Transcription factor c-jun regulates β3Gn-T8 expression in gastric cancer cell line SGC-7901

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Abstract. Aberrant glycosylation, a common feature of malignant alteration, is partly due to changes in the expression of glycosyltransferases, including β1,3-*N*-acetylglucosaminyltransferase 8 (β3Gn-T8), which synthesizes poly-*N*-acetyllactosamine (poly-LacNAc) chains on β1,6 branched *N*-glycans. Although the role of β3Gn-T8 in tumors has been reported, the regulation of β3Gn-T8 expression, however, is still poorly understood. In the present study, we used three online bioinformatic software tools to identify multiple c-jun binding sites in the promoter of the β3Gn-T8 gene. Using luciferase reporter assay, chromatin immunoprecipitation (ChIP) analysis, RT-PCR and western blot analysis, we revealed that c-jun could bind to and activate the β3Gn-T8 promoter, thus upregulating β3Gn-T8 expression. This was also confirmed by changes in β3Gn-T8 activity as demonstrated by flow cytometry, immunofluorescence and lectin blot analysis using LEA lectin. Moreover, expression of glycoprotein HG-CD147, the substrate of β3Gn-T8, was also regulated by c-jun. In addition, c-jun and β3Gn-T8 were more highly expressed in the gastric cancer tissues when compared to these levels in the adjacent non-tumor gastric tissues, and β3Gn-T8 expression was positively correlated with c-jun expression. These results suggest that c-jun plays a significant role in regulating the expression of β3Gn-T8 in the SGC-7901 cell line and may be involved in the development of malignancy via the activity of β3Gn-T8.

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Introduction

Glycosylation is one of the most common forms of post-translational modifications and is essential for many cellular functions. Changes in the composition of glycans added to glycoproteins and glycolipids are common events in malignancy (1,2), and these changes can affect the course of the disease (3-5). Poly-*N*-acetyllactosamine (poly-LacNAc) linkage on glycoconjugates is a unique glycan comprised of *N*-acetyllactosamine (LacNAc) repeats (Galb1-4GlcNAcb1-3)$_n$, and is associated with cancer progression (6). For example, it has been reported that β1,6-branched *N*-glycans containing poly-LacNAc correlate with a variety of malignant phenotypes of tumor cells, and affect cell proliferation (7) and metastatic potential (8-10). In addition, poly-LacNAc may be modified to carry important carbohydrate structures such as Lewis-related antigens (11-14) and human natural killer-1 (HNK-1) antigen (15). Poly-LacNAc and its related structures may alter structural and functional characteristics of proteins that carry them and play important roles in cell-cell interaction, cell-extracellular matrix (ECM) interaction (16) and metastatic capacity (17). Notably, highly metastatic colon cell lines were found to synthesize more *N*-glycans that contain poly-LacNAc than poorly metastatic colon cell lines (18).

The presence and elongation of poly-LacNAc have been attributed to the overexpression of a number of β1,3-*N*-acetylglucosaminyltransferases (β3Gn-Ts). These enzymes have different tissue distribution and different receptor substrate specificity (19), but all utilize UDP-*N*-acetylglucosamine (UDP-GlcNAc) as the donor to transfer LacNAc to the non-reducing terminus of Gal to form β1,3 linkage. Among these enzymes, β3Gn-T8 was first cloned by our laboratory and was responsible for the synthesis of poly-LacNAc chains on β1,6 branched *N*-glycans (20,21). Our previous studies confirmed that β3Gn-T8 is abnormally expressed in a variety of tumor cells, and influences cancer invasion and metastasis ability by regulating matrix metalloprotein 2 (MMP2) expression (22,23). We also found that another glycoprotein

CD147 was modified by β3Gn-T8 and was associated with cancer metastatic potential in colon cancer cells (24). CD147 has been shown to be a glycoprotein that carries a large amount of poly-LacNAc chains on its *N*-glycans by LC/MS techniques in a variety of cancer cell lines (25). However, how β3Gn-T8 expression is regulated has not yet been reported. Bioinformatic studies revealed that most glycosyltransferase genes have TATA-less, CpG-associated promoters (26) and we found oncogenic transcription factor Ets-1 and c-jun binding sites within the β3Gn-T8 promoter region. In our preliminary experiments, no definite evidence was obtained that β3Gn-T8 was regulated by the transcription factor Ets-1 in gastric cancer cells (data not shown). Therefore, we investigated whether β3Gn-T8 expression was mainly regulated by the transcription factor c-jun in the SGC-7901 gastric cancer cell line.

In the present study, three bioinformatics online software tools (AliBaba 2.1, TESS and PATCH, data not shown) were employed to predict the binding sites of transcription factors to the β3Gn-T8 promoter. One transcription factor, c-jun, emerged as a potential regulator of β3Gn-T8 expression. Luciferase reporter assay, chromatin immunoprecipitation (ChIP) assay and point mutation analysis were used to confirm the binding of c-jun on the β3Gn-T8 promoter. Meanwhile, we also found that c-jun could regulate the expression and enzymic activity of β3Gn-T8 and *N*-glycans of HG-CD147 in the SGC-7901 cells. In addition, we further demonstrated that c-jun is positively correlated with β3Gn-T8 in human cancer tissues.

