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Original Article

Extracellular galectin-3 facilitates colon cancer cell migration and is related to the epidermal growth factor receptor

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Abstract: We previously found that galectin-3 enhanced DLD-1 cell migration through the K-Ras-Raf-Erk1/2 pathway, but the effect of extracellular galectin-3 on cancer cell migration and its interaction with the epidermal growth factor receptor (EGFR) remained unknown. We aimed to determine the effect of extracellular galectin-3 on colon cancer cell migration and its correlation with the EGFR expression. Western blotting was performed to analyze galectin-3 secretion, shRNA was used to stably knock down galectin-3 expression and a migration assay was performed to evaluate colon cancer cell migration. Tissues from eighty patients with four different stages of colon cancer were obtained and compared to normal colon tissue. The galectin-3 knockdown colon cancer cells exhibited decreased migration, which was restored by recombinant galectin-3. An EGFR blocking antibody decreased colon cancer cell migration. The addition of recombinant galectin-3 increased phosphorylated EGFR expression within minutes and enhanced the internalization of the EGFR from the cell membrane to the cytoplasm, particularly upon EGF stimulation. Extracellular galectin-3 increased colon cancer cell migration, which correlated with the EGFR. Targeting galectin-3 may have a synergistic effect on EGFR-targeted therapy.

Keywords: Colorectal carcinoma, galectin-3, EGFR, cell migration

Introduction

The endogenous β-galactoside-binding protein galectin-3 is a member of a widely distributed carbohydrate-binding protein family that has been implicated in cell growth, differentiation, adhesion, and malignant transformation. Several studies have suggested that galectin-3 plays a pivotal role in the transformation and metastasis of human tumors, including prostate cancer, breast cancer, thyroid cancer and gastric cancer [1-4]. Alterations in galectin-3 production have been independently correlated with the malignant behavior of human colon cancer cells [5]. High galectin-3 expression has been shown to be correlated with colon cancer metastasis [6, 7]. Galectin-3 is thought to regulate metastasis by binding to cell adhesion-related molecules and promoting cell-cell and cell-matrix interactions [8], thus increasing cancer cell migration and promoting metastasis [9, 10].

Several reports have suggested that galectin-3 participates in the metastatic progression of human colon cancer, but the mechanism by which galectin-3 modulates colon cancer cell migration remains unclear. We have previously shown that overexpression of intracellular galectin-3 enhanced colon cancer cell migration through the K-Ras-Raf-Erk1/2 pathway, and galectin-3 knockdown decreased colon cancer

cell migration [11]. Gao showed that circulating galectin-3 promoted cell migration via the calcium-sensitive MAPK/ERK1/2 pathway [12]. Galectin-3 has been independently correlated with the malignant behavior of human colon cancer, although the mechanism is not well understood.

Galectin-3 was recently shown to interact with the epidermal growth factor receptor (EGFR) to induce stable lattice cluster formation, cell-surface glycoprotein organization and signal transduction [13, 14]. EGFR is a member of the ErbB family (ErbB1-4) of receptor tyrosine kinases (RTKs) and mediates cellular responses to EGF and transforming growth factor α (TGF-α). The EGFR-mediated pathways are important for a variety of cellular processes, including cancer cell migration and invasion [15, 16]. Whether extracellular galectin-3 enhances colon cancer cell migration and the interaction between galectin-3 with EGFR remains an interesting unsolved question. We hypothesize that extracellular galectin-3 enhances colon cancer cell migration by altering EGFR expression and that the extracellular addition or targeting of galectin-3 could enhance or decrease colon cancer cell migration.

Materials and methods

Human colon cancer cell lines and culture

The Caco2 and DLD-1 human colon cancer cell lines were purchased from the Bioresource Collection and Research Center (BCRC, Taiwan) and were derived from ATCC (CCL-221) and ATCC (CCL-222), respectively. The cells were cultured in RPMI medium (Gibco Life Technologies, Grand Island, NY, USA) supplemented with 5% fetal bovine serum (FBS) (Gibco Life Technologies) and 1% antibiotics (Gibco Life Technologies) at 37°C with 10% CO_2. Both floating and attached cells were harvested for the subsequent analysis.

Antibodies and reagents

The EGF, EGFR and β-actin antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The Galectin-3 small hairpin RNAs (shRNA) and the transfection reagent Lipofectamine™ 2000 were purchased from Invitrogen (Carlsbad, CA, USA). Dulbecco's modified Eagle's medium, RPMI-1640 medium, Opti-MEM reduced serum medium, fetal bovine serum, and antibiotics were purchased from Invitrogen. Fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG and the CellTracker™ fluorescent cell-tracking dyes Green CMFDA and Orange CMRA (appears red in color) were purchased from Invitrogen (Carlsbad, CA, USA). Anti-tubulin (1:1000) was obtained from Santa Cruz. Lactose and sucrose were obtained from Sigma-Aldrich, the galectin-3 antibody (B2C10) was purchased from Santa Cruz, and the galectin-3 antibody was a gift from FT Liu. [6] Modified citrus pectin (MCP) was obtained from EcoNugenics, Inc. (CA). Cetuximab was purchased from Merck (Serono, Germany). The small hairpin (sh) RNA was designed as described in our previous publication [11].

