Galectin-3 Increases Gastric Cancer Cell Motility by Up-regulating Fascin-1 Expression

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BACKGROUND & AIMS: Galectin-3 is a β-galactoside–binding protein that increases gastric cancer cell motility in response to integrin signaling and is highly expressed in gastric tumor cells. Galectin-3 induces cytoskeletal remodeling to increase cell motility, but the mechanisms of this process are not understood. We investigated the effects of galectin-3 on fascin-1, an actin-bundling protein. **METHODS:** We collected malignant and normal tissues from gastric cancer patients and examined the expression levels of galectin-3 and fascin-1. We silenced galectin-3 expression in human gastric cancer cell lines using small interfering RNA and lenti-viral constructs and determined the effects on fascin-1 expression, cell motility, and invasion. **RESULTS:** Malignant gastric tissues expressed high levels of galectin-3 and fascin-1, compared with normal gastric tissues. Silencing of galectin-3 resulted in altered cancer cell morphology, reduced fascin-1 expression, decreased cell motility, and reduced malignant cell invasion. Galectin-3 overexpression reversed these effects. Silencing of fascin-1 also reduced cell motility and caused changes in cell shape, as did silencing of galectin-3. Furthermore, galectin-3 silencing inhibited the interaction between glycogen synthase kinase (GSK)-3β, β-catenin, and T-cell factor (TCF) 4, and the binding of β-catenin/TCF-4 to the *fascin-1* promoter. Nuclear localization of GSK-3β and β-catenin were not detected when galectin-3 was silenced. Overexpression of mutated galectin-3 (with mutations in the GSK-3β binding and phosphorylation motifs) did not increase fascin-1 levels, in contrast to overexpression of wild-type galectin-3. **CONCLUSIONS: Galectin-3 increases cell motility by up-regulating fascin-1 expression. Galectin-3 might be a potential therapeutic target for the prevention and treatment of gastric cancer progression.**

Keywords: Galectin-3; Fascin-1; Metastasis; Gastric Cancer.

High expression of galectin-3, a 31-kilodalton member of carbohydrate-binding proteins, correlates with poor prognosis and/or metastasis in gastric cancer patients.[1–3] Galectin-3 shows pleiotropic biological functions, in cell growth, apoptosis induction, tumor progression, and pre–messenger RNA (mRNA) splicing among others.[4–6] Galectin-3 is reported to regulate metastasis by binding to cell adhesion–related molecules and inhibiting cell–cell and cell–matrix interactions,[7] thereby augmenting the detachment of cancer cells, and promoting metastasis.[8,9] Also, galectin-3 clusters at cell–cell contact sites by interaction with integrins α1β1, involved in endothelial cell adhesion.[10] Highly metastatic human breast cancer cells also show higher levels of expression of galectin-3 and significantly increase adhesion to endothelial cells.[11] Moreover, overexpression of galectin-3 enhances cell motility and invasiveness in vitro in lung cancer cells,[12] suggesting that endogenous galectin-3 regulates cell metastasis in a variety of cancers.[4,13] Recently, it was shown that the functions of galectin-3 depend on its subcellular localization. For example, strong nuclear immunoreactivity of galectin-3 was detected in malignant regions of gastric cancer patients, whereas little or no nuclear immunoreactivity was seen in adjacent epithelial cells.[14] However, the exact role played by galectin-3 in influencing metastasis is still undefined. To understand how galectin-3 regulates gastric cancer metastasis, we knocked-down the expression of galectin-3 in gastric cancer cells with small interfering RNA (siRNA), and monitored changes in gene expression using DNA microarray analysis (unpublished data). We found that the expression of several genes changed after galectin-3 silencing. For example, matrix metalloproteinase (MMP)-3, hyaluronan-mediated motility receptor, MMP-1, and fascin-1 were down-regulated and serine protease in-

Abbreviations used in this paper: siRNA, small interfering RNA; GSK-3β, glycogen synthase kinase–3 beta; MMP, matrix metalloproteinase; RT-PCR, reverse-transcription polymerase chain reaction; TCF, T-cell factor.

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hibitor 1 and tissue inhibitors of MMP 2 were up-regulated after galectin-3 siRNA treatment.

In this study we focused on fascin-1, an actin-binding protein, because of the following reasons: (1) it is localized along the entire length of filopodia,[15,16] (2) it is high expressed in several types of cancer including lung and esophagus cancers,[17–19] (3) it induces membrane protrusions and increases cell motility,[20] (4) its knock-down using siRNA leads to a substantial reduction of filopodia, and (5) its overexpression significantly increases cell migration,[18,21,22] all of which suggest that fascin-1 may promote cancer cell motility and metastasis by participating in filopodia formation.[18,23] We examined whether there is any clinical correlation between galectin-3 and fascin-1 levels in gastric cancer patients and whether galectin-3 augments cancer cell motility and regulates fascin-1 expression. Based on our findings we propose that galectin-3 is a critical molecule that promotes gastric cancer metastasis through up-regulation of fascin-1.

