β3GnT8 regulates oxaliplatin resistance by altering integrin β1 glycosylation in colon cancer cells

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Abstract. Correlations between drug resistance and glycosylation changes have been analyzed intensively in the field of tumor biology. The present study was aimed to investigate the glycan and glycogene alterations involved in oxaliplatin resistance in human colon cancer cells. Using the lectin microarray for glycan composition and FITC-lectin binding for glycan profiling, we found that polylactosamine-type N-glycans were significantly increased in oxaliplatin-resistant SW620R cells. Using real-time PCR for quantification of glycogenes, we targeted β-1,3-N-acetylglucosaminyltransferase 8 (β3GnT8), which was overexpressed in SW620R cells. Using an RNA interference strategy, we revealed that the silencing of β3GnT8 in SW620R cells resulted in increased chemosensitivity to oxaliplatin. Conversely, the engineered overexpression of β3GnT8 in SW620 cells enhanced resistance to oxaliplatin. Further data revealed that manipulation of β3GnT8 was able to modify polylactosamine chains on integrin β1 and to regulate the integrin β1 downstream signaling pathway. These results revealed that β3GnT8 may play a key role in the development of oxaliplatin resistance in colon cancer cells possibly through the alteration of the glycosylation of integrin β1. These findings may be valuable for overcoming drug resistance in colon cancer.

Introduction

Colon cancer remains the most commonly diagnosed cancer and the leading cause of cancer-related deaths in the world (1,2). Current therapeutic strategies for colon cancer include surgery, radiotherapy, chemotherapy or a combination of these therapies. Drug resistance is often regarded as the cause of the failure of chemotherapeutic drug treatments in patients with colon cancer. Oxaliplatin is a third-generation platinum drug that is currently used as the front line treatment of colon cancer (3). To date, there have been extensive studies on the mechanisms of oxaliplatin resistance. Several factors including alterations in transport, detoxification, DNA damage response and repair, cell death, and epigenetic processes have been described to induce oxaliplatin resistance (4). However, little is known about the effects of glycosylation on the development of oxaliplatin resistance in colon cancer.

As the most common post-translational modifications of proteins, glycosylation is an effective way to induce diversity due to the structural variations of glycans. Glycans are more diverse in terms of chemical structure and information density than are DNA and proteins (5). The attachment of glycans usually occurs during or after the process of protein synthesis, which involves several glycogenes and isoforms of these enzymes. Cancer cells frequently express glycans at atypical levels or with different structural attributes than those found in normal cells (6). Correlations between drug resistance and glycosylation changes have been extensively studied. For example, Zhang *et al* demonstrated that N-glycans were associated with adriamycin resistance in human leukemia cells (7). Ma *et al* found that the alterations of glycogenes in breast cancer cells was correlated with tumor sensitivity to chemotherapeutic drugs (8). Kudo *et al* revealed that N-glycan alterations contributed to the drug-resistant phenotype in human hepatocellular carcinoma (9). Wu *et al* reported that N-glycosites and site-specific glycoforms of secreted proteins in drug-resistant cell lines were distinctly different from those in the parental cell lines (10). Therefore, it is of interest to clarify the relationship between aberrant glycosylation and oxaliplatin resistance in colon cancer and to find therapeutic targets for effective medical treatment.

Lectin microarray has been used as a robust, high-throughput, exceedingly sensitive, and reliable quantitative method for obtaining whole-cell glycan signatures (11,12). In the present study, we utilized this high-throughput glycomics technology to profile specific glycans from an oxaliplatin-resistant colon cancer cell line and its parental cell line. We also investigated whether glycogenes participated in the regulation of oxaliplatin resistance. In addition, the possible pathways underlying glycosylation-mediated oxaliplatin resistance were further explored.

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Materials and methods

Cell culture and establishment of an oxaliplatin-resistant cell line. The human colon cancer cell line SW620 was purchased from the cell library of the Chinese Academy of Sciences (Shanghai, China). An oxaliplatin-resistant cell line SW620R was established by continuous exposure of its parental cell line SW620 to gradually increasing concentrations of oxaliplatin (Sigma-Aldrich, St. Louis, MO, USA). Both cell lines were cultured in RPMI-1640 medium containing 10% fetal bovine serum (Gibco-BRL, Carlsbad, CA, USA) The SW620R cell line was incubated in the presence of 0.1 μg/ml oxaliplatin to maintain the drug-resistant phenotype.

Drug sensitivity assay of various anticancer drugs using CCK-8. A total of $1x10^3$ cells/well were seeded into 96-well plates and incubated overnight to allow for full adherence. Then, varied concentrations of anticancer drugs including oxaliplatin (0-0.5 μg/ml), etoposide (0-1 μg/ml), vincristine (0-0.1 μg/ml) and cisplatin (0-1 μg/ml) were added to each well. After 72 h, cell proliferation was determined by Cell Counting Kit-8 (CCK-8; Beyotime Institute of Biotechnology, Jiangsu, China). Then, the absorbance at 450 nm was assessed using a microplate reader (Molecular Devices, Sunnyvale, CA, USA). The 50% inhibition concentration (IC_{50}) was calculated based on individual cytotoxicity plots.