Western blotting

Protein extracts from wild-type and galectin-3 knockdown DLD-1 cells were subjected to western blotting. Equal amounts of protein (30 μg) under different culture conditions were loaded into each lane, electrophoresed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and transferred onto a polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA) according to the manufacturer's protocol. The membranes were incubated with the primary antibody overnight at 4°C, followed by an incubation with the secondary antibody and detection using Western Lightning Plus-ECL, Enhanced Chemiluminescence Substrate (PerkinElmer Inc., Waltham, MA, USA). The membranes were stripped and re-probed using antibodies against housekeeping genes (as internal controls), and then, detection was performed using enhanced chemiluminescence.

Migration assay

The cells were cultured as confluent monolayers, synchronized in the absence of serum for 24 h, and wounded by removing a 300- to 400-um-wide strip of cells across the well using a culture insert. The wounded monolayers were washed twice to remove the non-adherent cells. After washing with PBS, the wounded regions were allowed to heal for 24 h in serum-free medium prior to analysis. Wound healing was quantified as the ratio of the remaining cell-free area to the area of the initial wound (calculated as a mean percentage) using the public domain software ImageJ (http://rsbweb.nih.gov).

Infrared fluorescent protein (iRFP)

Stable transfected cell line: piRFP (Plasmid #31857, Addgene) was transfected using the Lipofectamine™ 2000 Transfection Reagent (Invitrogen, Carlsbad, CA, USA). To transfect the scramble or shGal3 DLD-1 cells with the plasmid vector, the cells were plated into 6-well plates (7*105 cells per well) and allowed to adhere for 0/N. Lipofectamine™ 2000 was used for the transfection. Then, the medium was replaced with fresh medium supplemented with 10% FBS.

In vivo studies: In total, sixteen 6- to 8-week-old female SCID mice (BioLASCO, Taipei, Taiwan) were maintained on a standard chow diet with ad libitum access to water. All procedures and protocols were approved by the Institutional Animal Care and Use Committee of Chang Gung Memorial Hospital. The mice were categorized into the following four groups (n = 4/group): group 1 (sham control); group 2 (non-injection); group 3 (scramble); and group 4 (shRNA galectin-3). To measure the tumor growth, stable galectin-3 knockdown DLD-1 cells and scramble DLD-1 cells (5×10^6 cells) labeled with piRFP were injected into the peritoneum of three mice. After the mice were injected intraperitoneally (I.P.), images were acquired at the post-injection (p.i.) time point of 24 hr using a Pearl Impulse Imager (LI-COR). The images are displayed using a pseudocolor output. All images were normalized to the same exposure time in each channel. The in vivo imaging was performed using 2-2.5% isoflurane and oxygen inhalational anesthesia flowing at 2 l/min on a 37°C bed. The tumors were measured using calipers once per week.

Immunoprecipitation: The target proteins were immunoprecipitated using a Catch and Release Immunoprecipitation Kit (Millipore). In total, 500 μg cell lysate were incubated with 5 μg specific capture antibody and 10 μl affinity ligand (Millipore) on a rotator at 4°C overnight. After the incubation, the complexes were washed with washing buffer at 5000 rpm for 30 seconds. The target protein extracts were eluted in 70 μl elution buffer and saved for western blotting.

In situ proximity ligation assay (PLA)

The interaction between Gal-3 and EGFR was measured using a Duolink reagent kit (Olink Biosciences, Uppsala, Sweden). In total, 1×10^3 cells were seeded onto a chamber slide. The cells were fixed in 1% paraformaldehyde and washed with washing buffer A. The cells were blocked with blocking solution at 37°C for 30 min and incubated with the primary antibodies (Mouse anti Galectin-3 (Santa Cruz) and Rabbit anti EGFR (Epitomics)) at 37°C for 60 min. After the incubation, the cells were washed and incubated with each PLA probe (anti mouse PLUS and PLA probe anti rabbit MINUS) at 3°C for 60 min. The probes were ligated with ligation solution and amplified using amplification solution. The slides were imaged by fluorescence microscopy (Axio Observer Z1, Carl Zeiss MicroImaging, Inc., Welwyn Garden City, UK).

Isolation of membrane and cytoplasmic proteins

The DLD-1 Scramble and DLD-1 shGal-3 cells (2×10^7 cells) were treated with 30 μg/ml rGal-3 for 20 min. The membrane proteins were isolated using a Mem-PER Plus Membrane Protein Extraction Kit (Thermo Fisher Scientific, USA). EGFR expression was detected by performing a western blot analysis.

Flow cytometry

The scramble and ShGal3 DLD-1 cells were seeded 1×10^6 in six-well plate in condition medium. After 6 hours, the serum-free medium was replaced, and the cells were treated with or without Gal 3 (30 μg/ml) for 10 mins and prepared for the cellular membrane EGFR analysis. The staining reaction was incubated with the primary antibodies for 60 mins, washed with PBS, and incubated with the secondary antibodies and their corresponding isotypes at 4°C for 30 minutes. After performing the staining procedure, the cells were resuspended in 200 ml PBS containing 2% paraformaldehyde for the flow cytometry analysis using FACSCalibur (Becton Dickinson, San Jose, CA). The surface expression of EGFR was calculated as the relative mean fluorescence intensity (rMFI) normalized to the fluorescence intensity of the isotype control cell population.

