly or V, presence of lymphatic or vascular invasion.

and secondarily spreading into the neighbouring structures. Four of the patients underwent pancreatoduodenectomy, and two underwent ampullectomy. Two representative paraffin-embedded tissue blocks for each patient were obtained, and haematoxylin and eosin (H&E)-stained sections were screened by microscopy.

Macroscopic and histologic classification of the tumours. Pathologic examination included assessment of the macroscopic type, tumour size (in diameter), depth of the tumour, presence of vascular invasion, lymphatic invasion and lymph node metastasis. Classification was based on the 6th edition of the UICC TNM Classification of Malignant Tumors.

The ampulla of vater adenocarcinomas were classified as intestinal or pancreatobiliary according to the proposal of Albores-Saavedra *et al*. Intestinal type adenocarcinoma resembles small and large intestinal adenocarcinomas with well-formed tubular to elongated glands. The individual cells are columnar, and the nuclei are oval and arranged in a pseudostratified configuration. Brush border and interspersed goblet cells are usually present. Pancreatobiliary type adenocarcinoma resembles pancreatic ductal adenocarcinoma or cholangiocarcinoma. It has simple or branching glands with focal solid nests and papillary and micropapillary formations, and the cuboidal to low columnar cells are generally arranged in a single layer without pseudostratification of the nuclei.

Antibodies. Immunohistochemistry (IHC) for *MUC5AC* was carried out with a monoclonal antibody against *MUC5AC* (1:200; Novocastra Laboratories, Newcastle upon Tyne, UK). IHC for *SOX2* was carried out with a polyclonal anti-*SOX2* antibody (1:200; Chemicon, Temecula, CA, USA).

IHC. A Dako LSAB kit (Dako, Carpinteria, CA, USA) was used for the immunohistochemical analyses. In brief, paraffin-embedded sections were deparaffinised in xylene and rehydrated through graded ethanols. Sections were then microwaved in concentration buffer for 15 min to retrieve antigenicity. After endogenous peroxidase activity was blocked with 3% H_2O_2-methanol for 10 min, sections were incubated with primary antibodies. Sections were treated consecutively with each primary antibody for 8 h at 4˚C followed by sequential 10-min incubations with biotinylated anti-rabbit or anti-mouse IgG and peroxidase-labeled streptavidin. Staining was completed after a 10-min incubation

Table II. Expression of *MUCSAC* in several components of ampulla vater carcinoma.

Case no.	Mucosal area	Central area	Ly or V	Lymph node
1	f+	f+	-	-
2	f+	*	*	*
3	f+	f+	d+	d+
4	-	*	*	*
5	f+	f+	f+	d+
6	f+	-	-	-

Mucosal area, supreficial components of the tumour not beyond the Oddi's muscular propria; central area, malignant tubles located in the centre of the tumour; Ly or V, lymphatic or vascular invasive area; lymph node, metastatic lymph node; f+, focally positive; d+, diffuse positive; -, negative; *, not obtained.

Table III. Expression of *SOX2* in several components of ampulla vater carcinoma.

Case no.	Mucosal area	Central area	Ly or V	Lymph node
1	-	-	-	-
2	-	*	*	*
3	f+	f+	d+	d+
4	f+	*	*	*
5	-	f+	d+	d+
6	f+	-	-	-

Mucosal area, supreficial components of the tumour not beyond the Oddi's muscular propria; central area, malignant tubles located in the centerofthe tumour; Ly or V, lymphatic or vascular invasive area; lymph node, metastatic lymph node; f+, focally positive; d+, diffuse positive; -, negative; *, not obtained.

