c-Jun-mediated β-1,3-N-acetylglucosaminyltransferase 8 expression: A novel mechanism regulating the invasion and metastasis of colorectal carcinoma cells

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Abstract. β-1,3-N-Acetylglucosaminyltransferase 8 (β3GnT8) is a key enzyme that catalyzes the formation of polylactosamine glycan structures by transferring GlcNAc to tetra-antennary β1-6-branched N-glycans, and it has been reported to participate in tumor invasion and metastasis by regulating the expression of matrix metalloproteinases (MMPs), cluster of differentiation 147 (CD147) and polylactosamine. By contrast, the role of transcription factor c-Jun in cell cycle progression has been well established. c-Jun has an important role in tumor cell invasion and metastasis. However, the precise molecular mechanisms by which c-Jun regulates these processes in colorectal carcinoma cells are not fully elucidated. In the present study, c-Jun had a significant effect on the invasive and migratory abilities of SW480 and LoVo cells. Additionally, overexpression of c-Jun was able to increase the expression of β3GnT8, MMPs, CD147 and polylactosamine. Similarly, knockdown of c-Jun was able to decrease the expression of β3GnT8, MMPs, CD147 and polylactosamine. These results suggest that c-Jun is able to regulate colorectal carcinoma cell invasion and metastasis via β3GnT8. A chromatin immunoprecipitation assay indicated that c-Jun is able to bind directly to the promoter regions of β3GnT8 in SW480 and LoVo cells. This leads to transcriptional activation of β3GnT8, which in turn regulates the expression of tumor invasion and metastasis-associated genes. The results of the present study demonstrate a novel mechanism underlying colorectal carcinoma cell invasion and metastasis, where β3GnT8 is transcriptionally activated via c-Jun binding to its promoter.

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Introduction

Glycans in glycoconjugates including glycoproteins and glycolipids participate in a number of important biological events, including cell-cell interactions, inflammation and tumor progression (1). Poly-N-acetyllactosamine (polylactosamine), carried on N- or O-glycans, is an important glycan structure containing repeats of the N-acetyllactosamine unit (Gal1-4GlcNAc1-3)*n* (2). The polylactosamine structure has key roles in mediating molecular interactions during embryogenesis, tumorigenesis and tumor metastasis (3), and is synthesized by members of the β-1,3-N-acetylglucosaminyltransferase (β3GnT) family.

β3GnT8 is a member of the β3GnT family (4). When β3GnT8 was first cloned, it was named β3GalT7 and mapped to chromosome 19q13.2 in our laboratory. β3GnT8 was renamed β3GnT8 on the basis of subsequent enzymatic study (2). β3GnT8 is a polylactosamine synthase and transfers GlcNAc to the non-reducing terminus of the tetra-antennary β1-6-branched N-glycans of Galβ1-4GlcNAc (2). Previously, it was reported that β3GnT8 is highly expressed in various types of tumor tissues, including colon cancer, gastric cancer and laryngeal carcinoma (2), which suggests a possible role for β3GnT8 in tumor malignancy. Our recent study demonstrated that β3GnT8 is able to regulate the metastasis of colorectal cancer cells by altering the β1,6-branched polylactosamine sugars of cluster of differentiation 147 (CD147) (5). The extracellular region of CD147 contains three Asn glycosylation sites, and the N-glycosylation sites make similar contributions to both high and low glycoforms of CD147 (HG-CD147 and LG-CD147, respectively) (6). A number of studies have confirmed that modulation of CD147

is associated with the expression of matrix metallopeptidases (MMPs) in normal and tumor tissues (7-9). High glycoforms of CD147 (HG-CD147) stimulate the production of matrix metalloproteinase (6,7). Additionally, increased HG-CD147 glycosylation has been attributed to β1-6-branched N-glycan to form polylactosamine structures (7,8). Consistent with these results, our previous study demonstrated that β3GnT8 may have an important role in the CD147 signal transduction pathway as an upstream modulator of MMP2 production in tumor cells (9). Although the functions of β3GnT8 in tumor invasion and metastasis are well documented, how β3GnT8 expression is regulated in tumor cells or tissues remains largely unclear.

Transcription factor c-Jun (c-Jun) is a well-known cellular transcription factor belonging to the activator protein 1 (AP-1) family that is able to promote cell cycle progression and cell proliferation (10,11). c-Jun regulates the expression of a number of genes that affect tumor invasion and metastasis by binding to their promoters (12,13). Considering the known associations between β3GnT8 and c-Jun in tumor malignancy, the aim of the present study was to investigate whether β3GnT8 acts as a downstream target gene of c-Jun to regulate tumor cell invasion. In the present study, the overexpression of c-Jun was demonstrated to be able to increase β3GnT8 expression in colorectal carcinoma cell lines. By contrast, knockdown of c-Jun resulted in a decrease in β3GnT8 expression. Notably, c-Jun was able to bind with β3GnT8 gene promoters and activate β3GnT8 transcription, which is consistent with the initial hypothesis. The results of the present study indicate a novel molecular mechanism underlying c-Jun-mediated colorectal carcinoma cell invasion and metastasis.