Materials and methods

Materials. Gastric cancer cell line SGC-7901 was preserved in our laboratory. Plasmid pCI-neo, pGL3-basic-luc (pGL3) pRL-SV40 and Dual-Luciferase Reporter Gene Assay kit were purchased from Promega (Madison, WI, USA). RPMI-1640 medium was obtained from Gibco-BRL (USA), and transfection reagent Lipofectamine 2000 and primers were procured from Invitrogen. ChIP assay kit was purchased from Beyotime Institute of Biotechnology (China) and c-jun antibody from Abcam (Hong Kong).

Patients and samples. A total of 97 patient specimens were obtained from the First and Second Affiliated Hospitals of Soochow University between January 1, 2008 and March 31, 2010. In all cases, the specimens obtained were inspected independently by two pathologists according to the classification of gastric cancer by Lauren's system. The clinical and pathological data collected included gender, age, clinical stage (AJCC, American Joint Committee on Cancer), histological grade, histological type (Lauren), depth of invasion, and presence of lymph node metastasis. Ethical approval was obtained from the First and Second Affiliated Hospitals and the study was approved by the Soochow University Research Ethics Committee.

Cell culture. The SGC-7901 cell line was cultured in RPMI-1640 medium containing 10% fetal calf serum, 100 μg/ml penicillin and 100 μg/ml streptomycin in a water-saturated, 5% CO_2 atmosphere at 37˚C.

Cloning and plasmid construction. The putative promoter region of the human β3Gn-T8 gene was amplified by PCR and cloned into a pGL3 vector, to construct the recombinant vectors pGL3-luc (-1449/+107), pGL3-luc (-947/+107), pGL3-luc (-760/+107), pGL3-luc (-561/+107), pGL3-luc (-503/+107), pGL3-luc (-393/+107), pGL3-luc (-248/+107) and a mutant plasmid pGL3-luc (-561/+8). Point mutations were generated with the QuickChange II XL Site-Directed Mutagenesis kit (Stratagene) using pGL3-561/+8-luc as a template. All plasmids were confirmed by DNA sequencing.

Transient transfection and dual-luciferase activity assay. The cells were cultured in 24-well plates at $1x10^5$ cells/well on the day prior to transfection. Plasmids were extracted, measured for concentration, and transfected into SGC-7901 cells using Lipofectamine 2000. The cells were co-transfected with 500 ng pCI-neo-jun vector, 500 ng pGL3-β3Gn-T8-promoter vectors and 50 ng pRL-SV40 vector. After transfection (48 h), the cells were lysed with 500 μl of lysis buffer. Dual-luciferase activity assays were performed according to the Dual-Luciferase Reporter Assay System technical manual. The relative luciferase activity (firefly luciferase/*Renilla* luciferase) of the transfected cells in each group was determined with the Thermo Scientific Fluoroskan Ascent FL.

Chromatin immunoprecipitation assays. The SGC-7901 cells were used for the ChIP assays. We used the Beyotime Chip Assay kit and followed the manufacturer's instructions. The ChIP analysis was conducted using antibodies against c-jun and IgG. After the ChIP assessment, the samples were purified using the PCR/DNA purification kit and products were subjected to PCR amplification with the following primer sequences: sense, 5'-TGTACGCGTGAGGCACATGGCAAAGG-3' and antisense, 5'-GTTCTCGAGAGTGGGGAGGAAGTGGT-3'. The PCR products were subjected to 1.5% agarose gel electrophoresis, and a gel imaging system was used to analyze the bands.

RT-PCR. Total RNA from each experimental group of cells was extracted using TRIzol (Gibco-BRL) according to the manufacturer's instructions. cDNA was generated from total RNA using M-MLV Reverse Transcriptase (Fermentas, USA). Amplification was performed for >28 cycles. PCR products were separated by electrophoresis on a 1.5% agarose gel and stained with ethidium bromide to visualize the bands. Primer sequences and expected product sizes are listed in Table I.

Western blot analysis. Western blot analysis was performed as previously described (23,24). In brief, the cells were lysed with lysis buffer and 40 μg of protein from each sample was resolved by 10% SDS-PAGE gel and transferred to a nitrocellulose membrane (Millipore, Bedford, MA, USA). The membrane was blotted with antibodies β3Gn-T8, GAPDH, c-jun and CD147 (all purchased from Santa Cruz Biotechnology, Santa Cruz, CA, USA).

Flow cytometric analysis. For poly-LacNAc chain analysis, biotin-labeled LEA (Sigma, USA), which is specific for poly-LacNAc residues, was used. Cells were harvested and stained with 10 μg/ml LEA PBS (containing 0.5% BSA and 0.05% sodium azide) at 37˚C for 1 h. After being washed three

Table I. Primer sequences for RT-PCR analysis.