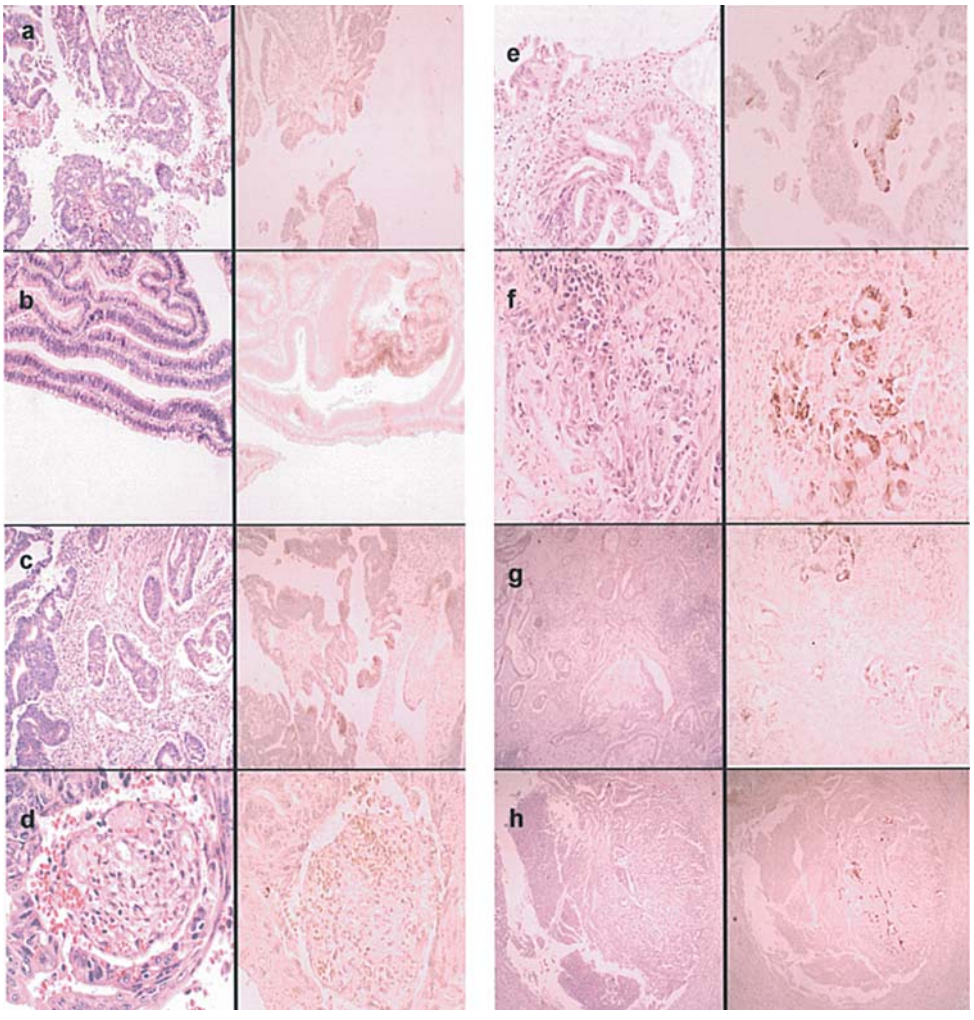

Figure 1. Expression of *MUC5AC* in several components of ampulla of vater adenocarcinoma. Original magnification is x200 unless otherwise stated. Representative H&E stained sections are shown in the left column and corresponding immunohistochemically stained sections are shown on the right. (a-d) Expression in intestinal type carcinoma. (a) Weak and focal expression of *MUC5AC* is present in mucosa (case 1). (b) In intestinal type adenocarcinoma associated with tubilovillous adenoma, focal expression of *MUC5AC* is present only in the carcinoma components (case 2). (c) Slight expression of *MUC5AC* is visible in the neoplastic tubules of invasive components (case 6). (d) *MUC5AC* is not expressed in the vascular invasive lesion (case 1). (original magnification, x400). (e-h) Expression in pancreatobiliary type carcinoma. (e) In the mucosal neoplastic epithelium, *MUC5AC* is slightly expressed (case 3). (f) Strong expression of *MUC5AC* is present in the invasive lesion. The surrounding abundant fibrous stroma does not express *MUC5AC* (case 5). (g) In the metastatic lymph node, *MUC5AC* is strongly expressed (case 5). (original magnification, x100). (h) Lymphatic invasive lesion with marked necrosis. Dense expression of *MUC5AC* is present.

with the substrate-chromogen solution. Sections were counterstained with 0.1% haematoxylin. Results of the antibody staining were graded according to the percentage of stained target cells. Staining intensity was scored as follows: -, no staining or <10% positive-stained tumour cells; f+, 10-50% of tumour cells stained; and d+, >50% of tumour cells stained.