Lectin microarray scanning. A total of $1x10^6$ cells were collected, washed twice with ice-cold PBS, and labeled with CFDA-SE cell tracking dye (Life Technologies, Carlsbad, CA, USA) at 10 μM for 30 min at 4˚C. Then, the cells were resuspended in PBS with 1% BSA (Sigma-Aldrich), 0.5 mM $CaCl_2$ and 0.1 mM $MnCl_2$. The lectin microarrays with 30 lectins (BC Biotechnology, Guangdong, China) were blocked for 1 h at room temperature with 1% BSA. Subsequently, the cells were allowed to bind on lectin microarrays in the dark for 1 h. The excess and/or unbound cells were gently removed with washing buffer (PBS with 0.5% Tween). GenePix 5.0 (Molecular Devices) was used for extracting binding signals from the scanned images (13).

Lectin flow cytometry. A total of $1x10^6$ cells were collected and washed with fluorescence-activated cell sorting (FACS) buffer (PBS with 20 mg/ml bovine serum). Then, cells were stained with one of the 6 FITC-lectins (Vector Laboratories, Inc., Burlingame, CA, USA) at a final concentration of 10 μg/ml for 1 h at 4˚C in the dark. After three washes with FACS buffer, the cells were centrifuged at 1,000 x g for 5 min and resuspended in 200 μl of PBS. The samples were analyzed using FACScan flow cytometry (Becton-Dickinson, Mountain View, CA, USA). Ten thousand cells were analyzed for each condition.

Analysis of glycogenes. A total of $1x10^6$ cells were harvested for real time RT-PCR analysis. Total mRNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and then reversed transcribed to cDNA with the Superscript First Strand synthesis system (Invitrogen) according to the manufacturer's instructions. All PCR reactions were performed on an ABI PRISM® 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) for 40 cycles (5 sec at 95˚C, 30 sec at 60˚C and 30 sec at 72˚C). The SYBR-Green Real-Time PCR Master Mix kit (Toyobo, Osaka, Japan) was used. Primer sequences are listed in Table I. The expression level of each glycogen was normalized to GAPDH. The results were calculated using the $2^{-\Delta\Delta Ct}$ method.

Small interference RNA (siRNA) transfection. The siRNA directed against β3GnT8 and nontargeting negative siRNA (NC) were designed and synthesized by GenePharma (Shanghai, China). The following sequences were used: β3GnT8 sense, 5'-CAUUCGGCUCUGGAAACAAdTdT-3' and antisense, 5'-UUGUUUCCAGAGCCGAAUGCTT-3'; NC sense, 5'-UUC UCCGAACGUGUCACGUdTdT-3' and antisense, 5'-ACG UGACACGUUCGGAGAATT3' (14). SW620R cells were transfected with 100 nM siRNA using Lipofectamine 3000 (Invitrogen) as suggested by the manufacturer.

Plasmid construction and transfection. The full coding sequence of β3GnT8 was cloned into the pEGFP-C1 eukaryotic expression vector (Clontech, Heidelberg, Germany). Primers 5' and 3' for PCR were 5'-AATCTCGAGTAATGC GCTGCCCCAAG-3' and 5'-GCGGAATTCTCAGCACTG GAGCCTT3-3'. After being identified by digestion with restriction enzymes *Xho*I and *Eco*RI (MBI, Fermentas, Vilnius, Lithuania), the pEGFP-c1-β3GnT8 plasmid was transfected into SW620 cells using Lipofectamine 3000 reagent. The empty vector pEGFP-C1 was also transfected into SW620 cells as a mock control.

Western blotting. Proteins were extracted and quantified with a BCA protein assay kit (Beyotime Institute of Biotechnology). Equal amounts of the protein samples were separated by 10% SDS-PAGE and transferred onto a PVDF membrane (Millipore, Bedford, MA, USA). The membrane was blocked with 5% non-fat milk solution at room temperature for 1 h. After being incubated with primary antibodies at 4˚C overnight, the blots were incubated with HRP-conjugated corresponding secondary antibodies at room temperature for 1 h, followed by detection with an ECL kit (Beyotime Institute of Biotechnology). All the antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The antibodies and dilutions were as follows: pY397 FAK (1:800; mouse monoclonal; cat. no. sc-81493), FAK (1:1,000; mouse monoclonal; cat. no. sc-271126), pY118 paxillin (1:800; mouse monoclonal; cat. no. sc-365020) and paxillin (1:1,000; mouse monoclonal; cat. no. sc-365059).

Lectin blotting. Proteins were separated by 10% SDS-PAGE and transferred onto a PVDF membrane as aforementioned for western blotting. The membrane was blocked with Carbo-Free Blocking Solution (Vector Laboratories, Inc.) at room temperature for 1 h. After incubation with 2 μg/ml biotinylated LEA (Vector Laboratories, Inc.) at 4˚C overnight, the blots were incubated with HRP-conjugated streptavidin (Beyotime Institute of Biotechnology) at room temperature for 1 h, followed by detection with an ECL kit.

Lectin immunoprecipitation (IP). A total of $1x10^6$ cells were collected and lysed. Then, cell lysates were incubated with

Table I. Sequences of the primers used for real-time RT-PCR.

Primer name	Sequences (5'-3')
β3GnT8	F: GTCGCTACAGTGACCTGCTG
	R: GTCTTTGAGCGTCTGGTTGA-3
GnT-III	F: ATGAAGATGAGACGCTACAAGC
	R: GCTGGACACCAGGTTAGGG
GALNT1	F: TTCCCAGCGACTCCAGAAACAC
	R: TGGGATAACCTGCATCCACGGA
ST6GaL1	F: GTGGGCACAAAAACTACCAT
	R: GGCTCTGGGCTCATAAACTG
FUT8	F: CCTGGCGTTGGATTATGCTCA
	R: CCCTGATCAATAGGGCCTTC
GnT-V	F: CTTCACTCCGTGGAAGTTGTC
	R: TGGATGGTAAAGTGCAGAAGC
GADPH	F: CCAACCGCGAGAAGATGA
	R: CCAGAG GCGTACAGGGATAG

F, forward; R, reverse.

