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Expression of Sialyl-Tn Antigen is Correlated with Survival Time of Patients with Gastric Carcinomas

Xiao Chun Ma, Nobukuni Terata, Masashi Kodama, Snezana Jancic, Youhei Hosokawa and Takanori Hattori

Expression of sialyl-Tn antigen (STN) was examined by an immunohistochemical method in 85 primary gastric carcinomas. The STN expression occurred in 53 (62.4%) cancers, and the positive staining was correlated with degree of gastric wall and lymph vessel invasion, lymph node metastasis, and stage of tumour. Five-year survival rates of patients with STN-positive cancers (47.2%) were significantly lower than those with STN-negative cancers (84.4%) ($P < 0.01$), and patients with STN-positive cancers at stage III and stage IV had a worse prognosis. In the cancers with serosal invasion, patients with STN-positive cancers disclosed a significantly poorer prognosis than those with STN-negative cancers ($P < 0.01$). Therefore, it is suggested that a careful follow-up study and intensive postoperative therapy are needed for patients with advanced gastric cancers with positive STN expression.
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INTRODUCTION

NEOPLASTIC TRANSFORMATION is often associated with changes in glycosylation of glycolipids or glycoproteins in cell membranes. Recently, a novel monoclonal antibody recognising a core structure of mucin-type carbohydrate chain has been made by Kjeldsen *et al.* [1]. This antibody (TKH2) directed to the tumour-associated *O*-linked sialyl Tn 2-6-α-*N*-acetylgalactosaminyl [sialyl-Tn(STN)] epitope was made by immunisation with ovine submaxillary mucin. Immunohistochemical studies have demonstrated that STN is expressed in human bladder cancer [2], ovarian cancer [3], and gastrointestinal cancer cells [4–6], whereas its expression in normal adult tissues is highly restricted [1, 4, 6, 7]. In this study, we examined the expression of STN in gastric cancers of different stages, and determined whether the STN expression might influence the prognosis of patients with gastric carcinomas.

MATERIALS AND METHODS

Patients and pathological studies

85 patients with primary gastric cancers were selected. The patients had undergone gastrectomy in the Department of Surgery, Shiga University of Medical Science Hospital, from 1981 to 1986. Tissue from the resected stomachs were fixed in formalin and embedded in paraffin. Serial paraffin sections of 5 microns were cut, and they were stained by routine histopathological techniques. Histological classification was made according to the criteria of the WHO International Histological Classification of Tumours [8] and stages of cancers were determined according to the Japanese Research Society for Gastric Cancer [9], and TNM classification was also recorded (stage I: T1,2, 3N0M0, stage II: T1,2,3N1M0, stage III: T1,2,3N2M0, stage IV: T1,2,3,4N3,4M1).

Immunoperoxidase staining

The expression and localisation of STN in tissues was determined immunohistochemically by the avidin–biotin–peroxidase complex (ABC) method using 10% formalin-fixed and paraffin-embedded sections [10]. All steps were conducted at room temperature. After deparaffinisation, sections were rehydrated, incubated with freshly prepared 3% hydrogen peroxide in methanol for 30 min, and then washed three times with phosphate-buffered saline (PBS). Then, 10% normal horse serum in PBS was applied to the sections for 30 min. The primary monoclonal antibody TKH2 (Otsuka Assay Labs, Tokushima, Japan) was applied to the sections at a dilution of 1:10 in PBS for 2 h. After rinsing in PBS, the sections were incubated for 30 min with biotin-labelled horse anti-mouse IgG (Vector Labs.). The sections were then treated with avidin–biotin com-

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plex for 30 min (Vector Labs.) and were washed three times with PBS. Finally, sections were reacted with 0.1% 3,3'-diaminobenzidine-tetrahydrochloride containing 0.02% hydrogen peroxidase in PBS, and counterstained with methyl-green. Negative controls were prepared by substituting normal mouse serum or PBS for primary antibody which resulted in no detectable staining. The staining of the cancers was regarded as positive when the stained cells/tissues occupied more than 5% of the observed fields.

Statistical analysis

Statistical analyses of the results between different groups were made on actual case numbers, using the χ^2 test or Student's *t*-test. Overall survival curves of different groups were calculated with the Kaplan–Meier method and the generalised Wilcoxon tests. For multivariate regression analysis, Cox's proportional hazards model was used to determine the effect of several prognostic factors on survival.