Gene	Primer sequences	Size (bp)
c-jun	Sense: 5'-GCCTCAGACAGTGCCCGAGAT-3' Antisense: 5'-GTTTAAGCTGTGCCACCTGTTCC-3'	245
β3Gn-T8	Sense: 5'-CCCTGACTTCGCCTCCTAC-3' Antisense: 5'-GGTCTTTGAGCGTCTGGTTGA-3'	362
GAPDH	Sense: 5'-TGAACGGGAAGCTCACTGG-3' Antisense: 5'-TCCACCACCCTGTTGCTGTA-3'	307

times with PBST (PBS + 0.05% Tween-20), the cells were then stained with 10 μg/ml phycoerythrin (PE)-conjugated streptavidin (Sigma) at 37˚C for 1 h and washed another three times with PBST. The fluorescence intensity of the stained cells was measured with a flow cytometer and analyzed with CellQuest (BD Biosciences, USA).

Immunofluorescence staining. Biotin-labeled LEA was used in this experiment to examine poly-LacNAc chains on the cell surface. In brief, cells were incubated with biotinylated *Lycopersicon esculentum* (tomato) lectin (20 μg/ml) for 2 h at room temperature and then incubated with streptavidin-R-phycoerythrin (0.4 μg/ml, Sigma) for 1 h at room temperature. Images were obtained using an inverted fluorescence microscope combined with a digital camera.

Lectin blot analysis. The levels of poly-LacNAc were analyzed by lectin blot analysis. In brief, the cells were lysed and cell extracts were separated using 10% SDS-PAGE gel electrophoresis, and transferred onto nitrocellulose membranes. The membranes were incubated with biotinylated LEA (1:400 dilution) for 1 h and then incubated with streptavidin-HRP (1:1,000 dilution) for 1 h. The protein bands on the membranes were visualized using an ECL kit (GE Healthcare).

Tissue microarrays and immunohistochemistry. Tissue microarrays were constructed using 97 gastric adenocarcinoma specimens paired with 89 adjacent non-tumor gastric mucosa 5 cm away from the adenocarcinoma (eight samples of adjacent non-tumor gastric tissues were lost). Immunohistochemical staining was performed on 4-μm sections of paraffin-embedded tissue samples to detect the expression levels of β3Gn-T8, c-jun, MMP2, and tissue inhibitors of matrix metalloproteinases 2 (TIMP2) protein. In brief, the slides were incubated in β3Gn-T8, c-jun MMP2, and TIMP2 antibodies diluted to 1:100-300 at 4˚C overnight. The subsequent steps were performed using the EnVision™ FLEX High pH 9.0 visualization system (Dako, Demark).

Statistical analysis. The results are presented as means ± SD. $P<0.05$ was considered to indicate statistically significant differences. SPSS 13.0 was used for statistical analysis. The intensities of the protein expression levels in gastric cancer and adjacent non-tumor gastric tissue were compared with the chi-square test (McNemar's test). The relationship between the intensity of protein expression and clinical pathological parameters was analyzed with the chi-square test.

Results

c-jun regulates β3Gn-T8 promoter activity by directly binding to the β3Gn-T8 promoter. To map the c-jun binding site on the β3Gn-T8 promoter, a series of β3Gn-T8 promoter segments were generated and analyzed by co-transfection with c-jun. Promoter fragments ranging from -1449, -947, -760, -561, -503, -393, -248, -561 to +107 (related to the transcription start site) were cloned into a pGL3 vector upstream of a luciferase reporter gene and assessed for their transcriptional activity in the SGC-7901 cell line. As shown in Fig. 1A, compared with other promoter segments, -561/+8 caused the biggest changes in the reporter activity, and with the increasing amount of exogenous c-jun, the reporter activity of the -561/+8 deletion mutant was also gradually increased (Fig. 1B). Furthermore, point mutations within this element were constructed and luciferase assays were performed again (Fig. 1C). When we mutated the TGAGTCA/TTAATCA conservative sequence (-160~-154), which is critical for the binding of c-jun transcription factors, the promoter activity was markedly reduced in the SGC-7901 cells. These results suggest that -561/+8 is a potential c-jun binding sequence on the β3Gn-T8 promoter. We next carried out a ChIP assay to examine the *in vivo* relevance of c-jun's binding to the β3Gn-T8 promoter. With an anti-c-jun antibody, immunoprecipitated chromosomal DNA was subjected to RT-PCR. The results showed that c-jun indeed interacted with the β3Gn-T8 promoter region in the SGC-7901 cell line (Fig. 1D).

Correlation between c-jun and β3Gn-T8 expression in SGC-7901 cells. Next we explored whether c-jun could regulate β3Gn-T8 transcription and protein expression in the SGC-7901 cells. Firstly, stable cell lines with overexpression and interference-expression of c-jun were established in the SGC-7901 cell line. As shown in Fig. 2, the c-jun mRNA and protein levels in the SGC-7901 cells were measured by RT-PCR and western blot analysis, respectively. When compared with control groups, c-jun expression was significantly decreased in the interference vector-transfected cells (7901/c-junSi) and increased in the pCI-neo-c-jun expression vector-transfected cells (7901/c-jun). Notably, c-jun overexpression upregulated β3Gn-T8 expression, and silencing downregulated β3Gn-T8 expression. These results indicated that β3Gn-T8 expression was at least partially regulated by c-jun.