Immunohistochemical staining

This study was conducted at the Kaohsiung Chang Gung Memorial Hospital after the study protocol was approved by the Institutional Review Board of the hospital. The immunohisto-

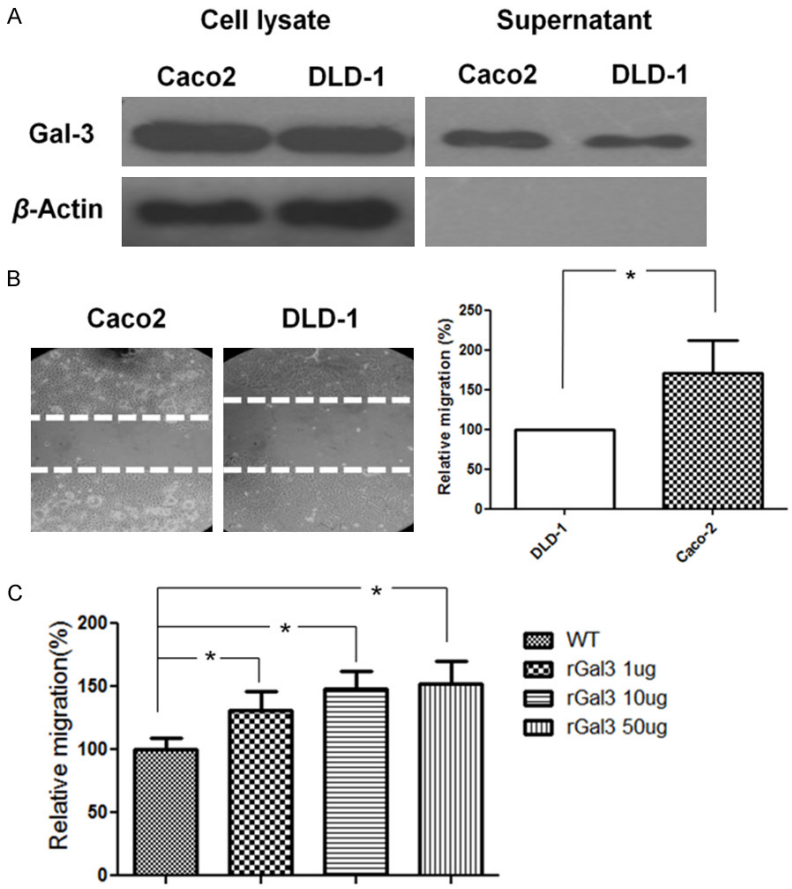

Figure 1. Extracellular galectin-3 correlated with the migration of different colon cancer cell lines and facilitated colon cancer cell migration. A. Caco2 cells secreted more galectin-3 than DLD-1 cells according to the western blot analysis; B. Caco2 cells migrated faster (as detected by performing a wound healing assay) than the DLD-1 cells. Statistical analysis was performed using the Student's t-test and the statistical significance is indicated by "*" which denote a *P* value of < 0.05. C. Recombinant galectin-3 dose-dependently enhanced DLD-1 cell migration. Statistical analysis was performed using the Student's t-test and the statistical significance is indicated by "*" which denote a *P* value of < 0.05. Bars represent the mean ± SD of three independent experiments.

chemical staining of galectin-3, EGFR, and CEA in colon cancer tissues from patients was performed using a Vectastain ABC kit and a DAB substrate kit according to the manufacturer's instructions (Vector Laboratories, Burlingame, CA, USA). Twenty pairs of colon cancer tissues at different stages were obtained from a tissue bank and compared to normal colon tissues. The sample size was chosen based on a power of 0.8, a 2-sided alpha level of 0.05, and a 50% higher expression of galectin-3 in the advanced colon cancer tissues. The H-score method was adopted to assign a continuous score to each patient based on the percentage of cells at different staining intensities. The staining intensity was scored according to the following four categories: 0, no staining; 1+, weak staining; 2+, moderate staining; and 3+, strong staining. The H-score was calculated using the following formula: 1 × (percentage of 1+ staining) + 2 × (percentage of 2+ staining) + 3(percentage of 3+ staining); the H-scores ranged from 0 to 300. The median H-score was chosen as the cut-off value to divide the patients into the high or low expression groups.

Results

Extracellular galectin-3 correlates with the migration ability of different colon cancer cell lines and facilitates colon cancer cell migration

To determine whether the extracellular galectin-3 expression was associated with colon cancer cell migration, we examined colon cancer cell lines with high and low levels of galectin-3 secretion. We found that the Caco2 cells secreted more extracellular galectin-3 than the DLD-1 cells by performing a western blot analysis of the extracellular galectin-3 secretion (**Figure 1A**). Furthermore, the Caco2 cells migrated faster (according to the migration assay) than the DLD-1 cells ($P < 0.05$) (**Figure 1B**). To determine whether extracellular galectin-3 was involved in DLD-1 cell migration, we added recombinant galectin-3 to DLD-1 colon cancer cells and found that the recombinant galectin-3 dose-dependently enhanced DLD-1 cell migration ($P < 0.05$) (**Figure 1C**).