RESULTS

Of 85 patients with gastric carcinomas, 51 were male and 34 were female (Table 1), and histological classifications, stages of the cancers and other clinicopathological features are shown in Table 1. Fifty three (62.3%) of the cancers were STN-positive and 32 (37.6%) were STN-negative. In papillary and tubular adenocarcinomas, STN was located preferentially on cell membranes of cancer cells and intraluminal contents of cancerous glands, and occasionally in a few cytoplasmic organelles (Fig. 1). In signet ring cell carcinomas, staining for STN was usually seen in cytoplasm (Fig. 2). In several cases of this cancer type, extracellular mucin lakes were also positively stained. No obvious relationship was found between the STN positivity and age or sex of patients. Of 85 patients, the STN positive cases were 7 out of 19 cancers (37%) in stage I, 9 out of 17 cancers (53%) in stage II, 23 out of 33 cancers (70%) in stage III, and 14 out of 16 cancers (88%) in stage IV. The stage I and stage II cancers showed significantly lower frequencies of the STN expression, compared with more advanced cases ($P < 0.05$). The STN expression was observed in 9 out of 23 early cancers whose invasion was limited to the mucosa and submucosa, and the rate of expression became high in advanced cancer; the rate of expression seemed to be proportional to the depth of invasion ($P < 0.01$). The STN positivity was also correlated with a higher incidence of lymph vessel invasion and lymph node metastasis ($P < 0.01$). A difference in tumour sizes between STN-positive and -negative cancers was also noted ($P < 0.05$).

Table 1. Clinicopathological characteristics of patients with positive and negative STN-stained gastric cancers (n = 85)

	STN staining	
	Negative (%)	Positive (%)
Sex		
Male	19 (37)	32 (63)
Female	13 (38)	21 (62)
Mean age (year)	55.55	57.64
Tumour size (cm)	4.27	5.38*
Stage		
I	12 (63)	7 (37)*
II	8 (47)	9 (53)
III	10 (30)	23 (70)
IV	2 (13)	14 (88)
Histological type		
Papillary adenocarcinoma	1 (25)	3 (75)
Tubular adenocarcinoma	17 (43)	23 (57)
Signet-ring cell carcinoma	14 (36)	25 (64)
Mucinous adenocarcinoma	0 (0)	2 (100)
Depth of invasion		
Mucosa + submucosa	14 (61)	9 (39)†
Muscularis propria	7 (54)	6 (46)
Serosa	11 (22)	38 (78)
Invasion into lymphatics		
Negative	16 (64)	9 (36)†
Positive	16 (27)	44 (73)
Metastases to the lymph nodes		
Negative	20 (54)	17 (46)†
Positive	12 (25)	36 (75)
Venous invasion		
Negative	23 (40)	35 (60)
Positive	9 (33)	18 (67)
Total	32 (37.6)	53 (62.3)

* $P < 0.05$, † $P < 0.01$. *P* value based on the χ^2 test or Student's *t*-test.

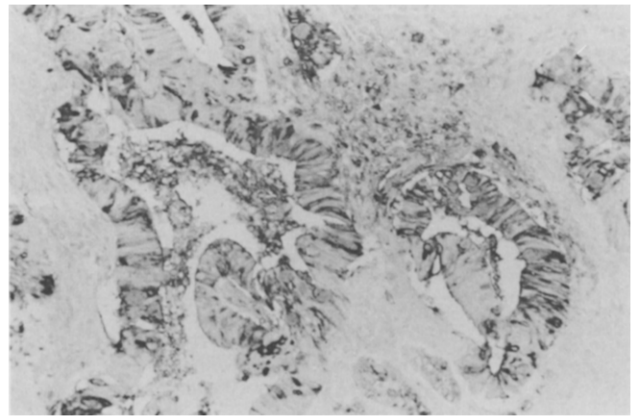

Fig. 1. Tubular adenocarcinoma stained with STN. STN bound to cell membrance and luminal contents, and some to cytoplasm. × 200.

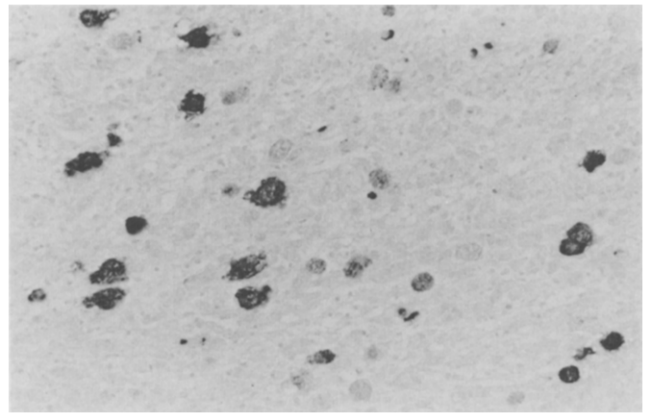

Fig. 2. Signet ring cell carcinoma stained with STN. STN mainly in the cytoplasm of cancer cells. × 400.