Materials and methods

Cell culture. SW480 and LoVo cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and were cultured in RPMI 1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), supplemented with 10% fetal bovine serum (HyClone; GE Healthcare Life Sciences, Logan, UT, USA) in a humidified atmosphere with 5% CO_2 at 37˚C.

Cell transfection. The pIRES2-EGFR plasmid, used as a mock control vector, was purchased from Suzhou GenePharma Co., Ltd. (Suzhou, China); the c-Jun-pIRES2-EGFR plasmid was constructed in our laboratory. The plasmids c-Jun-shRNA-pGPU6/GFP/Neo and negative control-shRNA-pGPU6/GFP/Neo (mock control) were purchased from Suzhou GenePharma Co., Ltd. Cells were seeded in 6-well plates at a density of $8x10^5$ cells/ml (2 ml/well). Following cell attachment, c-Jun-pIRES2-EGFR and pIRES2-EGFR plasmids (5 μg per well) were transfected into SW480 cells, and c-Jun-shRNA-pGPU6/GFP/Neo and NC-shRNA-pGPU6/GFP/Neo plasmids (5 μg per well) were transfected into LoVo cells, using Lipofectamine™ 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The effects of c-Jun-pIRES2-EGFR and c-Jun-shRNA-pGPU6/GFP/Neo transfection were confirmed by western blot analysis of c-Jun expression.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from the cells using TRIzol (Invitrogen, Carlsbad, CA, USA). A total of 1 μg RNA was reverse transcribed with the ReverTra Ace qPCR RT kit (Toyobo Co., Ltd., Osaka, Japan). RT-qPCR was performed using SYBR Green Real-Time PCR Master mix (Toyobo Co., Ltd.). The reaction mixture was heated to 95˚C for 1 min, followed by 40 cycles of 95˚C for 15 sec, 60˚C for 1 min. The primers were as follows: GAPDH forward, 5'-AGAAGGCTG GGGCTCATTTG-3' and reverse, 5'-AGGGGCCATCCACAG TCTTC-3', c-Jun forward, 5'-TCCAAGTGCCGAAAAAGG AAG-3' and reverse, 5'-CGAGTTCTGAGCTTTCAAGGT-3', β3GnT8 forward, 5'-GTCGCTACAGTGACCTGCTG-3' and reverse, 5'-GTCTTTGAGCGTCTGGTTGA-3', CD147 forward, 5'-ACCGTAGAAGACCTTGGCTC-3' and reverse, 5'-CGTCGGAGTCCACCTTGAAC-3', MMP2 forward, 5'-TATGGCTTCTGCCCTGAGAC-3' and reverse, 5'-CAC ACCACATCTTTCCGTCA-3' and MMP15 forward, 5'-TAC GAGTGAAAGCCAACCTG-3' and reverse primer, 5'-TCT CCGTGTAGTTCTGGATGC-3'. The data was analyzed with the ABI 7500 software (version 2.0.3; Applied Biosystems; Thermo Fisher Scientific, Inc.). GAPDH was used as an internal control, and the data were analyzed using the $2^{-\Delta\Delta Cq}$ method (14).

Western blot analysis. Cells were harvested and homogenized with lysis buffer (150 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS and protease inhibitor cocktail) (Roche Applied Science, Madison, WI, USA). Proteins (30 μg/lane) were resolved with SDS-PAGE (10% gel; Invitrogen; Thermo Fisher Scientific, Inc.) and transferred onto nitrocellulose membranes (EMD Millipore, Billerica, MA, USA). The membranes were blocked with 5% skimmed milk or 1% bovine serum album (BSA) in Tris-buffered saline (TBS; 10 mM Tris-HCl and 150 mM NaCl, pH 7.9) containing 0.05% Tween-20 at room temperature for 2 h. The proteins were analyzed using specific antibodies as indicated below. The membranes were incubated with the appropriate primary antibodies at 4˚C overnight. Following three washes in TBS containing Tween-20, the membranes were incubated at room temperature for 2 h with the appropriate peroxidase-conjugated secondary antibodies. Following three washes in TBS containing Tween-20, the protein bands on the membranes were visualized using an enhanced chemiluminescence kit (GE Healthcare Life Sciences, Shanghai, China). The antibodies, which were used at a dilution of 1:1,000, were as follows: Anti-CD147 (cat. no., sc13976), anti-MMP2 (Cat. sc-6838), anti-MMP15 (cat. no., sc-80213; all Santa Cruz, Dallas, TX, USA), anti-GAPDH (cat. no., AG019), and horseradish peroxidase-conjugated anti-rabbit (cat. no., A0208), anti-goat (cat. no., A0181) and anti-mouse (cat. no., A0216, all Beyotime Institute of Biotechnology, Haimen, China) secondary antibodies.