Extracellular lactose and galectin-3 antibody inhibit DLD-1 colon cancer cell migration

To confirm that the migration rate was related to the extracellular galectin-3 secretion, we in-

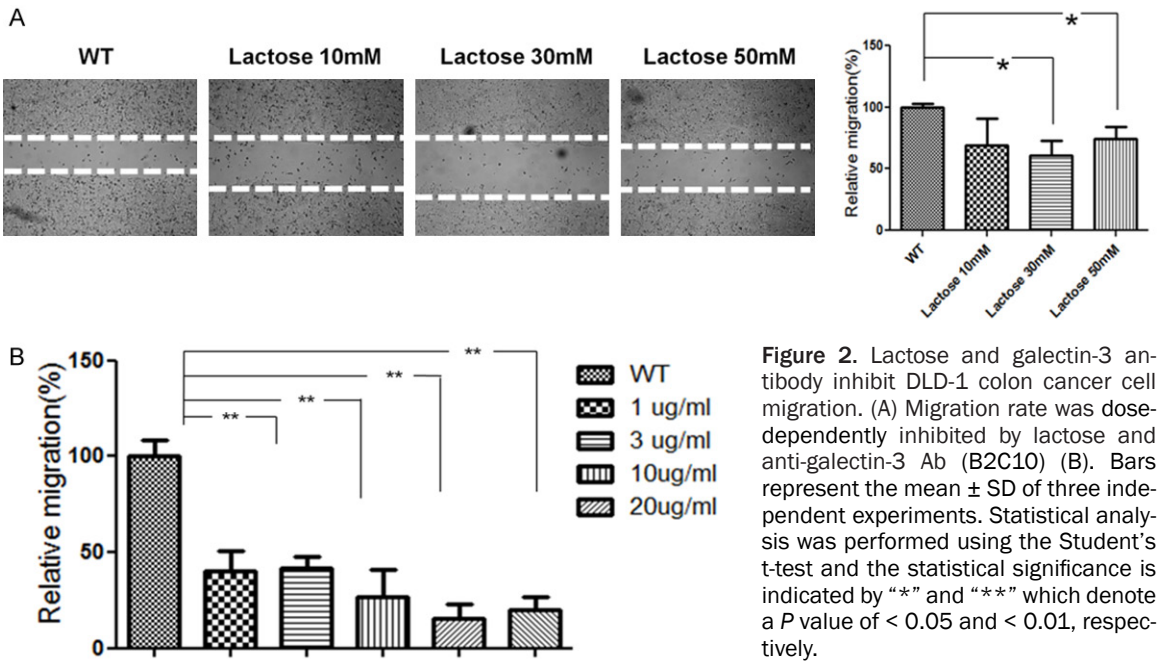

Figure 2. Lactose and galectin-3 antibody inhibit DLD-1 colon cancer cell migration. (A) Migration rate was dose-dependently inhibited by lactose and anti-galectin-3 Ab (B2C10) (B). Bars represent the mean ± SD of three independent experiments. Statistical analysis was performed using the Student's t-test and the statistical significance is indicated by "*" and "**" which denote a *P* value of < 0.05 and < 0.01, respectively.

hibited galectin using lactose and found that DLD-1 cell migration was inhibited in a dose-dependent manner (lactose 30 mM ($P < 0.05$), 50 mM ($P < 0.05$)) (**Figure 2A**). The migration rate was also inhibited by treating the cells with a neutralizing anti-galectin-3 antibody (B2C10) ($P = 0.001$) (**Figure 2B**).

Extracellular recombinant galectin-3 rescues galectin-3 knockdown DLD-1 cell migration

To block the influence of intracellular galectin-3, we used shRNA to stably knock down intracellular galectin-3 and performed immunocytochemical staining to observe the effect (**Figure 3A**). To determine whether extracellular galectin-3 was involved in DLD-1 cell migration without the influence of intracellular galectin-3, we investigated the effects of recombinant galectin-3 on shRNA galectin-3 DLD-1. We found that the stable knockdown of galectin-3 decreased the lamellipodia formation ($P < 0.05$) (**Figure 3B**), migration rate of the DLD-1 cells ($P < 0.01$) (**Figure 3C**). Tumor growth in animal study using iRFP method, we found after stable knockdown galectin-3, tumor growth was inhibited (Mann Whitney test, $P = 0.0286$) (**Figure 3D**), tumor weight decreased and no ascites found (data not shown). The recombinant galectin-3 (30 μg/ml) restored the galectin-3 knockdown-induced decrease in lamellipodia formation (**Figure 3B**) and cell migration ($P < 0.01$) (**Figure 3E**).

Inhibition of extracellular galectin-3 decreases colon cancer cell migration

Because galectin-3 can bind different membrane glycoproteins, we examined whether multiplex natural pectins, such as modified citrus pectin, block colon cancer cell migration. We found that the addition of recombinant galectin-3 enhanced the galectin-3 knockdown cancer cell migration and dose-dependently modified the citrus pectin blockade of galectin-3-induced DLD-1 cell migration ($P < 0.01$) (**Figure 4**).

Galectin-3 interacts with EGFR via carbohydrate recognition and effects migration

To investigate whether galectin-3 interacts with EGFR at the cell surface, we performed immunoprecipitation and immunoblotting to confirm their interaction. As shown in **Figure 5A**, we found that the anti-EGFR antibody co-precipitated EGFR and galectin-3. Reciprocally, the anti-galectin-3 antibody immunoprecipitation co-precipitated EGFR and galectin-3. Further studies involving an in situ proximity ligation assay (PLA) showed that upon the galectin-3 knockdown, the fluorescent resonance between galectin-3 and EGFR proximity ligation was