10 μg/ml of the LEA overnight at 4˚C on a rotating shaker. Protein A agarose beads (Thermo Fisher Scientific, Waltham, MA, USA) were added to the aforementioned complex and incubated for 4 h at 4˚C. Finally, the beads were washed, boiled, centrifuged and subjected to western blotting as previously described. Precipitated proteins were immunoblotted to detect integrin β1 with the anti-integrin β1 antibody (1:1,000; mouse monoclonal; cat. no. sc-13590; Santa Cruz Biotechnology).

Statistical analysis. Each assay was performed at least three times. The data were expressed as the mean ± SD. All analyses for statistically significant differences were determined by the Student's t-test. $P<0.05$ was considered to indicate a statistically significant result.

Results

Differential glycan composition is defined by lectin microarray in SW620 and SW620R cell lines. First, a CCK-8 assay was performed to determine the IC_{50} value of oxaliplatin in sensitive SW620 and resistant SW620R cells. As shown in Table II, the IC_{50} of oxaliplatin in SW620 and SW620R cells was 0.015±0.002 and 0.183±0.024 μg/ml, respectively ($P<0.05$). In addition to oxaliplatin, SW620R cells also exhibited cross-resistance to other chemotherapeutic drugs including etoposide, vincristine and cisplatin. The IC_{50} values for these drugs were greater in SW620R cells than those in SW620 cells. It suggested that the oxaliplatin-resistant cell line SW620R was successfully established and could be used for the successive experiments.

Then, lectin microarray assay was performed to detect the glycan signatures in SW620 and SW620R cell lines. Using this microarray containing 27 lectins, two negative controls (0.5% BSA and Cy5), and one positive control (Cy3), a cell binding map was generated (Fig. 1). SW620R cells had higher expression of four glycan structures recognized by lectins SNA, LCA, LEA, and L-PHA, and lower expression of two glycan structures recognized by PHA-E and Jacalin. Increased staining by SNA, LCA, LEA, and L-PHA indicated elevated levels of α2-6 sialylation, core fucosylation, polylactosamine-type N-glycans and β1,6-GlcNAc branched N-glycans in SW620R cells. Reduced staining by PHA-E and Jacalin indicated downregulation of bisecting GlcNAc and Galβ1-3GalNAcα-Ser/Thr (T antigen) in SW620R cells. These data revealed that differential glycan composition may be associated with colon cancer drug resistance.

Table II. Sensitivity and cross-resistance of SW620 and SW620R cells to anticancer drugs.

	IC_{50} (μg/ml)		
Drug	SW620	SW620R	P-value
Oxaliplatin	0.015±0.002	0.183±0.024	0.009
Etoposide	0.117±0.003	0.302±0.015	0.005
Vincristine	0.014±0.005	0.033±0.006	0.021
Cisplatin	0.109±0.027	0.577±0.062	0.018

Differential glycan profiles are defined by FITC-lectin flow cytometry in SW620 and SW620R cell lines. To analyze the glycan profiles of SW620 and SW620R cells, an FITC-lectin flow cytometric assay was performed. Differences in fluorescence intensity for SNA, LCA, LEA, L-PHA, PHA-E and Jacalin were evident when SW620 cells were compared to SW620R cells (Fig. 2). The SW620R cells exhibited higher fluorescence signal intensities of SNA, LCA, LEA and L-PHA but lower intensities of PHA-E and Jacalin, which were consistent with the results of the lectin microarray analysis.

Differential expression of glycogenes in SW620 and SW620R cell lines. Differences in glycan structures highlight the importance of glycosyltransferases encoded by glycogenes. A real-time RT-PCR analysis was performed to analyze the expression of glycogenes in colon cancer cells and paired oxaliplatin-resistant cells. Six genes were differentially expressed between the two cell lines, SW620 and SW620R. Two glycogenes, GnT-III and GALNT1 were expressed at an elevated level (i.e., >3-fold higher) in the SW620 cells compared with those in SW620R cells. Conversely, four glycogenes, ST6GaL1, FUT8, β3GnT8 and GnT-V were expressed at a higher level in the SW620R cells compared with those in the SW620 cells (i.e., >3-fold higher, Table III). Since it was revealed that knockdown of β3GnT8 could increase 5-fluorouracil sensitivity in SW620 cells, we then tested whether β3GnT8 affected the oxaliplatin resistance in the same cancer cells (15). Western blot analysis was performed to detect the expression of the β3GnT8 protein. The relative level of the β3GnT8 protein expression in the SW620 cell line was significantly lower than that in the SW620R cell line (Fig. 3). High expression of β3GnT8 also corresponded with high fluorescence intensity of LEA in SW620R cells (data not shown).