A rabbit anti-human β3GnT8 affinity polyclonal antibody was also used, produced in an earlier study as previously described (15). In brief, the antibody was purified from rabbit antiserum with 50% saturated ammonium sulfate and 33.3% saturated ammonium sulfate, followed by immunizing protein affinity purification. The purity of the antibody was determined by SDS-PAGE analysis. The specificity of the

antibody was confirmed previously via western blotting and/or immunochemical analysis of β3GnT8 protein in tumor cells and tissues (5,15,16).

Chromatin immunoprecipitation (ChIP) assay. ChIP was performed using a ChIP assay kit (cat. no., P2078; Beyotime Institute of Biotechnology) according to the manufacturer's protocol with a small number of modifications. Chromatin solutions were sonicated and incubated with an anti-c-Jun antibody (dilution, 1:2,000; cat. no., ab119944; Abcam, Cambridge, MA, USA) or mouse control IgG (dilution, 1:2,000; cat. no., A7028; Beyotime Institute of Biotechnology), and rotated overnight at 4˚C. The solution was washed for 3-5 min in each of the following from the ChIP assay kit: Low salt immune complex wash buffer, high salt immune complex, LiCl immune complex wash buffer and Tris-EDTA buffer. DNA-protein cross-links were reversed, and chromatin DNA was purified and subjected to PCR analysis with the Easy-Load PCR Master mix (cat. no., D7251; Beyotime Institute of Biotechnology). PCR was performed with 30 cycles of 95˚C for 35 sec, 60˚C for 45 sec and 72˚C for 1 min, followed by 72˚C for 10 min. Primers 5'-TGTACGCGTGAGGCA CATGGCAAAGG-3' (forward) and 5'-GTTCTCGAGAGT GGGGAGGAAGTGGT-3' (reverse) were used to amplify the β3GnT8 promoter sequence. Following amplification, PCR products were resolved on a 1.5% agarose gel and visualized by ethidium bromide staining.

Flow cytometric analysis. To detect polylactosamine structures of cell-surface glycoproteins, biotin-labeled *Solanum lycopersicum* (tomato) agglutinin lectin (LEA; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), which specifically binds polylactosamine residues, was used. Cells were detached with 0.25% trypsin-EDTA solution and subsequently washed three times with PBS. The cell density was adjusted to $3x10^6$ cells/ml, and the cells were stained with 10 μg/ml LEA in PBS (containing 0.5% BSA and 0.05% sodium azide) at 37˚C for 1 h. The cells were subsequently washed three times with PBST (PBS containing 0.05% Tween-20). Staining was performed with 10 μg/ml PE-conjugated streptavidin (Sigma-Aldrich; Merck KGaA) at 37˚C for 1 h, and the cells were washed three times with PBST. The fluorescence intensity of the stained cells was measured using a flow cytometer and analyzed with CellQuest software (version 5.2.1; BD Biosciences, Franklin Lakes, NJ, USA).

Wound healing assay. SW480 or LoVo cells ($1x10^5$) were plated in a 6-well plate and incubated overnight, yielding confluent monolayers. Wounds were made using a pipette tip, and cell motility was examined using a light microscope. Images were captured at 0 and 24 h after wounding. The plates were marked to ensure consistent photo documentation. Using ImageJ software (version 1.49; National Institute of Health, Bethesda, MD, USA), the area of each wound was calculated at each time point.

Transwell migration and invasion assays. The invasion assay was performed in 24-well cell culture chambers using Transwell inserts (Corning Life Sciences, Corning, NY, USA) with porous membrane (pore size, 8 μm) precoated with Matrigel (BD Biosciences). SW480 or LoVo cells ($1x10^5$) were plated in 200 μl serum-free RPMI 1640 medium in the upper chamber, and 500 μl RPMI 1640 medium with 10% FBS was added to the lower wells. After 48 h, the non-invading cells with Matrigel matrix were removed from the upper surface of the membrane by scraping with a cotton tipped swab. The cells on the lower surface of the filter were fixed for 30 min in 4% polyoxymethylene, air-dried briefly and stained with eosin staining solution (Beyotime Institute of Biotechnology, Haimen, China) at room temperature for 30 min. The number of invading cells was manually counted from 5 randomly selected microscopic fields at x100 magnification using a light microscope (IX-70, Olympus, Tokyo, Japan).