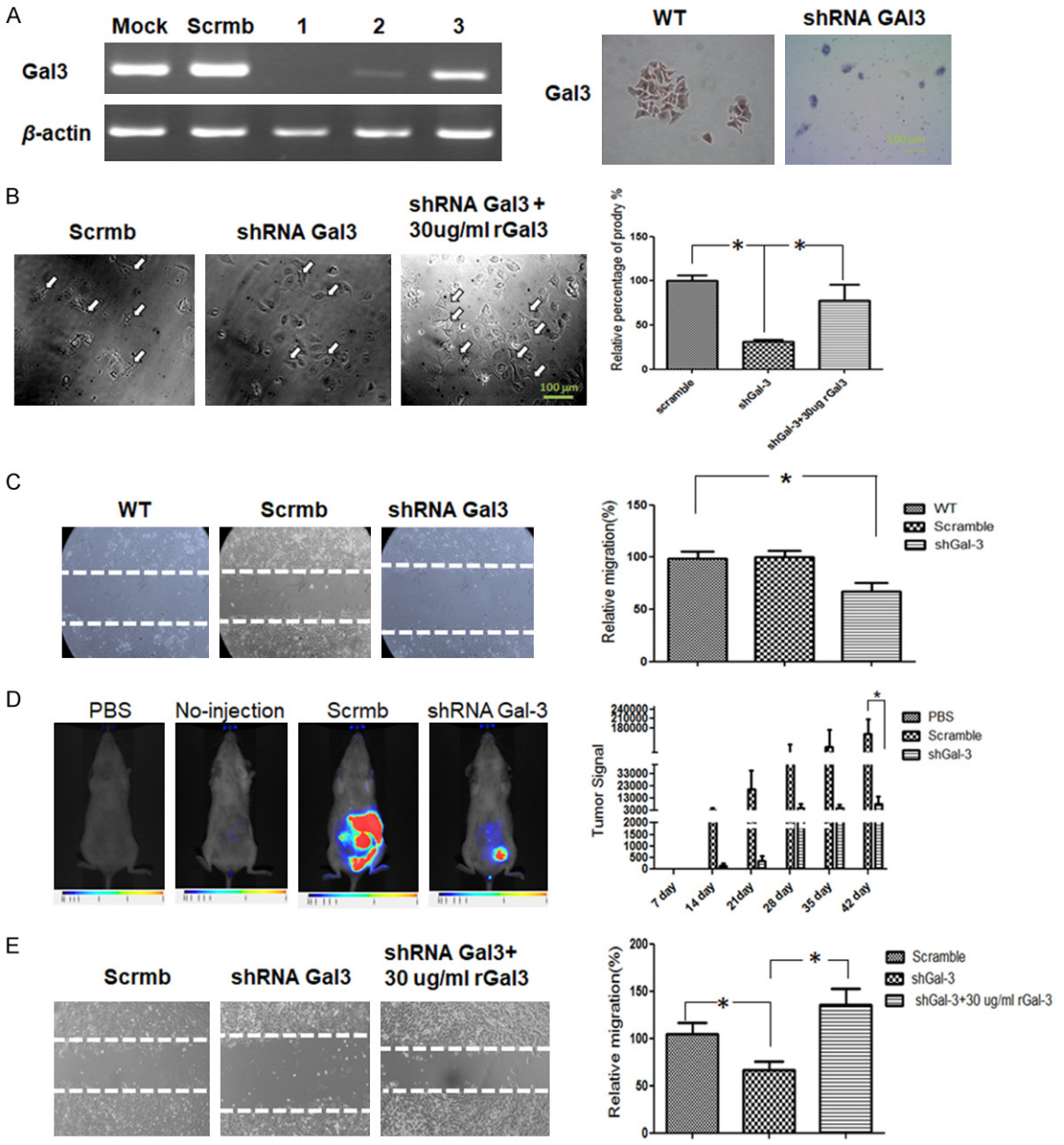

Figure 3. Extracellular recombinant galectin-3 rescues galectin-3 knockdown DLD-1 cell migration. (A) shRNA knockdown of galectin-3 shown by WB and ICC. (B) Stable knockdown of galectin-3 decreased the lamellipodia formation, migration rate (C) and tumor growth by iRFP (D). Recombinant galectin-3 restored the galectin-3 knockdown-induced decrease in lamellipodia formation (B) and cell migration (E). Bars represent the mean ± SD of three independent experiments. Statistical analysis was performed using the Student's t-test and the statistical significance is indicated by "*" which denote a *P* value of < 0.05.

absent, but the addition of extracellular recombinant galectin-3 induced the fluorescent resonance signal (pink color) of the proximity ligation between labeled galectin-3 and the EGFR antibodies (**Figure 5B**). The addition of recombinant galectin-3 increased EGFR phosphorylation within 10 minutes (**Figure 5C**). Moreover, we found that the EGFR antibody (cetuximab) significantly decreased DLD-1 cell migration (**Figure 5D**).