To evaluate the association between *MUC5AC* or *SOX2* and tumourigenesis of ampulla of vater adenocarcinoma, we divided each tumour into four components: superficial mucosal epithelium, centre of the tumour including neoplastic ducts, vascular or lymphatic invasive lesion and metastatic lymph node. Expression of *MUC5AC* or *SOX2* in each component was scored independently.

Results

Histopathologic evaluation of six resected cases of ampulla of vater adenocarcinoma. Data are summarised in Table I. The average tumour size was 4.8 cm (range, 3.0-6.0 cm) in diameter. Five of the six tumours showed the mass-forming type morphology. Histologically, the six cases included four well-differentiated adenocarcinomas, one papillary adenocarcinoma, and one moderately differentiated adenocarcinoma. Lymph node metastasis and vascular invasion were present in three of the six cases. Four cases were identified as intestinal type carcinomas, two of these were limited to the mucosa. The other two cases were identified as pancreatobiliary type adenocarcinomas accompanied by lymph node metastasis and vascular invasion.

Immunoreactivity and localisation of MUC5AC (Table II). IHC revealed that *MUC5AC* and *SOX2* were not expressed in normal papillary epithelium (data not shown). The expression of *MUC5AC* in the six cases was dependent on histologic subtype. In two of the four intestinal type carcinomas, slight expression was present only in the mucosal neoplastic epithelium (Fig. 1a and b). In the centre of the tumour, only

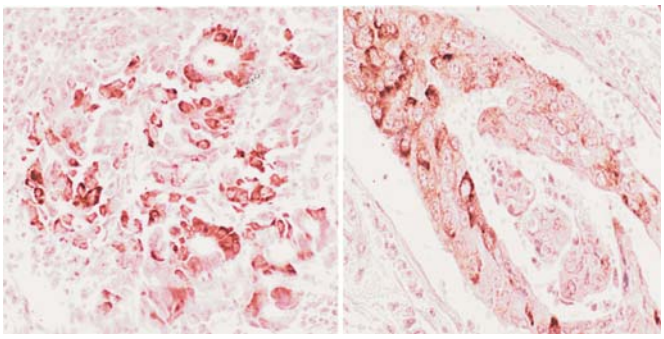

Figure 2. Cytoplasmic expression of *MUC5AC*. Heterogeneous and granular distribution is visible. Original magnification, x400.

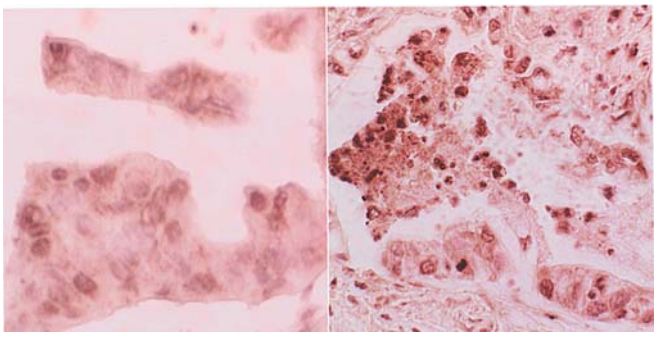

Figure 4. Nuclear expression of *SOX2*. Original magnification, x400.