Effect of c-jun on the poly-LacNAc expression in the SGC-7901 cells. β3Gn-T8 is involved in the synthesis of

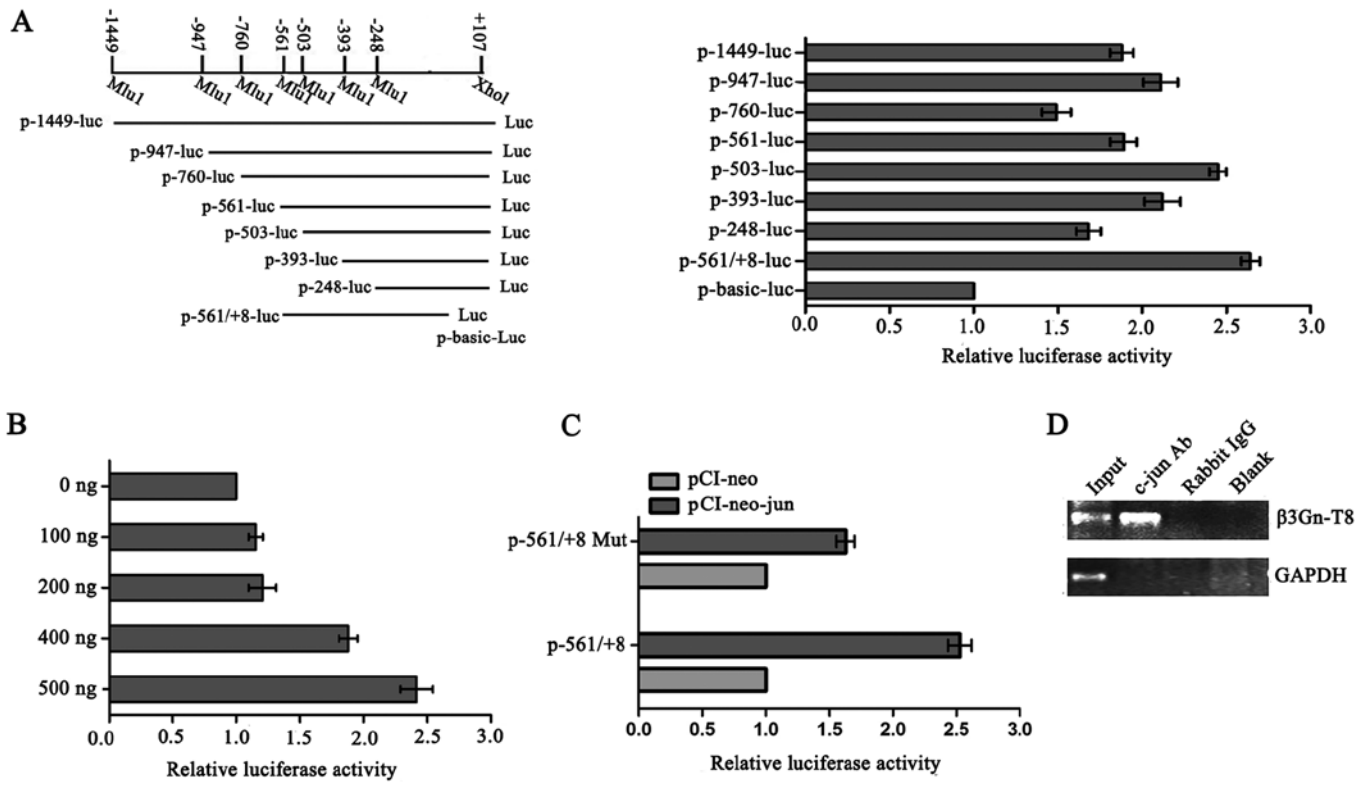

Figure 1. Direct binding of c-jun to the β3Gn-T8 promoter. (A) The putative promoter region of the human β3Gn-T8 gene was cloned into a pGL3 vector upstream of the firefly luciferase reporter gene. Luciferase activity (right) was normalized to *Renilla* luciferase activity. (B) Activation of the β3Gn-T8 promoter region (-561/+8) with different amounts of c-jun. (C) Activation of the β3Gn-T8 promoter region (-561/+8) mutant by c-jun. (D) ChIP analysis was performed with an anti-c-jun antibody using the SGC-7901 cells. RT-PCR was performed to quantify the amount of precipitated c-jun in the β3Gn-T8 core promoter region.