Recombinant-galectin-3 enhances EGFR internalization from the membrane to the cytoplasm

After showing that galectin-3 interacts with EGFR, we then studied the dynamic changes in

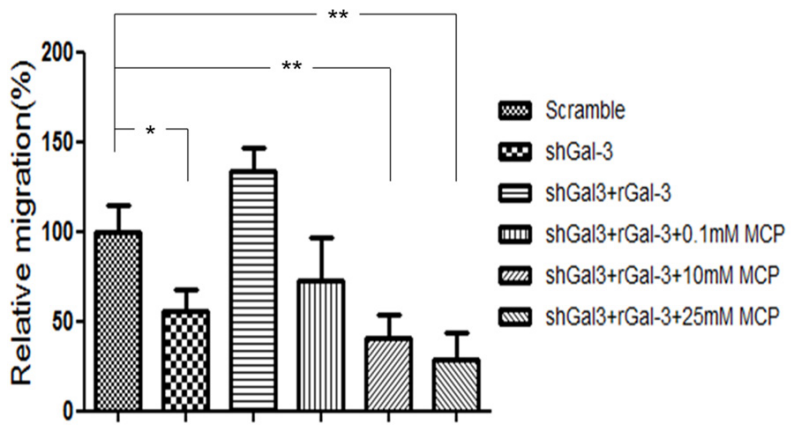

Figure 4. Inhibition of extracellular galectin-3 decreased colon cancer cell migration. Modified citrus pectin dose-dependently blocked galectin-3-induced DLD-1 cell migration. Bars represent the mean ± SD of three independent experiments. Statistical analysis was performed using the Student's t-test and the statistical significance is indicated by "*" and "**" which denote a *P* value of < 0.05 and < 0.01, respectively.

EGFR internalization in the presence of recombinant galectin-3. We found that recombinant galectin-3 enhanced EGFR internalization from the cell membrane to the cytoplasm, particularly upon EGF stimulation. The stable galectin-3 knockdown increased the membrane EGFR expression according to the western blot analysis, and the addition of recombinant galectin-3 to the galectin-3 knockdown DLD-1 cells decreased the membrane EGFR expression and increased the cytoplasmic EGFR expression (**Figure 6A**). The addition of recombinant galectin-3 to the galectin-3 knockdown DLD-1 cells decreased the membrane EGFR expression according to the flow cytometry analysis (**Figure 6B**).

Correlation between higher Gal-3 expression and augmented EGFR and CEA expression in advanced stages of colon cancer

To determine whether higher Gal-3 expression is associated with augmented EGFR and CEA expression in advanced stages of colon cancer, we collected 20 pairs of early and advanced stage clinical samples and performed immunohistochemical staining. As shown in **Figure 7**, we found that the tissues with the advanced stages of colon cancer had a higher Gal-3 expression than the tissues with the earlier stages of cancer or normal colonic epithelial cells. The higher Gal-3 expression in the advanced cancers was also associated with increased EGFR and CEA expression.

Discussion

Galectin-3 expression has been shown to increase the migration and/or invasion of many cancer cell types in vitro, including breast cancer, melanoma, lung cancer, pancreatic cancer and gastric cancer cells [4, 17, 18]. Endo et al reported that galectin-3 expression was a prognostic factor independent of Dukes' stage and lymph node metastasis in colon cancer patients [19], and other studies have shown that galectin-3 expression correlates with colon cancer progression and metastasis. Chen et al further showed that increased levels of circulating galectin-3 in cancer patients induced the secretion of several metastasis-promoting cytokines from the blood vascular endothelium that enhance endothelial cell activities in metastasis [20]. An increased galectin-3 expression was related to faster colon cancer cell migration [11]. According to the present study, colon cancer cell lines with higher extracellular galectin-3 secretion migrated faster, and the shRNA knockdown of galectin-3 decreased the galectin-3 secretion and colon cancer cell migration. We further showed that extracellular galectin-3 played an important role in colon cancer cell migration through its interaction with and dynamic changes to EGFR. Mayris et al showed that galectin-3 promoted cell migration during the re-epithelialization of corneal wounds and that endogenous galectin-3 regulated dendritic cell migration [21]. Our in vitro study further demonstrated that the addition of recombinant galectin-3 reversed galectin-3 knockdown cell migration. According to the immunoprecipitation and in situ proximity ligation assay, galectin-3 interacted with EGFR, induced EGFR phosphorylation and re-localized from the membrane to the cytoplasm. Lactose, but not the EGFR blocking antibody, inhibited the proximity ligation, suggesting that the interaction between EGFR and galectin-3 is mediated through sugar-lectin, but not protein, interactions. Altogether, our data suggest that extracellular galectin-3 binds EGFR and influences EGFR mem-