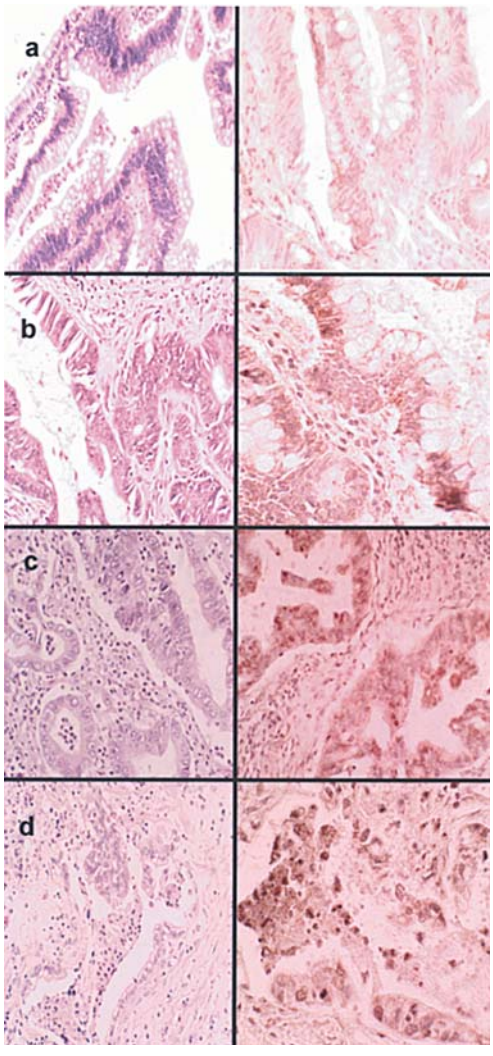

Figure 3. Expression of *SOX2* in several components of ampulla of vater adenocarcinoma. Representative H&E-stained sections are shown in the left column and corresponding immunohistochemically stained sections are shown on the right. (a) *SOX2* is not expressed in the mucosal epithelium of intestinal type carcinoma (case 1). (b) *SOX2* is focally expressed in the basal area of neoplastic tubules in intestinal type carcinoma (case 6). (c) Dense nuclear expression is present in invasive ducts of pancreatobiliary type carcinoma (case 5). (d) A vascular invasive lesion strongly expressed *SOX2* (case 3). Original magnification, x200.

one case (case 1) showed *MUC5AC* expression in the neoplastic duct (Fig. 1c). Metastatic lymph nodes and vascular invasive lesions did not express *MUC5AC* in four intestinal type carcinomas (Fig. 1d).

In contrast with the intestinal type adenocarcinomas, the two pancreatobiliary type carcinomas expressed *MUC5AC* in the invasive lesions, including neoplastic ducts (Fig. 1f), vascular invasive lesions (Fig. 1g), and metastatic lymph nodes (Fig. 1h). Some invasive components showed dense cytoplasmic staining arround the nuclei (Fig. 2).

Immunoreactivity and localisation of SOX2 (Table III). SOX2 is a transcription factor involved in gastric epithelial morphogenesis; therefore, only nuclear immunoreactivity was considered positive. In the four intestinal type carcinomas, mucosal neoplastic epithelium rarely showed positive staining (Fig. 3a). In two cases, focal expression was localised in basal components of the epithelium (Fig. 3b). Metastatic lymph nodes and vascular invasive lesions did not express *SOX2* in four intestinal type carcinomas. In contrast, *SOX2* staining was equal or more intense in the two pancreatobiliary type carcinomas than that in superficial neoplastic epithelium. (Fig. 3c and d).

Discussion

The two major findings in our study were: i) *MUC5AC* and *SOX2* were well expressed in a subset of cases of pancreatobiliary type ampullary carcinoma; and ii) in this subtype, expression was maintained and sometimes increased in several invasive components including vascular invasive lesions and lymph node metastases.

MUC5AC expression was reported to be associated with pancreatobiliary type ampullary carcinoma by Zhou *et al* (1), and our present findings are concordant. Furthermore, in the present study, some tumour cells in invasive components expressed higher levels of *MUC5AC* than those in superficial mucosal neoplastic lesions. This finding suggests *MUC5AC* is involved in later events in the development of pancreatobiliary type carcinoma, such as invasion and metastasis. For *SOX2*, strong nuclear expression was observed in metastatic lymph nodes of pancreatobiliary type carcinomas supporting equal suggestion (Fig. 4).