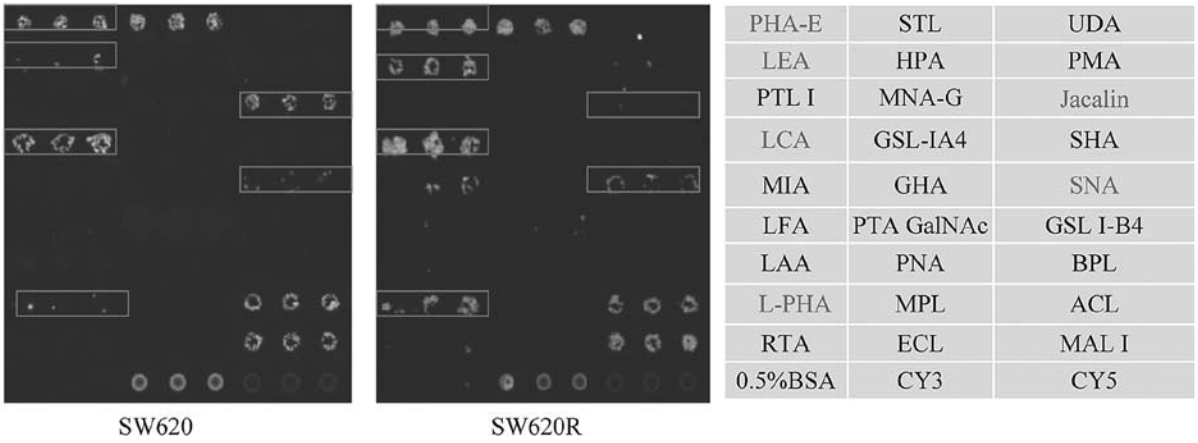

Figure 1. Differential glycan composition is detected by lectin microarray in SW620 and SW620R cell lines.

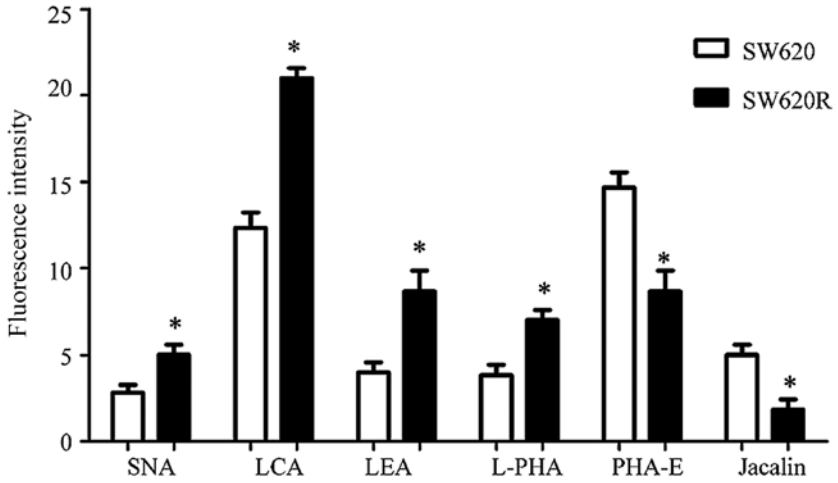

Figure 2. The fluorescence intensities are detected by flow cytometry in SW620 and SW620R cell lines using 6 different lectins. *P<0.05 compared with SW620 cells.

Table III. Differential expression of glycogenes in SW620 and SW620R cell lines.

	Ratio (>3-fold)		
Glycogene	SW620R/SW620	SW620/SW620R	Enzyme
ST6GaL1	4.61±0.25		GMP-NeuAc: Galactoside α-2, 6-sialyltransferase
FUT8	3.92±0.19		α1, 6-Fucosyltransferase 8
β3GnT8	5.57±0.14		β1, 3-N-Acetylglucosaminyltransferase 8
GnT-V	3.89±0.35		α-3-D-Mannoside-β1, 6-N-acetylglucosaminyltransferase V
GnT-III		4.16±0.12	α-3-D-Mannoside-β1, 4-N-acetylglucosaminyltransferase V
GALNT1		4.29±0.33	Polypeptide N-acetylgalactosaminyltransferase 1

Silencing of β3GnT8 in SW620R cells results in increased sensitivity to oxaliplatin. To elucidate the direct effect of β3GnT8 expression on the sensitivity of SW620R cells, we silenced β3GnT8 with siRNA. Compared with the negative siRNA-transfected cells, β3GnT8 expression at the mRNA and protein levels was downregulated in β3GnT8-siRNA transfected SW620R cells (Fig. 4A and B). To further evaluate the effect of β3GnT8 silencing on cell chemosensitivity, a CCK-8 assay was performed. The IC_{50} values of oxaliplatin in SW620R cells transfected with negative or β3GnT8 siRNA were 0.176±0.019 and 0.042±0.007 μg/ml, respectively (P<0.05) (data not shown). LEA lectin, which specifically recognizes polylactosamine chains (product of β3GnT8), was used to analyze the alteration of glycan structures. Fig. 4C

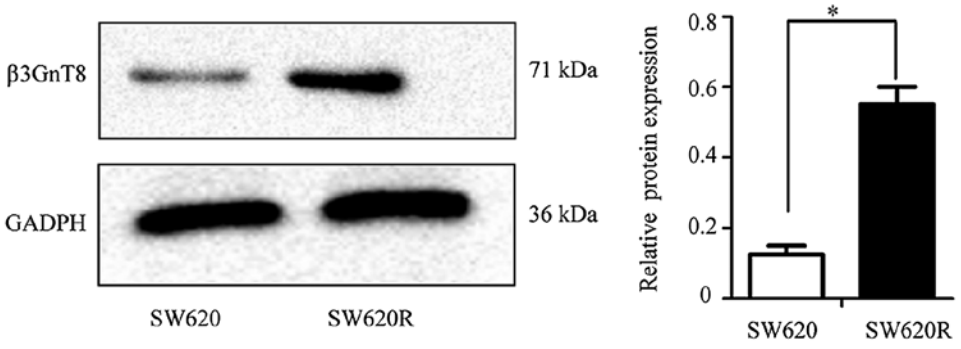

Figure 3. The protein expression levels of β3GnT8 are detected by western blotting in SW620 and SW620R cell lines. *P<0.05 compared with SW620 cells.