A cell migration assay was similarly performed, except without Matrigel. Cells were incubated at 37˚C for 24 h. Cells on the lower surface of the filter were stained and counted as previously described.

Statistical analysis. Statistical analysis was performed using SPSS software (version 22.0; IBM SPSS, Armonk, NY, USA). Each assay was performed ≥3 times. Results are presented as the mean ± standard deviation. Student's t-test was used to evaluate the significance of data. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Effects of c-Jun on the expression of the β3GnT8, CD147, MMP2 and MMP15. It is well known that the transcription factor c-Jun regulates the expression of numerous tumor invasion-associated genes (11,12). To determine the role of c-Jun in the regulation of β3GnT8, which is also involved in tumor invasion (5), the effects of c-Jun overexpression and knockdown on β3GnT8 expression were examined. Additionally, the effects of c-Jun overexpression and knockdown on the expression of a number of tumor metastasis-associated genes (CD147, MMP2 and MMP15) were investigated. As presented in Fig. 1A, overexpression of c-Jun in SW480 cells was able to significantly increase the mRNA expression of β3GnT8, CD147, MMP2 and MMP15 ($P<0.001$). By contrast, knockdown of c-Jun in LoVo cells resulted in a significant decrease in mRNA expression of these genes ($P<0.001$; Fig. 1B). Additionally, western blot analysis indicated that overexpression of c-Jun increased protein levels of β3GnT8, HG-CD147, MMP2 and MMP15 in SW480 cells (Fig. 2A). Similarly, the levels of all these proteins decreased when c-Jun was knocked down in LoVo cells (Fig. 2B). However, expression of LG-CD147 did not alter when c-Jun was overexpressed or knocked down (Fig. 2A and B). These results suggest that c-Jun may be one of the master regulators of colorectal carcinoma cell metastasis, and the alterations in the N-glycosylation level of CD147 may be due to the induction of β3GnT8 by c-Jun.

Effects of c-Jun on the level of polylactosamine. In order to determine whether c-Jun affects the structure of polylactosamine chain in colorectal carcinoma cells, a flow cytometric assay was performed to examine the level of polylactosamine in SW480 and LoVo cells. The results indicated that overexpression of c-Jun significantly promoted the polylactosamine level in SW480 cells (3.78 vs. 1.93; Fig. 3A). By contrast, knockdown

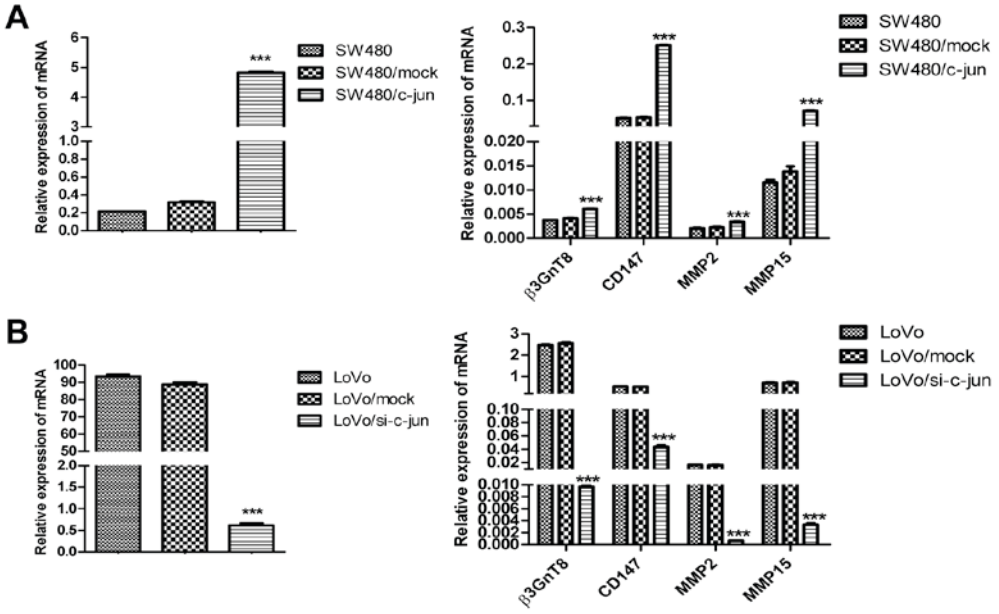

Figure 1. mRNA expression of c-Jun, β3GnT8, CD147, MMP2 and MMP15 using RT-qPCR. (A) Exogenous c-Jun plasmid vector and the empty vector were transfected into SW480 colon cancer cells with a low metastatic potential. RT-qPCR was performed to detect mRNA expression. (B) Exogenous c-Jun short hairpin RNA vector and the empty vector were transfected into LoVo colon cancer cells with a high metastatic potential. RT-qPCR was performed to detect mRNA expression. Results are the mean ± standard deviation representative of 3 independent experiments. ***P<0.001 vs. untreated control cells. c-Jun, transcription factor c-Jun; β3GnT8, β-1,3-N-acetylglucosaminyltransferase 8; CD147, cluster of differentiation 147; MMP, matrix metalloproteinase; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; si-c-jun, c-Jun short hairpin RNA.