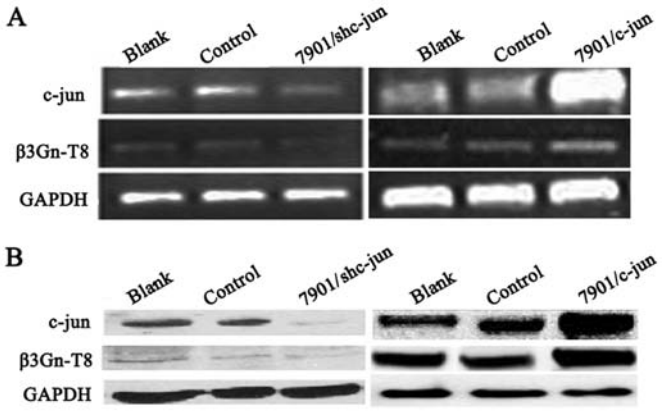

Figure 2. β3Gn-T8 transcription is regulated by c-jun. (A) c-jun was knocked down by siRNA transfection or overexpressed in the SGC-7901 cells. The total mRNA was extracted, and the expression levels of β3Gn-T8 and GAPDH mRNA were quantified by RT-PCR. (B) Cells were lysed and expression levels of β3Gn-T8 and GAPDH protein were quantified by western blot analysis.

poly-LacNAc chains, and hence, we investigated whether c-jun expression could influence poly-LacNAc chain formation. The level of poly-LacNAc on the cell membrane was detected by flow cytometric analysis. As shown in Fig. 3A, the level of poly-LacNAc in the SGC-7901/c-junSi cells was significantly decreased compared with the level noted in the control groups but was increased in the SGC-7901/c-jun cells ($p<0.05$). In order to confirm the results, immunofluorescence staining was also performed to examine the alteration of poly-LacNAc chains in the SGC-7901/c-junSi cells and we obtained similar results (Fig. 3B). To further confirm the relationship of c-jun and poly-LacNAc, lectin blot analysis was used to detect whole poly-LacNAc expression in the SGC-7901 cells. As shown in Fig. 3C, compared to the control group, c-jun overexpression upregulated the glycoprotein modified by poly-LacNAc, and c-jun silencing downregulated the glycoprotein modified by poly-LacNAc expression. In addition, the molecular size of glycoproteins regulated by c-jun ranged from 49 to 90 kDa. These results indicated that c-jun may affect poly-LacNAc expression and glycoprotein modified by poly-LacNAc ranged from 49 to 90 kDa through regulation of β3Gn-T8 expression and enzymatic ability.

Correlation between c-jun and CD147 expression in SGC-7901 cells. To study the co-expression relationship between β3Gn-T8 and CD147, western blot analysis was used to assess β3Gn-T8 and CD147 expression. As shown in Fig. 4A, the level of glycosylation of HG-CD147 was reduced apparently with silenced β3Gn-T8 expression when compared to the control group. The changes in the *N*-glycans of HG-CD147 indicated that β3Gn-T8 may be involved in the synthesis of poly-LacNAc on *N*-glycans of HG-CD147 in the SGC-7901 cells. To further study the correlation between c-jun and CD147 expression, c-jun was upregulated or downregulated in the SGC-7901 cell line. As shown in Fig. 4B, the level of glycosylation of HG-CD147 was