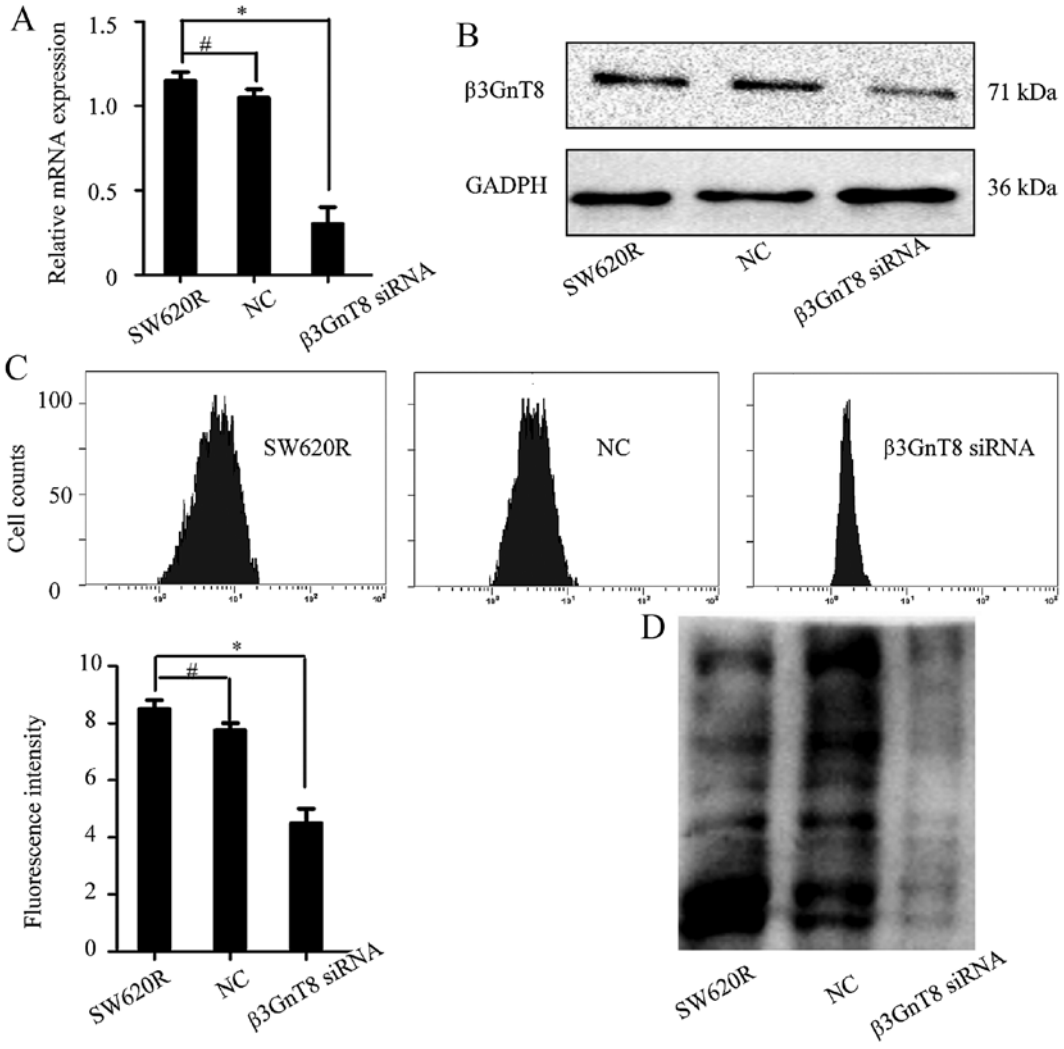

Figure 4. Effects of β3GnT8 knockdown on the synthesis of polylactosamine chains in SW620R cells. (A) The mRNA level of β3GnT8 was analyzed by real-time RT-PCR. (B) The protein expression level of β3GnT8 was analyzed by western blotting. (C) Results of the flow cytometric analysis. (D) Results of the lectin blotting analysis. Data were recorded as the mean ± SD of three independent experiments. *P<0.05, #P>0.05 compared with the untreated SW620R cells.

revealed that β3GnT8 knockdown resulted in a decrease of fluorescence intensity compared with the negative control cells. The results of lectin blotting were consistent with the FITC-lectin binding analysis (Fig. 4D). Thus, downregulation of β3GnT8 could enhance the sensitivity of SW620R cells to oxaliplatin by alteration of polylactosamine structures.