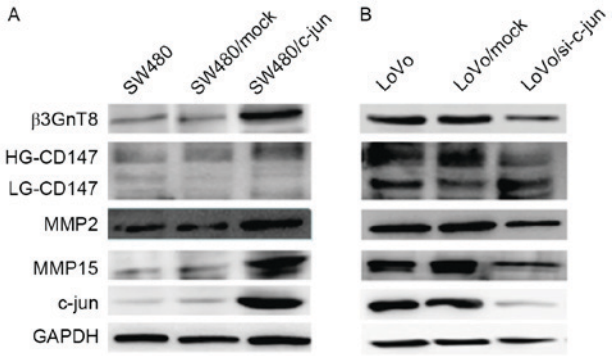

Figure 2. Western blot analysis of c-Jun, β3GnT8, CD147, MMP2 and MMP15. (A) Exogenous c-Jun plasmid vector and the empty vector were transfected into SW480 colon cancer cells with a low metastatic potential, and western blotting was performed to detect protein levels. (B) Exogenous c-Jun shRNA vector and the control vector were transfected into LoVo colon cancer cells with a high metastatic potential, and western blotting was performed to detect protein levels. c-Jun, transcription factor c-Jun; β3GnT8, β-1,3-N-acetylglucosaminyltransferase 8; CD147, cluster of differentiation 147; MMP, matrix metalloproteinase; HG, high glycoform; LG, low glycoform; si-c-jun, c-Jun short hairpin RNA.

of c-Jun in LoVo cells decreased the polylactosamine level (1.6 vs. 4.71; Fig. 3B). These results suggest that c-Jun has a significant effect on the structure of polylactosamine, and this may be mediated via β3GnT8, which is involved in biosynthesis of polylactosamine chain.

c-Jun directly binds to the β3GnT8 promoter. In order to determine whether there is interaction between c-Jun and β3GnT8, a ChIP assay was performed in SW480 and LoVo cells, and mouse IgG was used as a negative control. Immunoprecipitated chromosomal DNA with anti-c-jun antibody or mouse IgG was subjected to PCR. As presented in Fig. 4, compared to the mouse IgG control group, the β3GnT8 promoter sequence was detected by PCR in anti-c-Jun antibody-pulled down DNA. This result suggests that c-Jun is able to bind to the promoter region of β3GnT8 gene and may activate β3GnT8 transcription.

Effects of c-Jun expression on the migratory response of SW480 and LoVo cells. c-Jun has a role in the migration of tumor cells. In order to determine whether c-Jun affects the migration of SW480 and LoVo cells, a wound healing assay was performed and images were captured after 24 h. The results demonstrated that overexpression of c-Jun markedly increased the migration of SW480 cells compared with the control (Fig. 5A), whereas c-Jun knockdown markedly decreased migration of LoVo cells compared with the control (Fig. 5B). These results suggest that c-Jun is able to affect the migratory response of colorectal carcinoma cells *in vitro*.

Effects of c-Jun expression on the invasion and migration of SW480 and LoVo cells using a Transwell assay. The effect of c-Jun on metastasis abilities of colorectal carcinoma cells was assessed (Fig. 6). SW480 and LoVo cells were seeded into the upper compartment of the Transwell chamber. SW480 cells were incubated at 37˚C for 48 h, LoVo cells were incubated at 37˚C for 24 h, and cell migration was assessed by counting the number of cells that diffused through the membrane. As presented in Fig. 6A and C, overexpression of c-Jun in SW480 significantly increased cell migration and invasion. By contrast, c-Jun knockdown in LoVo cells inhibited cell migration and invasion (Fig. 6B and D), which suggests that c-Jun has an important role in tumor cell invasion and metastasis.