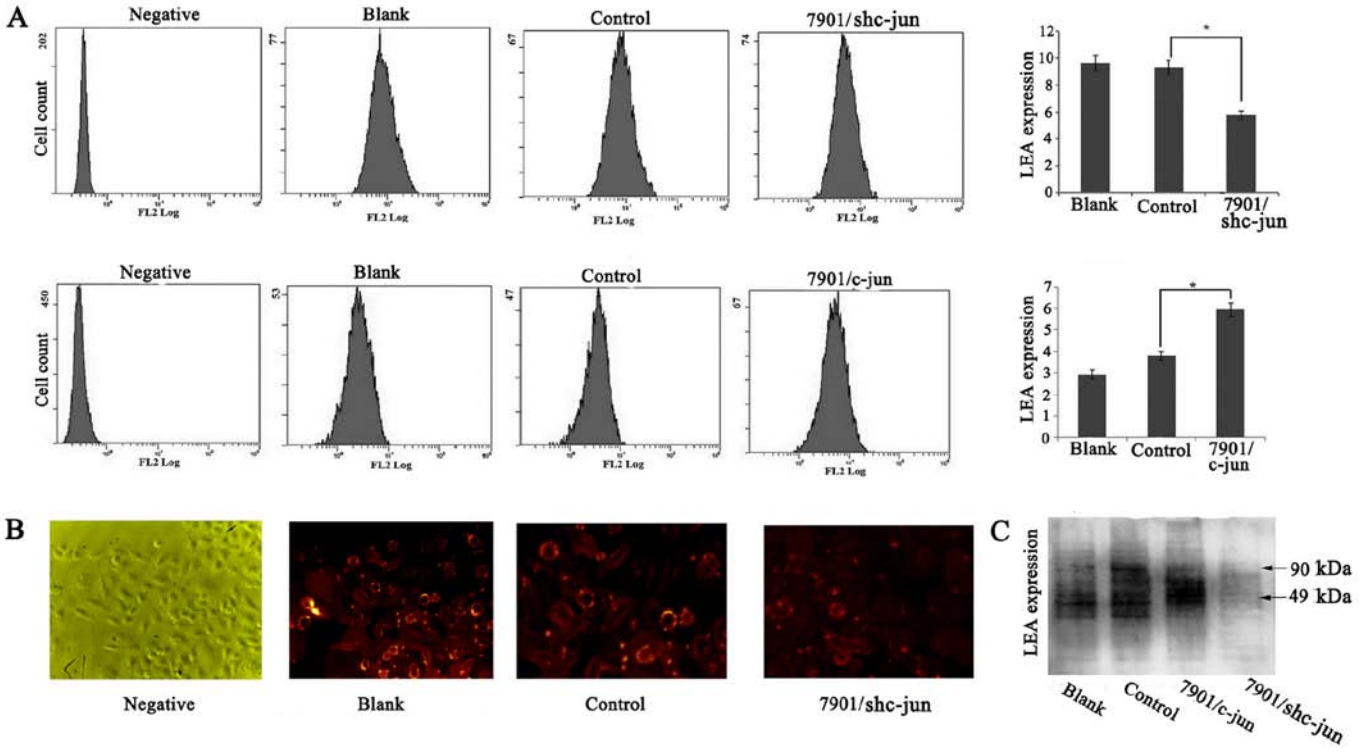

Figure 3. β3Gn-T8 enzymatic activity of synthesizing poly-LacNAc chains is regulated by c-jun. (A) Results of flow cytometric analysis and poly-LacNAc residues can be specifically identified by tomato lectin (LEA) (negative, non-treated; blank, non-transfected; control, control vector-transfected. *P<0.05). (B) Results of immunofluorescent staining and the red fluorescence intensity by phycoerythrin reflect the poly-LacNAc residues. (C) Poly-LacNAc chains on glycoproteins using lectin blot analysis. The molecular size of the glycoproteins modified by poly-LacNAc chains regulated by c-jun ranged from 49 to 90 kDa.

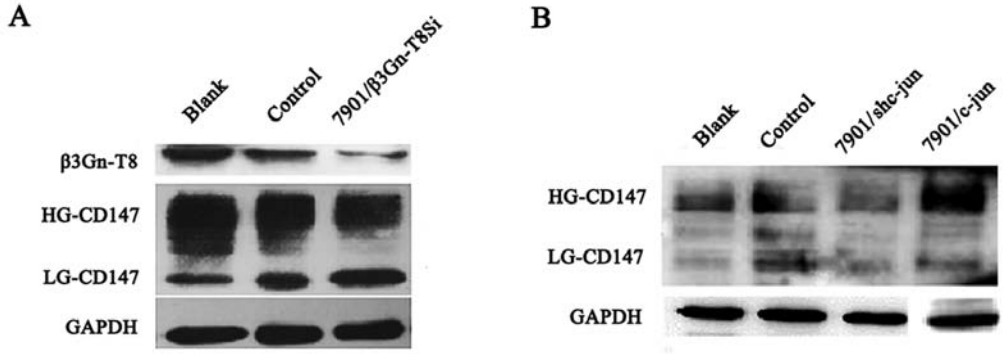

Figure 4. *N*-glycans of HG-CD147 is regulated by β3Gn-T8 and c-jun, respectively. (A) β3Gn-T8 was knocked down in SGC-7901 cells by siRNA transfection. Western blot analyis was used to detect β3Gn-T8 and CD147 expression (blank, non-transfected; control, control vector-transfected). (B) Under c-jun-depleted and -overexpressed conditions, the level of CD147 was quantified by western blot analysis. The expression levels of CD147 were normalized to the corresponding GAPDH levels (blank, non-transfected; control, control vector-transfected).

decreased with the silencing of c-jun expression compared with the control cells but increased in the c-jun-upregulated cells. The molecular size of HG-CD147 (55 kDa) was also in the range (49-90 kDa) of the glycoproteins modified by poly-LacNAc by the aforementioned lectin blot analysis. We speculated that c-jun affects *N*-glycans of HG-CD147 through regulation of β3Gn-T8 expression in the SGC-7901 cell line.

c-jun and β3Gn-T8 expression and clinicopathological features of gastric cancer. To investigate the clinical importance of c-jun and β3Gn-T8 in gastric cancer tissues, we performed immunohistochemical analysis in 97 human gastric cancer tissues and 89 matched adjacent tissues (eight adjacent tissues were lost). As shown in Fig. 5, the expression of c-jun and β3Gn-T8 in gastric cancer tissues was significantly higher than that noted in the adjacent tissues ($p<0.001$). Furthermore, c-jun and β3Gn-T8 expression was related to clinicopathological features. The characteristics of the 97 patients included in this study are described in Table II. c-jun and β3Gn-T8 expression were positively correlated with TNM stage (AJCC), depth of invasion and lymph node metastasis ($p<0.05$). There was no significant association between c-jun and β3Gn-T8 expression when comparing age, gender, histological grade and Lauren type ($p>0.05$).

We further investigated whether the expression of β3Gn-T8 was correlated with that of c-jun and invasion-related proteins

Table II. Relationship between expression of β3Gn-T8 and c-jun and clinicopathological parameters of the gastric cancer cases.