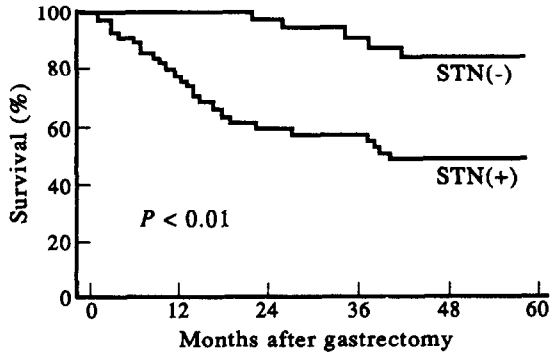

Fig. 3. Five-year survival of patients with gastric carcinoma.

Overall survival curves of patients with STN-positive and -negative cancers are shown in Fig. 3. Patients with STN-positive cancers had lower survival rates than did those with STN-negative cancers [47.2% vs. 84.4% ($P < 0.01$)]. When 5-year survivals were analysed in terms of tumour stages and the STN positivity (Fig. 4), patients with STN-positive cancers had a worse prognosis in stage III [43.5% vs. 70% ($P < 0.05$)] and stage IV [11.4% vs. 50% ($P < 0.05$)]. In Fig. 5, 5-year survival rates of patients with STN-positive and negative cancers in different invasion stages are shown. Figure 6 demonstrates 5-year survival rates of patients with different histological types of STN-positive and -negative cancers, in which 5-year survival was also worse in patients with STN-positive tumours ($P < 0.01$).

Multivariate regression analysis was performed to evaluate further potential prognostic factors. Cox regression analysis

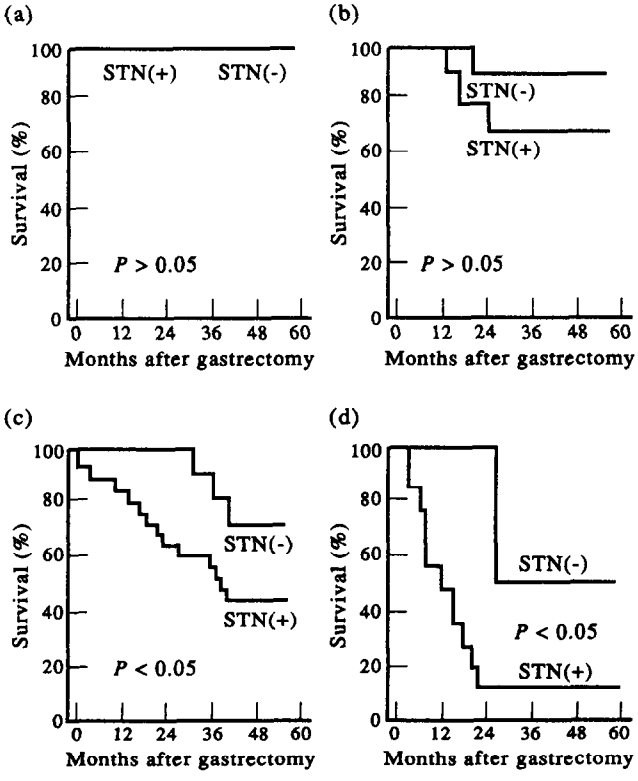

Fig. 4. Five-year survival of patients by stage and STN status. (a) stage I, (b) stage II, (c) stage III, (d) stage IV.

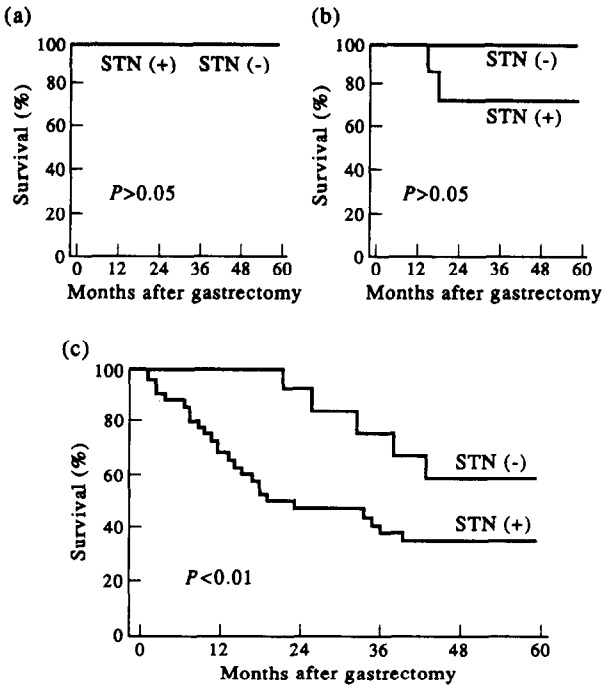

Fig. 5. Five-year survival of patients as a function of tumour invasion plus STN status. (a) Mucosa or submucosa, (b) muscularis propria, (c) serosa.