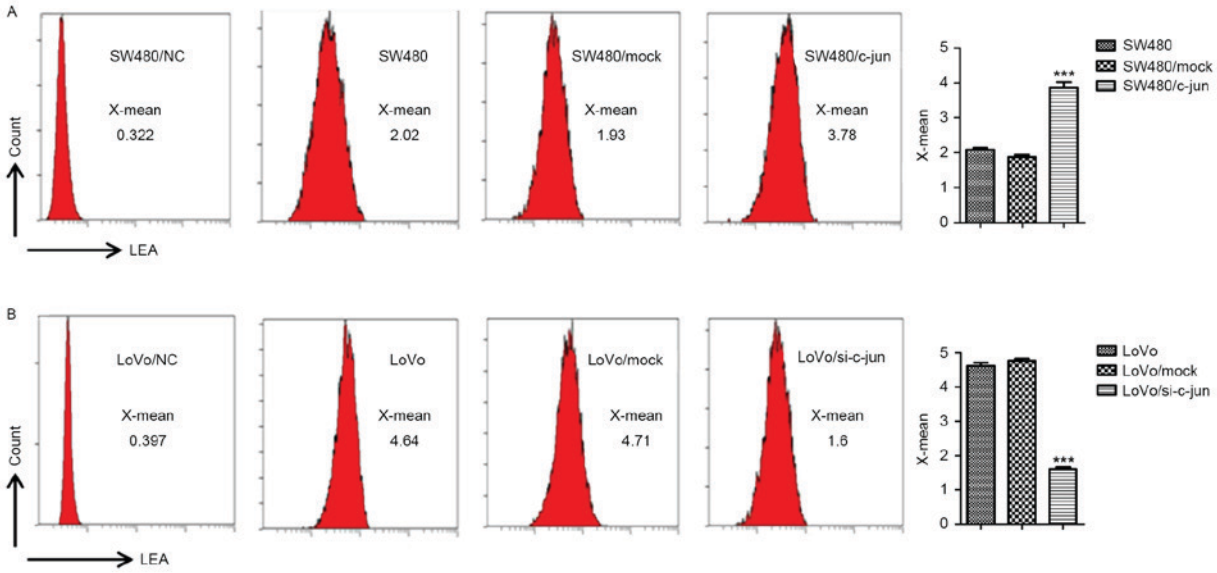

Figure 3. Flow cytometric analysis of polylactosamine residues in colorectal carcinoma cells. (A) Exogenous c-Jun plasmid vector and the empty vector were transfected into SW480 colon cancer cells with a low metastatic potential, and flow cytometric assay was performed to detect the level of polylactosamine residues. (B) Exogenous c-Jun shRNA vector and the control vector were transfected into LoVo colon cancer cells with a high metastatic potential, and flow cytometric assay was performed to detect the level of polylactosamine residues. NC represents the negative control groups without primary antibody. Results are the mean ± standard deviation representative of 3 independent experiments. ***P<0.001 vs. mock control. LEA, *Solanum lycopersicum* agglutinin; NC, negative control; X-mean, mean intensity of fluorescence; si-c-jun, c-Jun short hairpin RNA.

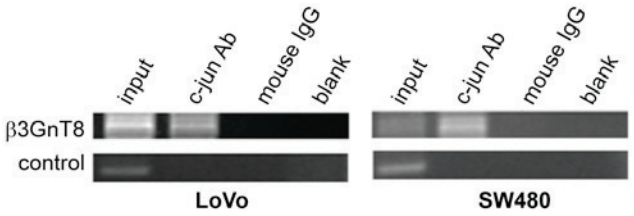

Figure 4. ChIP assay analysis of the interaction between c-Jun transcription factor and β3GnT8 promoter. A ChIP assay was performed to detect the binding of c-Jun to β3GnT8 promoter in LoVo and SW480 cancer cells. Input, sonicated chromotin samples; c-jun Ab, immunoprecipitation with anti-c-jun antibody; mouse IgG, immunoprecipitation with mouse IgG; blank, no immunoprecipitation. ChIP, chromatin immunoprecipitation; c-Jun, transcription factor c-Jun; β3GnT8, β-1,3-N-acetylglucosaminyltransferase 8; Ab, antibody.

Discussion

Glycosylation is one of the most common protein post-translational modifications. Glycans have important roles in a number of distinct cellular events, including cell migration, cell-cell adhesion, cell signaling and growth (1,3). However, aberrant glycosylation has been associated with various human diseases and particularly with tumors; glycosylation is considered a hallmark of cancer (3).

Colorectal cancer is one of the leading causes of cancer-associated mortality (17). A recent study has demonstrated associations between colorectal cancer progression and changes in the pattern of expression of N-glycans (18). The expression patterns of β1,6-branched N-glycans (the most common structure of N-glycans in colorectal cancer) are associated with increased replicative potential, tissue invasion and metastasis, characteristics of which are considered hallmarks of colorectal cancer progression (2).