Overexpression of β3GnT8 in SW620 cells resulting in increased resistance to oxaliplatin. To further investigate the effect of β3GnT8 expression on the sensitivity of SW620 cells to oxaliplatin, a SW620 cell line stably expressing β3GnT8 was established. As shown in Fig. 5A and B, β3GnT8 expression at the mRNA and protein levels was increased

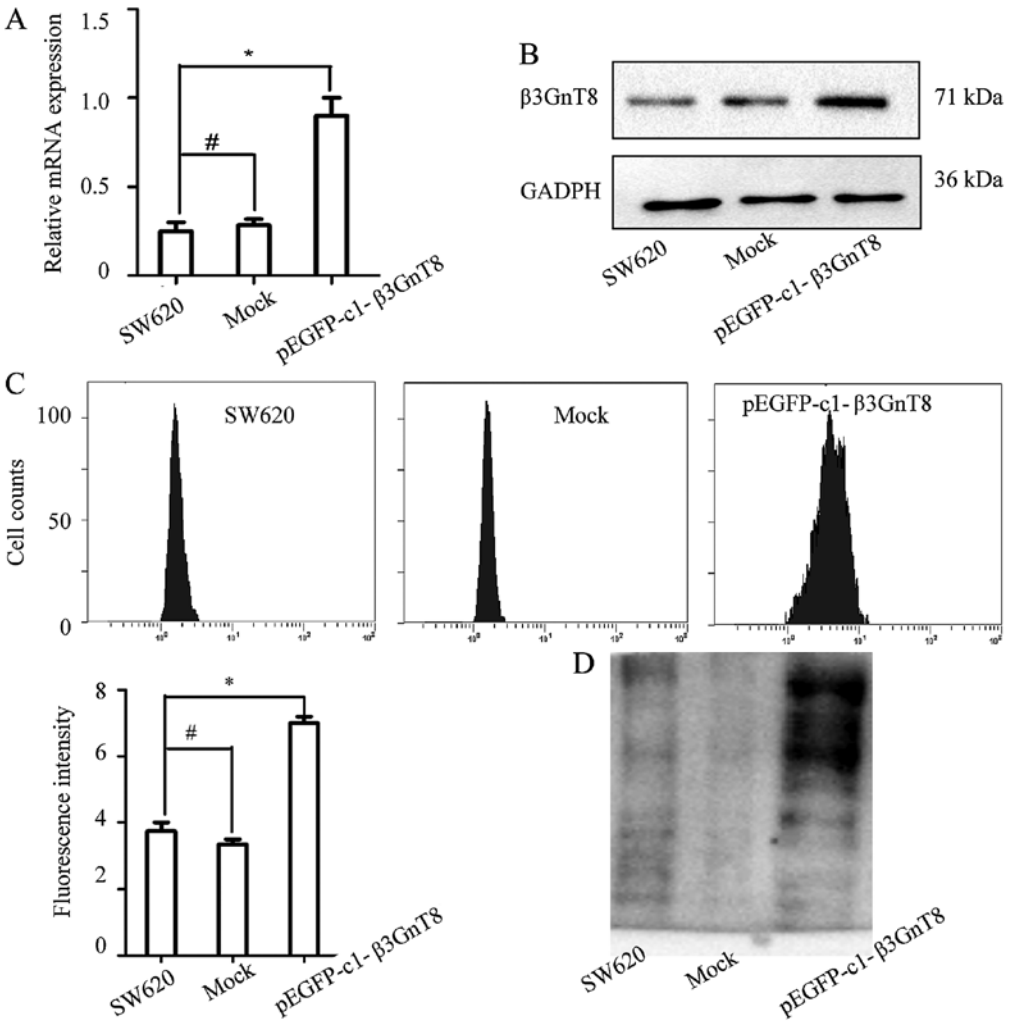

Figure 5. Effects of β3GnT8 overexpression on the synthesis of polylactosamine chains in SW620 cells. (A) The mRNA level of β3GnT8 was analyzed by real-time RT-PCR. (B) The protein expression level of β3GnT8 was analyzed by western blotting. (C) Results of the flow cytometric analysis. (D) Results of the lectin blotting analysis. Data were recorded as the mean ± SD of three independent experiments. *P<0.05, $^{\#}$P>0.05 compared with the untreated SW620 cells.

in the pEGFP-c1-β3GnT8 plasmid-transfected SW620 cells. The IC_{50} values of oxaliplatin in SW620 cells transfected with the pEGFP-c1-β3GnT8 plasmid or empty plasmid were 0.122±0.058 and 0.013±0.008 μg/ml, respectively (P<0.05) (data not shown). Fig. 5C revealed that β3GnT8 overexpression resulted in an increase of fluorescence intensity compared with the mock cells. The results of lectin blotting were consistent with the FITC-lectin binding analysis (Fig. 5D). These data clearly confirmed that β3GnT8 contributed to the development of oxaliplatin resistance via the regulation of polylactosamine chains.

Integrin β1 is a target of β3GnT8 in SW620R cells. Since integrin β1 is an extensively glycosylated glycoprotein, we further evaluated whether β3GnT8 modified glycan structures on integrin β1. Cell lysates were first incubated with LEA and LEA-bound glycoproteins were then immunoblotted for integrin β1. In line with the relative expression of β3GnT8, the binding of LEA to integrin β1 in SW620R cells was stronger than that in SW620 cells (Fig. 6A). However, there was no significant difference in the expression of the integrin β1 protein between SW620 and SW620R cells (Fig. 6B). These results revealed that integrin β1 was post-translationally modified by β3GnT8. Moreover, β3GnT8 overexpression in SW620 cells increased LEA binding to integrin β1, whereas β3GnT8 knockdown in SW620R cells decreased LEA binding to integrin β1 (Fig. 6C). To further confirm the existence of LEA-recognized carbohydrate structures on integrin β1, PNGase F (25 units; Sigma-Aldrich) was used to remove N-glycans from glycoproteins. As shown in Fig. 6D, the binding of LEA to integrin β1 was almost completely eliminated by PNGase F treatment in SW620R cells. All these data indicated that integrin β1 could be a target glycoprotein affected by β3GnT8.