Clinicopathological parameters	No.	High expression of c-jun n (%)	P-value	High expression of β3Gn-T8 (%) n (%)	P-value
Age (years)			0.662		0.879
≥60	63	54 (86)		53 (84)	
<60	34	28 (82)		29 (85)	
Gender			0.516		0.752
Female	29	25 (86)		24 (82)	
Male	68	57 (84)		58 (85)	
TNM stage (AJCC)			0.000[b]		0.001[b]
I-II	33	21 (64)		22 (66)	
III-IV	64	61 (95)		60 (93)	
Depth of invasion			0.001[a]		0.011[a]
T1 to T2	21	13 (62)		14 (66)	
T3 to T4	76	69 (91)		68 (89)	
Lymph node metastasis			0.017[b]		0.003[b]
Yes	70	63 (90)		64 (91)	
No	27	19 (70)		18 (66)	
Histological grade			0.107		0.305
High or moderate	34	26 (76)		27 (79)	
Low	63	56 (89)		55 (87)	
Lauren type			0.280		0.633
Intestinal	66	54 (82)		55 (83)	
Diffuse	31	28 (90)		27 (87)	

Correlation is significant at the [a]0.05 level (two-tailed) and [b]0.01 level (two-tailed). AJCC, American Joint Committee on Cancer.

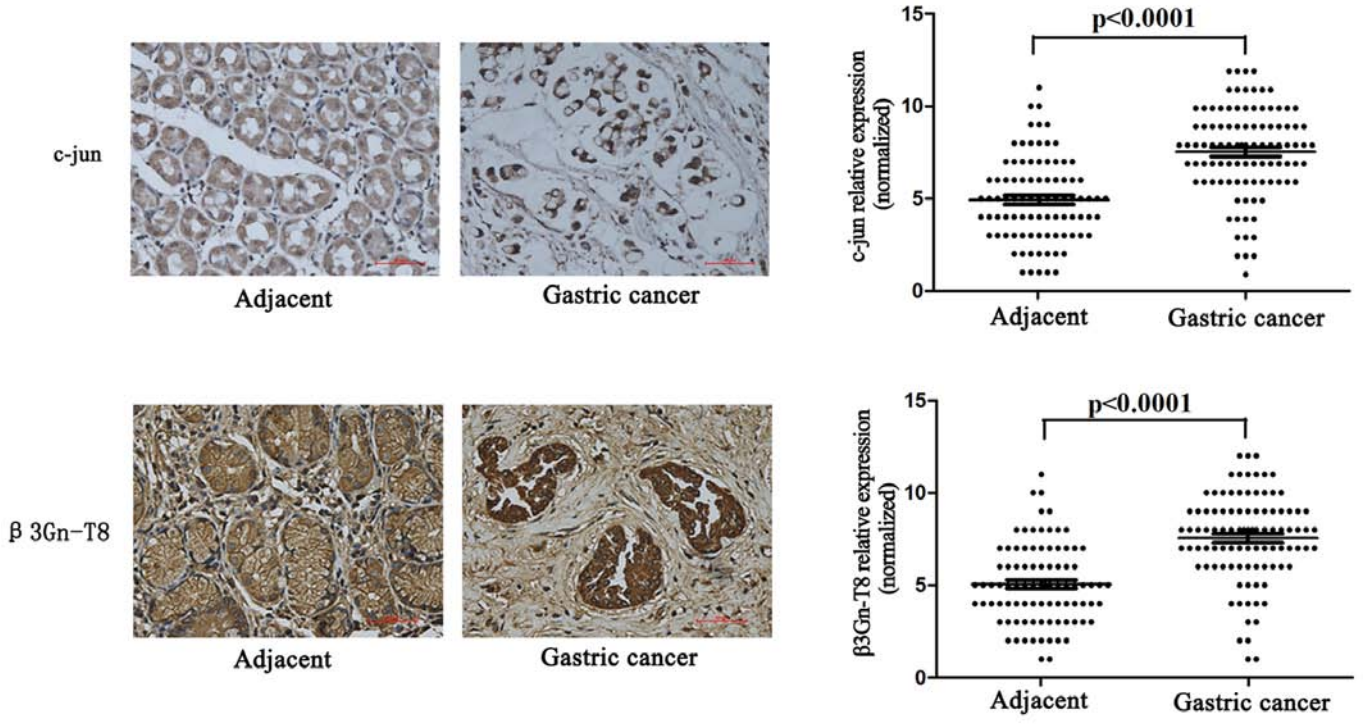

Figure 5. Expression of β3Gn-T8 and CD147 proteins in 88 samples of gastric cancer and adjacent non-tumor gastric tissues. Immunohistochemical analysis was used to detect the expression of c-jun and β3Gn-T8.

Table III. Correlation between β3Gn-T8 and c-jun, MMP2 and TIMP2 expression levels in gastric cancer.

		c-jun	MMP2	TIMP2
β3Gn-T8	r	0.842	0.703	-0.298
	P-value	0.011	0.000	0.021

MMP2 and TIMP2 in the gastric cancer tissue samples. As shown in Table III, the β3Gn-T8 protein expression level was significantly correlated with those of c-jun (r=0.842; P=0.01), MMP2 (r=0.703; P=0.000), and TIMP2 (r=-0.298; P=0.021).