It has been well established that U937 (human histiocytic lymphoma cells), ACHN (human kidney glandular cancer cells), MKN45 (human gastric cancer cells), A549 (human lung cancer cells) and Jurkat cells (acute T-cell leukemia) express a high level of N-glycans with polylactosamine residues (19). β3GnT8 is an enzyme involved in the biosynthesis of polyLac chains by transferring GlcNAc to the non-reducing terminus of Galβ1-4GlcNAc on β1,6-branched N-glycan. As overexpression of β3GnT8 in HCT15 colorectal cancer cells resulted in an increase in L-phaseolus vulgaris erythroagglutinin reactivity, the authors hypothesize that this enzyme may participate in tumor malignancy by synthesizing polylactosamine on β1,6-branched N-glycans (2). Our previous study demonstrated that overexpression of β3GnT8 in LS-174T cells increased the level of HG-CD147 and promoted tumor cell invasion and migration, whereas knockdown of β3GnT8 in LoVo cells had the opposite effect (5). These results suggest that β3GnT8 regulates the metastasis-associated behavior of colorectal cancer cells by altering the glycosylated forms of CD147. We have also previously demonstrated that β3GnT8 and polylactosamine residues on β1,6-branched N-oligosaccharides are associated with the metastatic potential of colorectal cancer cells and may promote the invasive and migratory abilities by modulating the N-glycosylated forms of CD147 (5). As a specific substrate of β3GnT8, CD147 exists in the glycosylated form and serves key roles in tumor invasion and metastasis. The glycosylated forms of CD147 are highly expressed on the cell surface of various types of tumor cell, including oral, breast, lung, bladder, kidney, laryngeal, pancreatic, gastric, colorectal cancer, glioma lymphoma and melanoma (20-22). Additionally, the glycosylated forms of CD147 are able to stimulate tumor cells to produce MMPs, particularly MMP2 and MMP9 (7,8,23). CD147 is able to induce MMP expression via phosphoinositide 3-kinase/protein kinase B (Akt)/inhibitor

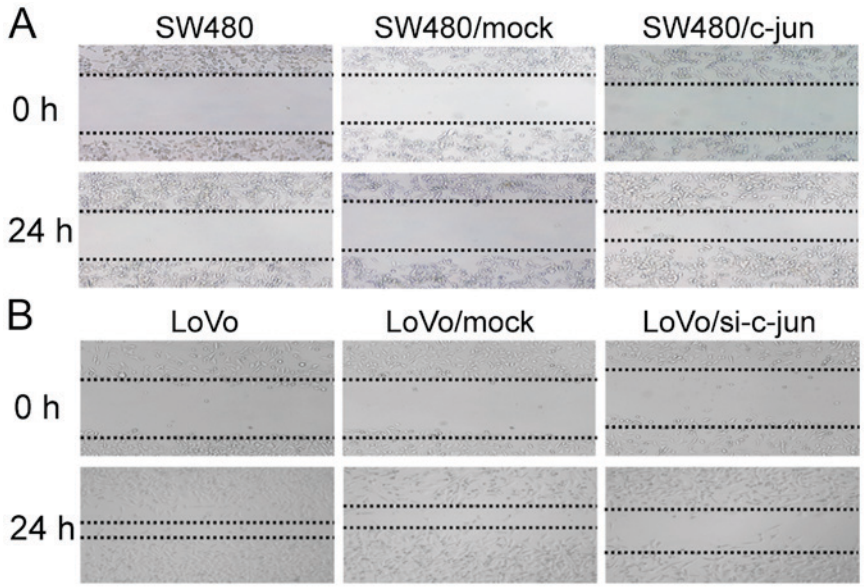

Figure 5. Analysis of cell migration using a wound healing assay. (A) SW480 cells were transfected with c-Jun plasmid vector. (B) LoVo cells were transfected with c-Jun short hairpin RNA vector. Cell motility was examined using a light microscope at 0 and 24 h after wounding. Magnification, x40. c-Jun, transcription factor c-Jun; si-c-jun, c-Jun short hairpin RNA.