β3GnT8 regulates the downstream signaling pathway of integrin β1. Since integrin β1 was involved in β3GnT8-mediated oxaliplatin resistance and β3GnT8 regulated the glycosylation of β1 integrin, we next examined whether β3GnT8 knockdown could regulate the expression of integrin β1 downstream signaling molecules. As shown in Fig. 7, β3GnT8 knockdown in SW620R cells reduced the phosphorylation of focal adhesion kinase (FAK) and paxillin. Conversely, β3GnT8 overexpression in SW620 cells enhanced FAK and paxillin phosphorylation.

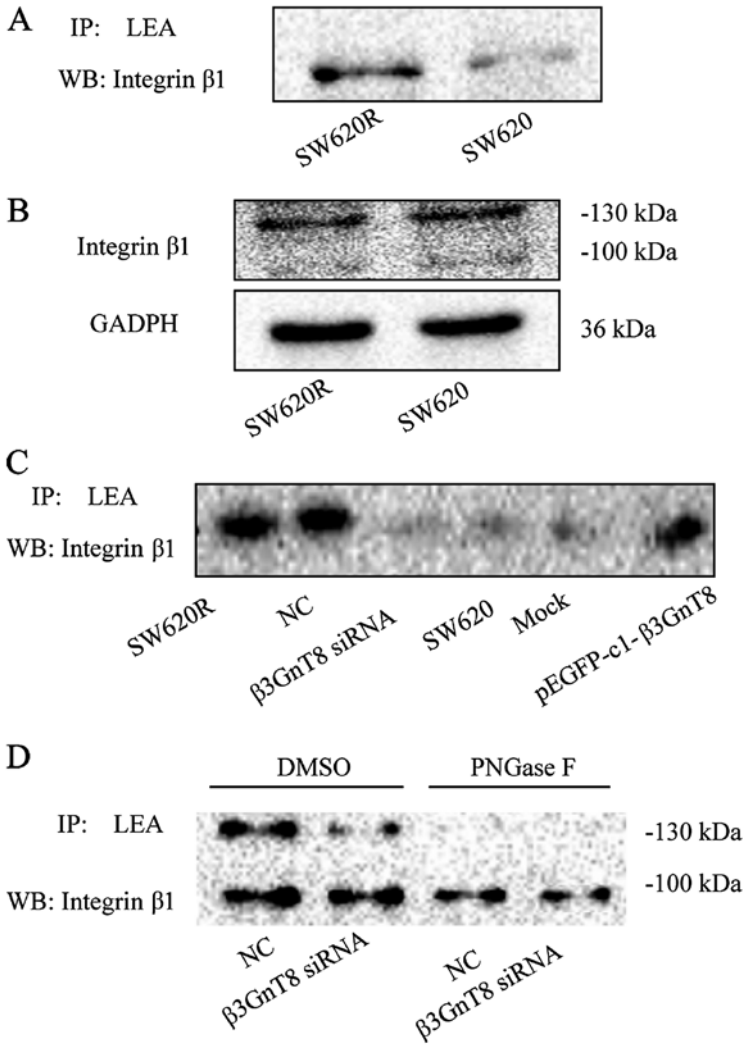

Figure 6. Effects of β3GnT8 overexpression or knockdown on the glycosylation of integrin β1 in SW620 and SW620R cell lines. (A) The glycosylation of integrin β1 was analyzed by lectin immunoprecipitation. (B) The protein expression level of integrin β1 was analyzed by western blotting. (C) The alteration of integrin β1 glycosylation was analyzed by lectin immunoprecipitation. (D) The existence of polylactosamine glycans in SW620 cells was analyzed by lectin immunoprecipitation using LEA. N-glycans were removed from peptide chains by PNGase F.

Discussion

In the present study, we investigated the possible correlations between oxaliplatin resistance and glycosylation changes in parental and oxaliplatin-resistant human colon cancer cell lines using lectin microarray, FITC-lectin binding and real time RT-PCR. Additionally, we confirmed that the glycogene β3GnT8 affected oxaliplatin resistance through modification of the polylactosamine structures on integrin β1.

Lectin microarray has been demonstrated to be useful in revealing glycan components and glycome structures of a biological sample (16). It can be exploited to identify aberrant glycosylation patterns, which in turn would help in enhancing the specificity of cancer diagnosis (17). For example, using lectin microarray analysis, Wu *et al* revealed that gastric cancer cells exhibited elevated levels of α2-6 sialylation, core fucosylation, and the Tn antigen (10). Zhou *et al* discovered that lectin RCA-I specifically bound to metastasis-associated cell surface glycans in triple-negative breast cancer (11). Moreover, the FITC-lectin flow cytometric analysis is an effective approach to validate the results of lectin microarray not only qualitatively but also quantitatively. Combined use of these two methods ensured identification of correct glycosylation status and led to several important findings. The integrated strategy employed in the present study revealed differential expression of α2-6 sialylation, core fucosylation, polylactosamine-type N-glycans, β1,6-GlcNAc branched N-glycans, bisecting GlcNAc and the T antigen (recognized by SNA, LCA, LEA, L-PHA, PHA-E, and Jacalin, respectively) between colon cancer cells SW620 and its oxaliplatin-resistant cells SW620R. In previous studies, polylactosamine structure alterations were reported to be involved in drug resistance of human colon cancer, breast cancer and leukemia (7,8,15). Our results further support that increasing polylactosamine chain expression may enhance colon cell oxaliplatin resistance.

