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Original Article

Knockdown of ST6Gal-I inhibits the growth and invasion of osteosarcoma MG-63 cells

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ABSTRACT

The upregulation of alpha-2,6-sialyltranferase 1 (ST6Gal-I) has been observed in several malignant tumors, including colon, ovarian and liver cancers, where its expression correlates with the invasion and metastasis of these tumors. However, the roles and molecular mechanisms by which ST6Gal-I mediates the growth and invasion of osteosarcoma cells still remain poorly unknown. In this study, we investigated the expression of ST6Gal-I in osteosarcoma MG-63 and Saos-2 cells which have different metastatic potential, and found that ST6Gal-I was highly expressed in MG-63 cells compared to Saos-2 cells. Downregulation of ST6Gal-I by shRNA in MG-63 cells significantly inhibited their malignant behaviors, including in cell proliferation and soft agar colony formation, as well as migration and invasion properties. In addition, we found that ST6Gal-I knockdown inhibited the expression levels of *N*-cadherin, vimentin, a-SMA, MMP-2, MMP-9 and VEGF. Together, our results suggest a role for ST6Gal-I to promote the growth and invasion of osteosarcoma cells through modulation of EMT-related molecules, and might be a promising marker for the prognosis and therapy of osteosarcoma.

1. Introduction

Osteosarcoma is the most common histological form of primary bone cancer and the most prevalent in children and young adults. It is characterized by high propensity for metastasis (specially to the lung), which is the main cause of death [1]. Despite current multidisciplinary treatments, there is currently no effective therapy to control the metastasis [2]. Therefore, exploring the molecular mechanisms underlying the metastasis of osteosarcoma may reveal new therapeutic targets for the treatment of osteosarcoma.

Alternations in cellular glycosylation play a key role in the tumorigenesis and development [3,4]. Aberrant glycosylation is driven by various enzymes responsible for the addition and removal of glycans, such as glycosyltransferases and glycosidases [5]. The ST6Gal-I is a sialyltransferase, which catalyzes the transfer of sialic acid from CMP-sialic acid to the termini of *N*-glycans [6].

It has been reported that ST6Gal-I is highly expressed in many tumor cells, such as breast, liver and colon etc [7–9]. Many studies indicated that ST6Gal-I overexpression positively correlates with the migratory and invasive properties of tumor cells [10–13]. In addition, ST6Gal-I is also involved in the apoptosis of tumor cells [14–16]. We recently reported that ST6Gal-I upregulation modulates the expression of α2,6-sialic acid on cell surface and is associated with the adhesion of hepatoma cells through FAK signalling pathway [17]. However, in osteosarcoma, the roles of ST6Gal-I and the molecular mechanisms by which it mediates cell growth and invasion remain poorly understood.

In this study, we first checked that the expression levels of ST6Gal-I in osteosarcoma cell lines, and then analyzed its knockdown effects on the growth and invasion ability of osteosarcoma cells. We found that ST6Gal-I expression positively correlates with the high metastatic potential, the growth and invasion abilities of tumor cells. In addition, we found that knockdown of ST6Gal-I resulted in the downregulation of *N*-cadherin, vimentin, a-SMA, MMP-2, MMP-9 and VEGF proteins expression. Together, our data suggest that ST6Gal-I may promote the proliferation, migration and invasion of osteosarcoma cells by modulating the expression of EMT-related proteins.

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2. Materials and methods

2.1. Cell culture

Human osteosarcoma cell lines, MG-63 and Saos-2, were purchased from the Cell Bank of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). MG-63 cells were maintained in 90% MEM (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), 1 × penicillin/streptomycin (Gibco) and 1 g/L sodium bicarbonate (Gibco). Saos-2 cells were maintained in 85% DMEM supplemented with 15% heat-inactivated fetal bovine serum, 1 × penicillin/streptomycin and 1 g/L sodium bicarbonate. All cells were cultured in a humidified incubator at 37 °C with 5% CO_2.

2.2. Real-time PCR analysis

Real-time PCR was performed as described previously [18]. Real-time PCR was performed with ABI PRISM 7900 detection system (Applied Biosystems) using SYBR Premix Dimer-Eraser Kit (TaKaRa). Total RNA was extracted using TRIzol reagent (Invitrogen), and cDNA synthesis was performed using PrimeScript RT reagent Kit (TaKaRa) according to manufacturer's instructions. Specific primers for ST6Gal-I and GAPDH were purchased (GenePharma). Relative changes in mRNA expression were normalized with GAPDH and calculated using $2^{-\Delta\Delta CT}$ method.