MUC5AC is known as a marker of diffuse type gastric carcinoma and is associated with a poor prognosis in patients with gastric carcinoma (10). Moreover, expression of *MUC5AC* in PanIN, pancreatic carcinoma and cholangiocarcinoma have been studied, and it has been suggested that expression of *MUC5AC* is correlated with a poor prognosis (9,11).

SOX2 is reported to play an important role in maintaining a gastric phenotype in the stomach and may be a marker of diffuse type carcinoma (12). In the present study, the localisation and distribution of *SOX2* were similar to those of *MUC5AC*. Prasad *et al* reported that gastric epithelial markers, including *MUC5AC*, *MUC6*, *GATA4/5/6* and *SOX2* may be involved in pancreatic ductal carcinogenesis (8).

On the basis of our present findings, we believe that gastric epithelial markers are involved not only in the carcinogenesis of pancreatobiliary phenotype carcinomas, but also in the invasion and metastasis that contribute to a poor prognosis. Further studies of other gastric transcription factors and detailed functional analyses are in progress in our laboratory.

References

1. Zhou H, Schaefer N, Wolff M and Fisher HP: Carcinoma of the ampulla of vater. Comparative histologic/immunohistochemical classification and follow-up. Am J Surg Pathol 28: 875-882, 2004.
2. Albores-Saavedra J, Mench HR, Scoazec JC, *et al*: Tumor of the gall-bladder and extrahepatic bile duct. In: WHO Classification of Tumors. Pathology and Genetics of the Digestive System. Hamilton SR and Aaltonen LA (eds). IARC Press, Lyon, pp203-218, 2000.
3. Albores-Saavedra J, Henson DE and Klimstrs DS: Tumor of the gallbladder, extrahepatic bile ducts, and ampulla of the vater. In: Atlas of Tumor Pathology. Third series, Fascicle 27. Rosai J and Sobin L (eds). Armed Forces Institute of Pathology, Washington DC, pp259-316, 2000.
4. Hansel DE, Maitra A, Lin JW, Goggins M, Argami P, *et al*: Expression of caudal-type homeodomain transcription factor CDX1/2 and outcome in carcinomas of the ampulla of vater. J Clin Oncol 23: 1811-1818, 2005.
5. Lau SK, Weiss LM and Chu PG: Differential expression of MUC1, MUC2, and MUC5AC in carcinoma of various sites. An immunohistochemical study. Am J Clin Pathol 122: 61-69, 2004.
6. Chu PG, Schwarz RE, Lau SK, Yen Y and Weiss LM: Immunohistochemical staining in the diagnosis of pancreatobiliary and ampulla of vater adenocarcinoma. Am J Surg Pathol 29: 359-367, 2005.
7. Tatematsu M, Tsukamoto T and Inada M: Stem cells and gastric cancer: role of gastric and intestinal mixed intestinal metaplasia. Cancer Sci 94: 135-141, 2003.
8. Prasad NB, Biankin AV, Fukushima N, Maitra A, Dhara S, *et al*: Gene expression profiles in pancreatic intraepithelial neoplasia reflect the effects of Hedgehog signaling on pancreatic ductal epithelial cells. Cancer Res 65: 1619-1626, 2005.
9. Kim GE, Bae HI, Park HU, Kuan SF, Crawlev SC, *et al*: Aberrant expression of MUC5AC and MUC6 gastric mucins and sialyl Tn antigen in intraepithelial neoplasms of the pancreas. Gastroenterology 123:1052-1060, 2000.
10. Kocer B, Soran A, Kiyak G, Erdogan S, Eroglu A, *et al*: Prognostic significance of mucin expression in gastric carcinoma. Dig Dis Sci 49: 954-964, 2004.
11. Boonla C, Wongkham S, Sheehan JK, Wongkhan C, Bhudhisawasdi V, *et al*: Prognostic value of serum MUC5AC mucin in patients with cholangiocarcinoma. Cancer 98: 1438-1443, 2003.
12. Tsukamoto T, Mizoshita T, Mihara M, Tanaka H, Takenaka Y, *et al*: Sox2 expression in human stomach adenocarcinomas with gastric and gastric-and-intestinal-mixed phenotypes. Histopathology 46: 649-658, 2005.