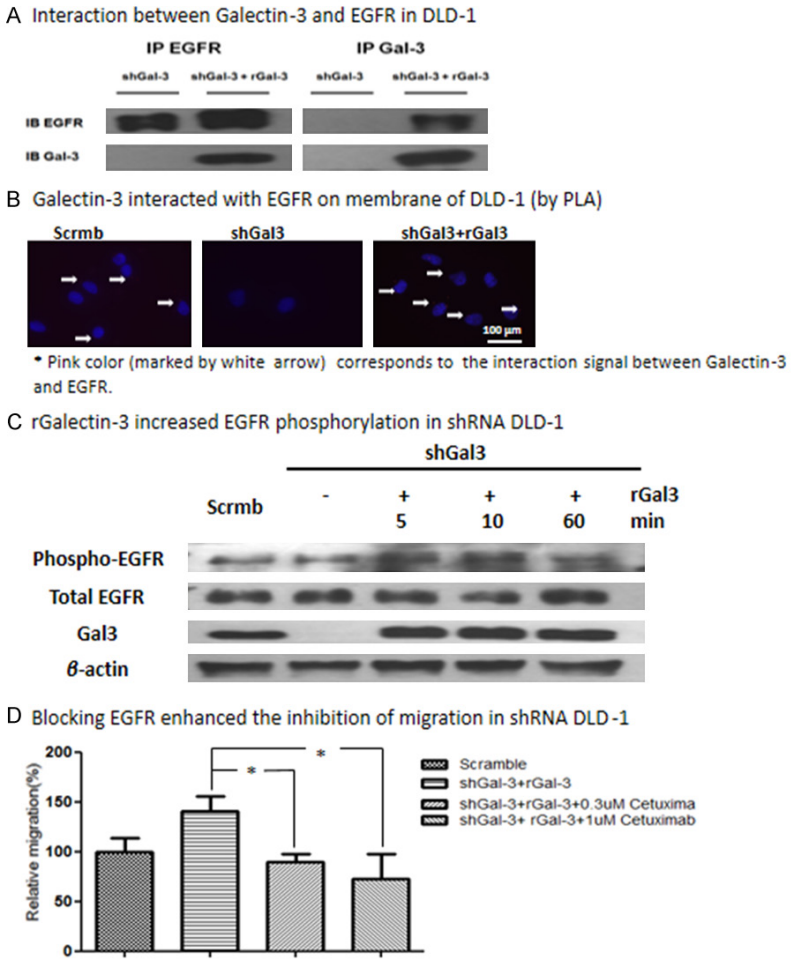

Figure 5. Galectin-3 interacts with EGFR via carbohydrate recognition and effects migration. A. Interaction between EGFR and galectin-3 was shown by IP, followed by IB. B. According to the in situ PLA, the addition of galectin-3 induced fluorescent resonance between EGFR and galectin-3, which was blocked by lactose but not the EGFR inhibitor. C. Addition of recombinant galectin-3 increased EGFR phosphorylation within 10 minutes. D. EGFR inhibitor significantly decreased DLD-1 cell migration. Statistical analysis was performed using the Student's t-test and the statistical significance is indicated by "*" which denote a *P* value of < 0.05.

brane dynamics to induce colon cancer cell migration.

The galectins lack a signal peptide that directs them to the classical secretory pathway; nevertheless, galectin-3 has been considered a secretory protein. Intracellular galectin-3 is secreted through the cell membrane to increase extracellular galectin-3 concentrations; thus, we used shRNA to stably knock down galectin-3 in colon cancer cells and decreased the extracellular galectin-3 secretion to block the influence of intracellular galectin-3 on colon cancer cell migration. We showed that extracellular galectin-3 enhanced colon cancer cell migration, which was specifically blocked by lactose and a neutralizing anti-galectin-3 antibody. We performed migration assays to evaluate colon cancer cell migration. To avoid the influence of proliferation on the cell migration assay, we used serum-free culture medium, added mitomycin C to inhibit proliferation and only recorded the migration distance within 24 hrs, which did not represent DLD-1 cell proliferation.

EGFR-mediated pathways are important in a variety of cellular processes, including cancer cell migration and invasion [22-24]. Galectin-3 and EGFR closely interacted on the cell membrane, and this interaction was associated with the dynamic changes in EGFR localization from the membrane to the cytoplasm. Gong et al showed a correlation between galectin-3 and EGFR by immunohistochemical staining in papillary thyroid carcinoma [25]. In addition to EGFR, extracellular galectin-3 can interact with multiple binding partners; in particular, polylactosamine-rich molecules in the extracellular matrix (ECM) or on the cell surface have been implicated in galectin's modulation of cancer metastasis. Partridge et al found that galectin-3 has a high affinity to β-1,6-N-acetylglucosamine branched glycans. Such interactions mediate lectin binding to many glycoproteins and glycolipids in the cell membrane, including carcinoembryonic antigen (CEA), mucin-1 and the glycosylated transmembrane tyrosine kinase receptors for epidermal growth factor [26, 27]. These data suggest that targeting galectin-3 may have multiple effects on the modulation of the cell-matrix and cell-cell interactions of cancer cells.

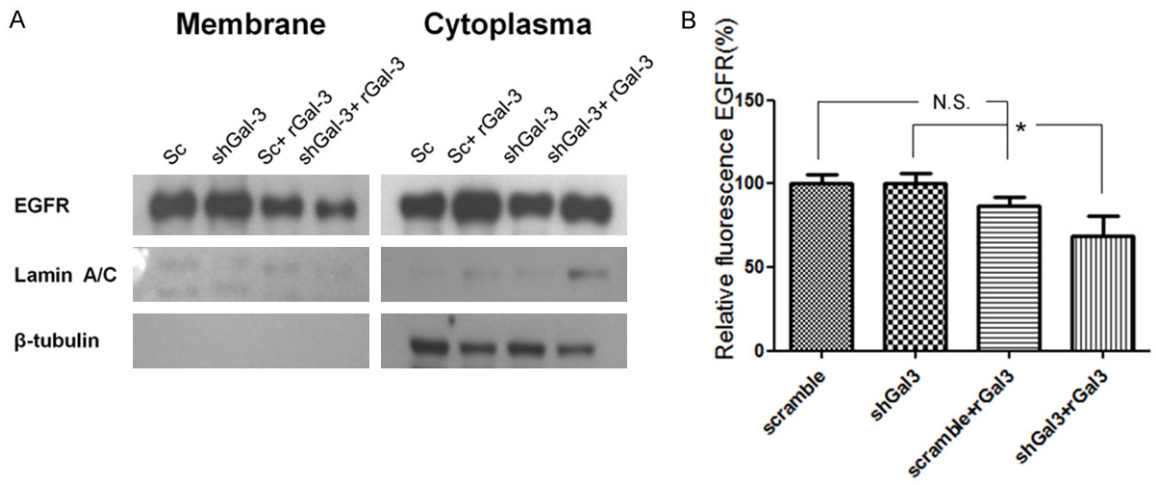

Figure 6. Recombinant-galectin-3 enhanced EGFR internalization. A. Recombinant galectin-3 promoted EGFR internalization from the membrane to the cytoplasm as shown by WB of the membrane and cytoplasm EGFR expression. B. Addition of galectin-3 to galectin-3 knockdown colorectal cancer cells decreased the membrane EGFR by flow cytometry. Bars represent the mean ± SD of three independent experiments. Statistical analysis was performed using the Student's t-test and the statistical significance is indicated by "*" which denote a *P* value of < 0.05. N.S.: not significant.