Glycogens play important roles in glycan synthesis and modification. In the present study, the expression profiles of glycogenes were analyzed using real-time PCR analysis. We determined that the glycogenes including ST6GaL1, FUT8, β3GnT8, GnT-V, GnT-III and GALNT1 were differentially expressed between the SW620 and SW620R cells. These data were consistent with the lectin microarray and FITC-lectin

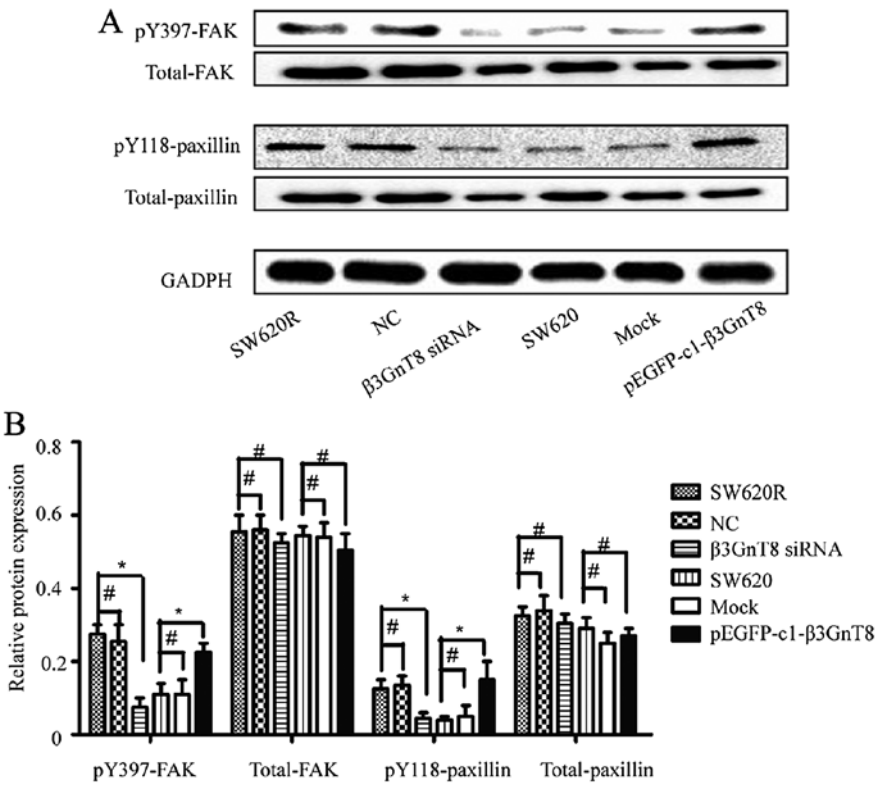

Figure 7. Effects of β3GnT8 overexpression or knockdown on the activation of β1 integrin downstream signaling pathways in SW620 and SW620R cell lines. (A) The protein expression was analyzed by western blotting. (B) Analysis of relative protein levels. Data were recorded as the mean ± SD of three independent experiments. *P<0.05, $^{\#}$P>0.05.

flow cytometric analysis. The glycogene β3GnT8 belongs to the family of β1, 3-N-acetylglucosaminyltransferase (β3GnT), which catalyze the biosynthesis of polylactosamine-type N-glycans (18). In the present study, we clearly demonstrated that the expression of β3GnT8 was increased in the oxaliplatin-resistant cells SW620R as compared to the parental cells SW620. In addition, we further demonstrated that the silencing of β3GnT8 in SW620R cells resulted in increased chemosensitivity to oxaliplatin. The product of β3GnT8 also decreased in β3GnT8-siRNA transfected SW620R cells labeled with LEA lectin. Conversely, a stable high expression of β3GnT8 in SW620 cells could increase resistance to oxaliplatin. The β3GnT8 product was also increased in pEGFP-c1-β3GnT8 plasmid-transfected SW620 cells. All these features suggest that β3GnT8 contributed to the development of oxaliplatin resistance by catalyzing the formation of polylactosamine-type N-glycans.

Integrin β1 is a highly N-glycosylated transmembrane protein that is composed of 12 potential N-glycosylation sites on its polypeptide backbone (19). Most studies of altered integrin β1 function have focused on either changes in integrin β1 expression or regulation of activity through 'inside-out' signaling mechanisms (20,21). However, there is growing evidence for the role of glycosylation in the regulation of integrin β1 function. For example, ST6Gal-I-mediated sialylation of integrin β1 enhanced cancer cell adhesion to, and migration on collagen I (22). Aberrant expression of β4GalT3 through alteration of integrin β1 activation and glycan structures may enhance cancer cell invasiveness (23). It has been reported that polylactosamine chains mainly appear on the N-glycans of integrin β1 in colon cancer cells (23). In the present study, altered glycosylation of integrin β1 was reveales in SW620R cells. We observed decreased and increased polylactosamine carried on integrin β1 with β3GnT8 knockdown and overexpression, respectively. Exogenous β3GnT8 induced marked alteration of the glycosylated forms on integrin β1. Integrin β1 could therefore be a target of β3GnT8 in SW620R cells.

Activation of FAK/paxillin is considered to be an important step in integrin β1 signaling (24,25). The present study confirmed that the resistant cell line SW620R exhibited higher FAK/paxillin activity than the sensitive one. Altered expression of β3GnT8 markedly regulated the activity of FAK/paxillin in colon cancer cells.

Collectively, our findings reveal new insights into the regulation of oxaliplatin resistance by aberrant expression of β3GnT8 through alteration of integrin β1 glycosylation in colon cancer. It also provides a potentially new biomarker for prediction of drug resistance in colon cancer patients.

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Competing interests

The authors declare that they have no competing interests.

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