Materials and Methods

Cell Culture and Transfection

Human gastric cancer cell lines were obtained from the Korea Cell Line Bank and maintained in RPMI 1640 with 5% fetal bovine serum and 1% antibiotics. Galectin-3 and fascin-1 siRNAs (galectin-3 siRNA 5′-AUAUGAAGCACUGGUGAGGUCUAUG-3′; and Fascin-1 siRNA 5′-UCCAGCAAGAAUGCCAGCUGCU ACU-3′) were obtained from Invitrogen (San Diego, CA) and transfected transiently in cells using Lipofectamine RNAiMAX reagent (Invitrogen) following the manufacturer's instructions. Cells were harvested 2 days after transfection.

RNA Isolation and Reverse-Transcription Polymerase Chain Reaction

Total RNA was isolated from cells and malignant gastric tissues obtained from the National Cancer Center (Korea). RNA isolation was performed using TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. A reverse-transcription polymerase chain reaction (RT-PCR) system (Promega, Madison, WI) was used. The primers used were for fascin-1: 5′-ACCTGTCTGCCAATCAGGAC-3′ and 5′-CCCATTCTTCTTGGAGGTCA-3′; LGAL3: 5′-ATGGCAGACAATTTTTCGCTCC-3′ and 5′-ATGTCACCAGAAATTCCCAGTT-3′; for β-actin: 5′-AGCCTCGCCTTTGCCGA-3′ and 5′-CTGGTGCCTGGGGCG-3′; and for glyceraldehyde-3-phosphate dehydrogenase: 5′-GGCTGCTTTTAACTCTGGTA-3′ and 5′-ACTTGATTTTGGAGGGATCT-3′. PCR was performed following Extaq (TaKaRa, Shiga, Japan) methods.

Western Blot Analysis and Immunoprecipitation

Cell lysate extractions for both Western blot and immunoprecipitation were performed in RIPA buffer containing protease inhibitor cocktail. Immunoblotting was performed with anti–Galectin-3, anti–Fascin-1, anti–glycogen synthase kinase-3β (GSK-3β), anti–T-cell factor (TCF)-4 (Santa-Cruz Biotechnology, Santa Cruz, CA), anti–GSK-3β [pS[6]] (Biosource, Camarillo, CA), anti–β-catenin, anti–Lamin A/C (Cell-Signaling, Danvers, MA), and anti-β-actin (Sigma-Aldrich, St. Louis, MO). Immunoprecipitation was performed with A/G agarose beads coated with anti–galectin-3 and anti–β-catenin. Proteins were detected using anti–Galectin-3, anti–β-catenin, anti–GSK-3β, and anti–TCF-4, using Western blot analysis. Mouse/rabbit immunoglobulin (Ig)G was used as the negative control.

Construction of Galectin-3 Expressing Lenti-Viral Vector and Infection

The full-length human galectin-3– expressing construct pcDNA3.1–NT-GFP-gal3 was amplified by PCR using 5′-ATGGCAGACAATTTTTCGCT-3′ and 5′-TTATATCATGGTATATGAAGCACTGGT-3′. The PCR fragment was cloned directly into pcDNA3.1–NT-GFP vector using TA cloning methods (Invitrogen). Lentiviral vector pLL3.7 (Addgene, Inc, Cambridge, MA) was obtained from Dr Kyung-Ho Choi of the National Cancer Center (Korea). Such modifications caused the lenti-viral vector to overexpress LacZ, gal-3 wild, and gal-3 S96A. pLL3.7 was generated by PCR amplification of the entire human gal-3 wild primers with *BamH1/Xho1* enzyme site: 5′-GGATCCATGGCAGACAATTTTTCGCTC-3′ and 5′-TCGAGTTATATCATGGTATATGAAGC-3′. Galectin-3 S96A is made of 2 fragments. The first fragment primers were as follows: 5′-GGATCCATGGCAGACAATTTTTCGCTC-3′ and 5′-GGACAGCCAGCAGCCACCGGAGCCTCACCTGCCACT-3′; the second fragment primers were as follows: 5′-TCCGGTGGCTGCTGGCTGTCCAGAAGATGGGTAGGC-3′ and 5′-CTCGAGTTATATCATGGTATATGAAGC-3′. Those 2 fragments were combined into 1 fragment using *BamH1/Xho1* enzyme. The lentivirus particles were generated using 3 plasmids, VSVG, RSV-REV, and PMDLg/pPRE, in which 293FT cells were cotransfected with pLL 3.7. The 293FT cells were transfected using Lipofectamine 2000 (Invitrogen) transfection reagent following the manufacturer's instructions. Two days after transfection, the 293FT cell cultured media was filtered using a 0.45-μm filter. Lenti-virus infections of lacZ, gal-3 wild, and gal-3 S96A were performed in media of SNU-638 cells.

Immunocytochemistry and Immunohistochemistry

Chamber slide cultured cells were fixed and blocked with 5% bovine serum albumin in phosphate-buffered saline (PBS) and then incubated with primary anti– galectin-3, anti–fascin-1, anti–GSK-3β, anti–β-catenin antibodies, diluted (1:200) in PBS. The cells were conjugated with the secondary antibodies labeled with fluorescein isothiocyanate, Cy5 (Zymed, South San Francisco, CA), or Texas Red-X phalloidin (Invitrogen), and diluted (1:200) in PBS. The samples were treated with