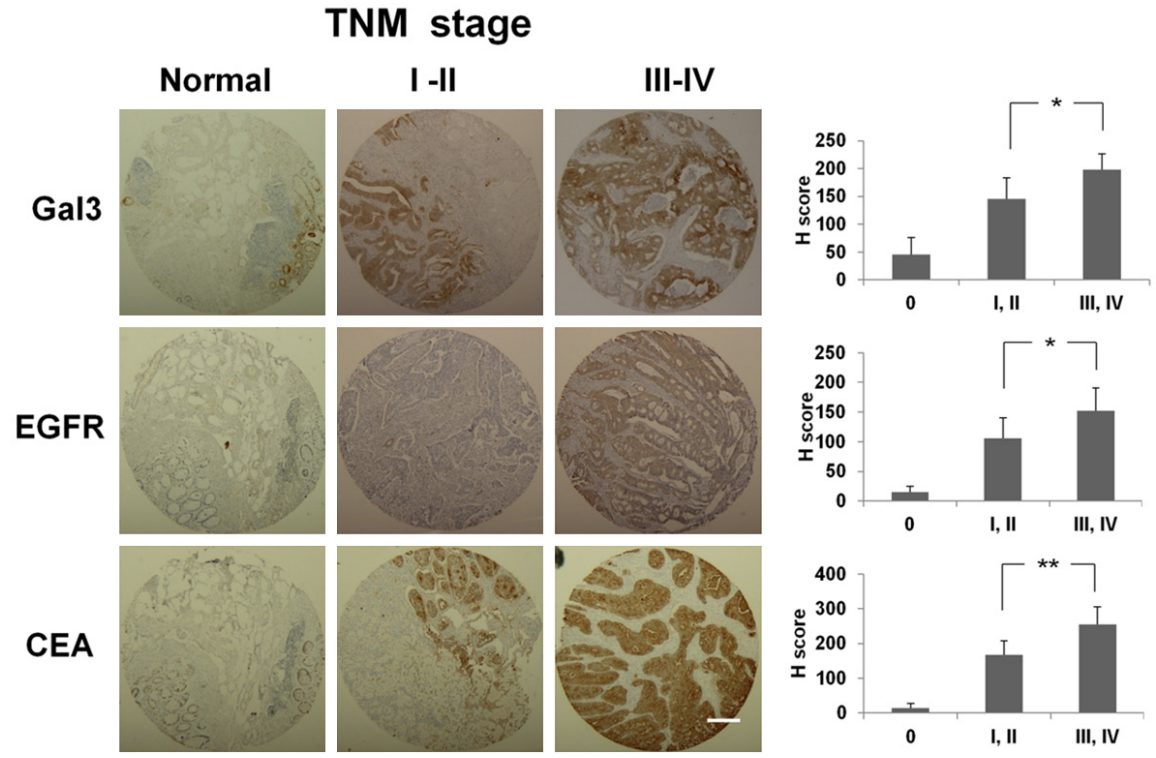

Figure 7. Correlation between higher Gal-3 expression and augmented EGFR and CEA expression in advanced stages of colon cancer. Advanced stages of colon cancer had higher Gal-3, EFGR, and CEA expression than earlier stage cancer or normal colonic tissue. White bar represents 100 μm. Statistical analysis was performed using one-way analysis of variance and subsequent Bonferroni post-hoc tests, the statistical significance is indicated by "*" and "**" which denote a *P* value of < 0.05 and < 0.01, respectively.

The lactose treatment and the neutralizing anti-galectin-3 antibody (B2C10) inhibited the galectin-3 enhancement of colon cancer cell migration. Extracellular galectin-3 oligomerizes to

form a pentamer through N-terminal connections, but B2C10 binds the N-terminus to inhibit pentamer formation and inhibit lattice formation. Moreover, we showed that extracellular galectin-3 binds to EGFR by proximity ligation of resonance fluorescence. These results suggest that galectin-3 recognizes EGFR through sugar-lectin interactions, and these interactions promote EGFR re-localization. The crosslink between galectin-3 and growth factor receptors, such as EGFR, through galectin-glycan clusters are termed lattices and have been implicated in modulating tumor cell behavior, such as adhesion, migration and metastasis [13, 14].

Our study has highlighted a promising potential to target galectin-3 through inhibitors or scavengers to interrupt lattice formation and inhibit tumor migration. Extracellular galectin-3 increased colon cancer cell migration, and the increased migration correlated with its interaction with EGFR and EGFR internalization from the cell membrane to the cytoplasm. Targeting galectin-3 could have a synergistic effect on EGFR targeted therapy.

Acknowledgements

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