2.3. Western blot analysis

Protein concentration was measured with BCA assay kit (Pierce). Equal amounts of denatured proteins were subjected to 10% SDS–PAGE and blotted onto nitrocellulose membranes (Pall Corporation). Antibodies against ST6Gal-I, *N*-cadherin, vimentin, a-SMA, E-cadherin, MMP-2, MMP-9, VEGF and GAPDH (Santa Cruz Biotech Inc.) were used as the primary antibodies. The detection was performed using ECL kit (Amersham Biosciences) according to manufacturer's instructions. Relative amount of protein was determined by densitometry using LabWorks software.

2.4. Construction of vectors and transfection

Construction of expression vector, including ST6Gal-I-specific small hairpin RNA (shRNA) sequences had been described previously [17]. The oligonucleotides targeting ST6Gal-I or the negative control were annealed and ligated into pGPU6/neo vector (GenePharma), respectively. MG-63 cells were transfected with the mixture of plasmids and Lipofectamine™ 2000 (Invitrogen) according to manufacturer's recommendation.

2.5. Lectin-blot assay

Cells were harvested, rinsed with PBS, and lysed with Membrane Protein Extraction Reagent Kit (Sigma–Aldrich). Cell lysates containing 30 μg of protein were boiled in SDS sample buffer with β-mercaptoethanol, loaded on 10% SDS–polyacrylamide gels, and then transferred onto a PVDF membrane. After being blocked with 5% BSA, the membrane was incubated with 1:1000 dilution of biotin-labelled SNA for 2 h at room temperature. The blots were developed using ECL detection system (Amersham Biosciences).

2.6. Cell viability assay

Cells (5×10^3 cells/well) were seeded in 96-well plates overnight. After 24 h, 10 μL of CCK8 solution (Cell counting kit-8, Dojindo, Gaithersburg, MD, USA) was added to each well following the manufacturer's instructions. The absorbance at wavelength 450 nm was measured for the supernatant of each well using the plate reader Multiskan EX. All experiments were repeated in triplicate.

2.7. Colony formation assay in soft agar

In brief, a bottom layer (0.6% low-melt agarose) was prepared with MEM media containing 10% fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin. A top layer (0.3% agar) was prepared with MEM and the same media as described above but containing 5000 indicated cells. Plates were incubated at 37 °C in 5% CO_2 in a humidified incubator for about two weeks. The cell colonies were stained for 20 min with a solution containing 0.5% crystal violet and 25% methanol. The plates were then scanned and photographed, and the number of colonies was quantified by Quantity one v.4.0.3 software (Bio-Rad, Hercules, CA).

2.8. Cell scratch-wound assay

Cells were seeded on 6-well plates and grown to a monolayer. Wounds were created using a 10 μL pipette tip. Subsequently free-floating cells and debris were removed using PBS, and FBS-free medium was added. At the indicated times (0 and 24 h), the wound areas were photographed using the light microscope and the wound healing rate was calculated.

Healing rate = (width of wound at 24 h – width of the wound at 0 h)/width of wound at 0 h.

Each experiment was repeated in triplicate.

2.9. Invasion assay

A Transwell chamber (Corning Corporation, MA, USA) with 8-μm polyester membrane filter coated with ECMatrix gel (Chemicon) was used for invasion assay. Cells (3×10^5) were harvested in serum-free medium containing 0.1% BSA and added into the top chamber. The bottom chambers were filled with MEM containing 10% FBS. After 24 h of incubation, the cells on the upper surface of the filter were completely removed by wiping with a cotton swab. Then, the filters were fixed in methanol and were stained with Wright–Giemsa. Cells that had invaded the Matrigel and reached the lower surface of the filter were counted using a light microscope. Triplicate samples were acquired and the data were expressed as the average cell number of five fields.

2.10. Statistical analysis

The data were expressed as the mean ± SD. Statistical analysis was performed with SPSS 13.0 software. One-way ANOVA with post-hoc Tukey's test was performed for experiments that involved more than two groups, and Student's *t*-test was performed for comparisons between two groups. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Expression of ST6Gal-I in osteosarcoma MG-63 and Saos-2 cells

To study the roles of ST6Gal-I in osteosarcoma, we firstly analyzed the expression of ST6Gal-I in two osteosarcoma cell lines, MG-63 and Saos-2, which have different metastatic potential [19]. Real-time PCR and western blot results showed that significant higher mRNA and protein levels of ST6Gal-I in MG-63 cells compared to Saos-2 cells (Fig. 1A–C). This indicates that