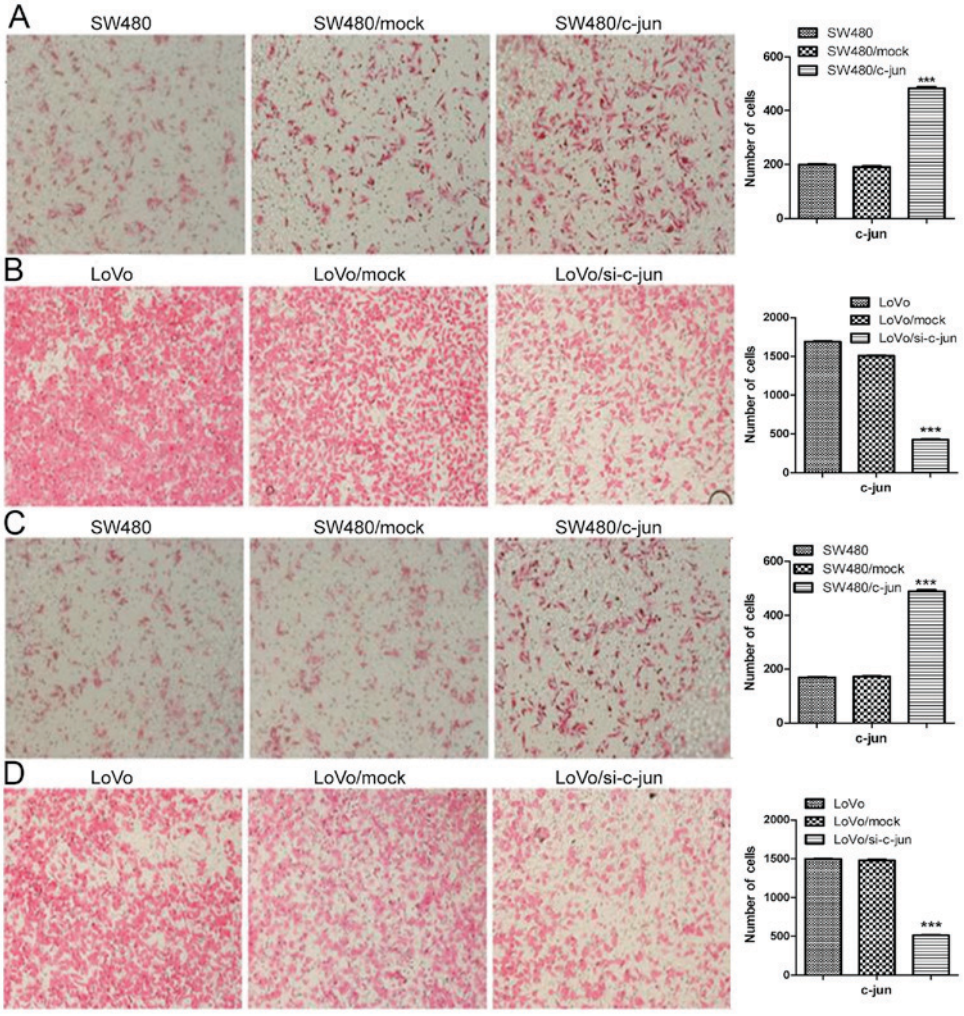

Figure 6. Transwell migration and invasion assays. For the migration assay, (A) SW480 or (B) LoVo cells were added to the upper chambers of 24-well cell culture chamber plates without Matrigel-coated polycarbonate membrane. For the invasion assay, (C) SW480 and (D) LoVo cells were added to the upper chambers of 24-well cell culture chambers containing a Matrigel-coated polycarbonate membrane. Following incubation for 24 or 48 h, the cells were fixed and stained with eosin staining solution and examined under a light microscope. Magnification, x100. Quantification results are the mean ± standard deviation of 3 independent experiments. ***P<0.001 vs. mock control. c-Jun, transcription factor c-Jun; si-c-jun, c-Jun short hairpin RNA.

of nuclear factor κB (NF-κB) (IκB) kinase-dependent IκB-α degradation, which is mediated by Ras-related C3 botulinum toxin substrate 1, NF-κB activation and by mitogen-activated protein kinase kinase 7/c-Jun N-terminal kinase-dependent AP-1 activation (20).

c-Jun is a protein encoded by the proto-oncogene JUN. c-Jun in association with c-Fos forms the early response transcription factor AP-1. AP-1 has been demonstrated to interact with a number of genes (12,13) and has important functions in various tumor types (11,24,25). In the present study, it was demonstrated that c-Jun is able to regulate the expression of β3GnT8, MMP2, MMP15, CD147 and polylactosamine chains in the colorectal carcinoma cell lines SW480 and LoVo by using gain- and loss-of-function assays. Notably, our previous studies revealed that β3GnT8 is able to regulate the expression of HG-CD147, MMP2 and MMP15 (5,9). Considering the results of these previous studies and those of the present study, it is hypothesized that c-Jun is able to regulate the expression of these genes, which is mediated partly through CD147 glycosylation catalyzed by β3GnT8.

In order to demonstrate whether c-Jun protein and β3GnT8 DNA interact, a ChIP assay was performed in SW480 and LoVo cells. It was identified that c-Jun is able to directly bind to the β3GnT8 gene promoter, which results in transcriptional activation of β3GnT8 and in turn regulates expression of other tumor invasion-associated genes including MMPs. In summary, the present study, to the best of our knowledge, is the first report of the functional and physical association between c-Jun and β3GnT8 and therefore provides a novel clue for elucidation of the molecular mechanisms regulating c-Jun-mediated tumor invasion and metastasis.

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