Discussion

Nearly all proteins that are expressed on the plasma membrane or secreted carry glycans that are involved in cell adhesion, recognition, molecular trafficking, clearance, and signaling (27). Aberrant glycosylation occurs in essentially all types of human cancer and appears to be an early event as well as a key factor in the induction of invasion and metastasis (1-5,28). Changes in glycosylation that occur in cancer can also alter molecular interactions with the immune system (29) and receptor signaling. Thus, increased expression of β3Gn-T8, which catalyzes the formation of poly-LacNAc glycans, may play an important role in the promotion and progression of cancer. β3Gn-T8 was found to be expressed in various human tissues. Notably, Ishida *et al* (21) reported that expression of β3Gn-T8 is quite low in normal colon tissues, but increases markedly in colon cancer tissues. Our results indicated that the enzyme was expressed significantly higher in some tumor tissues than in normal tissues (30). Knockdown of β3Gn-T8 expression by RNAi reduced the tumorgenicity of gastric cancer cells in nude mice (31). Moreover, overexpression of β3Gn-T8 promoted cancer invasion and metastasis ability in AGS gastric cancer (22), U251 glioma (23), LS-174T and LoVo colon cancer cells (24).

To date, little is known concerning the regulation of β3Gn-T8 expression in gastric cancer cells. Analysis of the promoter region of β3Gn-T8 identified binding sites for the ubiquitous transcription factor c-jun predicted by three bioinformatics softwares, AliBaba 2.1, TESS and PATCH (data not shown). AP-1 is a sequence-specific transcriptional factor composed of Fos and Jun family members, which form homodimers or heterodimers to recognize the AP-1 site or related sequence. As one of the major subunits of the AP-1 complex, c-jun was reported to be upregulated in various human cancers (32). Recent studies suggest that the AP-1 signaling pathway plays an important role in the regulation of cell proliferation, apoptosis and malignant transformation, and is also involved in tumor formation, invasion and metastasis (33-35). In the present study, luciferase assay and ChIP analysis showed that β3Gn-T8 promoter activity was regulated by c-jun in a dose-dependent manner in the region of -561/+8 (Fig. 1). Furthermore, to investigate whether c-jun actually regulates β3Gn-T8 transcription, c-jun expression was upregulated or downregulated and the β3Gn-T8 expression was also increased or decreased accordingly (Fig. 2). In addition, this change in expression also led to changes in the formation of poly-LacNAc chains on glycoconjugates (Fig. 3). All these results suggest that β3Gn-T8 expression may be regulated by c-jun.

It has been reported that CD147 is a cell surface transmembrane glycoprotein carrying β1,6 branched poly-LacNAc chains on its *N*-glycans (36) and may act as the substrate for β3Gn-T8 in colon cancer cells (24). CD147 is highly expressed in various human carcinoma tissues and cell lines, and is correlated with tumor progression under experimental and clinical conditions (37). It has been confirmed that all CD147 glycosylation is *N*-linked. A high-glycosylated form HG-CD147 (~40-60 kDa) contains complex-type carbohydrates, while the low-glycosylated form LG-CD147 (~32 kDa) contains the high-mannose form (36). It has been reported that HG-CD147 plays an important role in the induction of MMPs, thereby leading to extracellular matrix degradation and increased tumor growth and metastasis (38). In addition, HG-CD147 was found to contribute to lymphatic metastasis potential in mouse hepatocarcinoma cells by altering the level of *N*-glycans (39). Moreover, *N*-glycans of HG-CD147 mainly carry β1,6-branched structures, which are formed by GnT-V. The GnT-V product is the preferred substrate for extension with poly-LacNAc chains (40). In the present study, the level of glycosylation on HG-CD147 was greatly reduced with silencing of β3Gn-T8 expression when compared to the wild-type and mock group in the SGC-7901 cell line ($p<0.05$) (Fig. 4A), indicating that the *N*-glycans of CD147 contain β1,6-branched poly-LacNAc catalyzed by β3Gn-T8 in SGC-7901 cells. Notably, *N*-glycans of HG-CD147 were decreased with silenced c-jun expression compared with the control cells but increased in the c-jun-upregulated cells (Fig. 4B). All of these results suggest that c-jun affects *N*-glycans of HG-CD147 through the regulation of β3Gn-T8 expression in the SGC-7901 cells.

In summary, the present study demonstrated that the transcription factor c-jun could bind to the β3Gn-T8 promoter and activate β3Gn-T8 expression, and further regulate the *N*-glycans of HG-CD147 in the SGC-7901 cell line. Furthermore, c-jun and β3Gn-T8 were both upregulated in the gastric cancer tissues, and their expression also had a positive correlation with each other. Therefore, it can be concluded that the significance of c-jun in malignant potential such as tumor cell invasion can be ascribed at least partially to the increased expression of β3Gn-T8. Prevention of β3Gn-T8 as well as c-jun activity would provide a novel strategy for gastric cancer therapy.

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