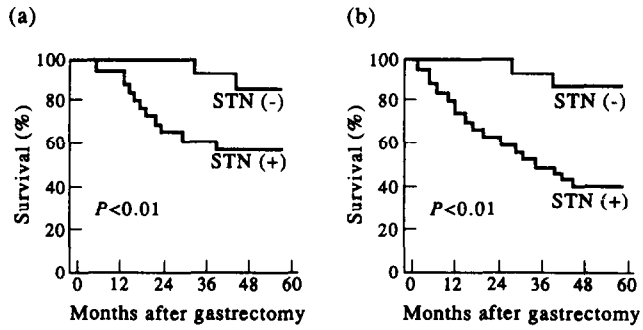

Fig. 6. Five-year survival of patients by histological category and STN status. (a) Tubular adenocarcinoma. (b) Signet-ring cell carcinoma.

revealed that stage, degree of tumour invasion, STN staining and lymph node metastasis were correlated with survival, and STN was shown to be independently correlated with prognosis (Table 2).

Table 2. Proportional hazards model of survival time

Variables	β	S.E.	χ^2	*P*
Age	0.05751	0.08617	0.445	0.5056
Stage	0.31782	0.12210	6.775	0.0109
Histology	−0.05804	0.09651	0.363	0.5492
Degree of invasion	0.21257	0.10119	4.413	0.0387
STN status	0.30681	0.11329	5.335	0.0285
Lymph nodes metastasis	0.12371	0.06201	1.995	0.0439

SE: standard error.

DISCUSSION

Neoplastic transformation is almost invariably associated with altered glycosylation of glycoproteins or glycolipids in cell membranes [11]. The change in cell surface carbohydrate occurs during tumour progression, and such a change can be detected by studying lectin binding patterns. The qualitative and quantitative change of cell surface glycoproteins or glycolipids is thought to be associated with altered cell adhesion ability of cancer cells, which is possibly related to invasive and metastatic properties [12]. Such a change is clearly observed in the synthesis of *O*-linked oligosaccharides of the polypeptide backbone, this is an incomplete synthesis associated with precursor accumulation. The initial step in the synthesis of *O*-linked oligosaccharides is the addition of *N*-acetylgalactosamine to serine or threonine residues, thus forming the Tn antigen. This substance then receives additional carbohydrate residues, such as sialic acid, resulting in formation of the sialyl-Tn antigen, or galactose, in formation of the T antigen. These three antigens T, Tn and sialyl-Tn, have been detected in a majority of cancers, whereas their expression in normal adult tissues is highly restricted. These substances have been speculated to accumulate as a result of blocking of the further glycosylation of T antigen and/or premature 2–6 sialylation of Tn antigen in cancer tissues [6].

Since the first observation of STN expression on tumour tissues by Kjeldsen *et al.* [1] many histochemical and serological studies have been reported. Several studies have revealed a correlation between the STN expression and histological grades, tumour sizes and advancing stages [13–15]. In the present study, we demonstrated that 53 out of 85 advanced gastric cancers (62%) showed STN expression. The gastric cancers in early stages, such as in stage I and II, showed a significantly low frequency of STN expression, compared with more advanced cancers, and a highly positive expression was observed following advance of the disease. Thus, STN expression may be a marker of invasion, possibly reflecting the cancer cells aggressiveness.

The finding that the STN positivity is correlated strongly with lymph vessel invasion and lymph node metastasis suggested that the cancer cells may retain, with STN molecules, an affinity for cells of lymphatics. A similar phenomenon was reported by Tabuchi *et al.* [16], in that the gastric cancer cells with positive CA19-9 antigen expression disclosed a marked lymphatic involvement. On the other hand, we also studied a circulating serum level of STN (unpublished data, 1991), and found that the serum STN level is more increased in those patients with gastric cancers with lymph node and lymphatic involvement than patients of gastric cancers without lymph node and lymphatic involvement.

The present study demonstrates that patients with STN-positive gastric cancers had a shorter survival time than those with STN-negative cancers. Similar results have been shown in colonic and ovarian carcinomas [14, 17]. The lower survival rate of patients with higher STN expression may reflect their higher tumour burden. The apparently better prognosis of patients with early gastric cancers can be simply interpreted as being due to the lower tumour burden, but it is difficult to explain a reason why the STN-negative, advanced cancers showed a relatively longer survival time. This cannot be simply attributed to the sizes or histological types of tumour.

Most of the patients with STN-positive cancers in stage III and IV died during the early postoperative period. In the present study, the expression of STN has been shown to be independently associated with prognosis, and staining power of STN in tissues or serum STN level can be a predictor of survival time. STN may be of considerable importance in deciding whether postresection patients require further therapy or not.

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