Introduction of the integrin β1- but not the β3-subunit in GE11 cells induces an epithelial-to-mesenchymal-transition (EMT)-like phenomenon that is characterized by the loss of cell-cell contacts, cell scattering, increased cell migration and RhoA activity, and fibronectin fibrillogenesis. Because galactose-binding lectins (galectins) have been implicated in these phenomena, we investigated whether galectins are involved in the β1-induced phenotype. We examined 9 galectins and, intriguingly, found that the expression of galectin-3 (Gal-3) is specifically induced by β1 but not by β3. Using β1-β3 chimeric integrins, we show that the induction of Gal-3 expression requires the hypervariable region in the extracellular domain of β1, but not its cytoplasmic tail. Furthermore, Gal-3 expression does not depend on RhoA signaling, serum factors, or any of the major signal transduction pathways involving protein kinase C (PKC), p38 mitogen-activated protein kinase (p38MAPK), extracellular signal-regulated kinase-1/-2 (ERK-1/2), phosphatidylinositol-3-OH kinase (PI3-K), or Src kinases. Instead, Gal-3 expression is controlled in an epigenetic manner. Whereas DNA methylation of the Lgals3 promoter maintains Gal-3 silencing in GE11 cells, expression of β1 causes its demethylation, leading to transcriptional activation of the Lgals3 gene. In turn, Gal-3 expression enhances β1 integrin-mediated cell adhesion to fibronectin (FN) and laminin (LN), as well as cell migration. Gal-3 also promotes β1-mediated cell adhesion to LN and Collagen-1 (Col)-1 in cells that endogenously express Gal-3 and β1 integrins. In conclusion, we identify a functional feedback-loop between β1 integrins and Gal-3 that involves the epigenetic induction of Gal-3 expression during integrin-induced EMT and cell scattering.

Integrins are transmembrane receptors composed of an α- and a β-subunit that connect the extracellular matrix (ECM) to the cytoskeleton, thus integrating the cell interior with its environment. In this way, integrins regulate cell adhesion and cell spreading, as well as cell migration, proliferation, differentiation, and ECM remodeling (1, 2). Upon integrin-ligand binding, a variety of proteins associate with the cytoplasmic tail of the β-subunit, initiating cytoskeletal reorganization, assembly of macro-molecular adhesion complexes such as focal adhesions (FAs), and signaling through Rho GTPases and kinase-regulated pathways (3–6). However, individual integrins can trigger very distinct cellular responses, even if they bind the same ligand.

We have previously characterized the cellular phenotypes induced by the β1 versus the β3 subunit in the β1-deficient murine epithelial cell line GE11 (7, 8). GE11 cells grow in well-defined epithelial islands and express αvβ3 at relatively low levels. Introduction of the β1-subunit, which results primarily in cell-surface expression of the fibronectin (FN) receptor α5β1, causes dramatic morphological changes reminiscent of an epithelial-to-mesenchymal transition (EMT), including the loss of cell-cell contacts, cell scattering, and a contractile, fibroblast-like phenotype with high cytoskeletal tension, large peripheral FAs, and multiple protrusions. The β1-induced phenotype is associated with high RhoA activity, fast but random cell migration, FN fibrillogenesis, and the assembly of fibrillar adhesions distributed along FN fibrils (7, 8). In contrast, overexpression of the β3 subunit, leading to cell-surface expression...
of the alternative FN-receptor αvβ3, increases cell spreading but induces only a modest loss of cell-cell contacts and a pancake-like morphology with many small, randomly distributed FAs (8, 9). Moreover, β3 stimulates Rac but not Rho activity and promotes directional rather than random migration. GE11 cells therefore constitute a unique model to study differential effects of integrins on Rho GTPase activation, cell migration, and other integrin-regulated processes.

Several of the β1-induced phenomena are associated with the actions of a family of matricellular proteins, the β-galactoside-binding lectins (galectins). The galectins represents 15 highly conserved proteins that bind through a carbohydrate-recognition domain to N-glycosylated proteins at the cell surface and in the ECM, including integrins and several of their ligands such as laminin (LN) and FN (10–14). Galectins can thus modulate integrin-mediated events, and this has been most extensively documented for galectin-1 (Gal-1), galectin-3 (Gal-3), and galectin-8 (Gal-8). For instance, Gal-1 stimulates integrin-mediated adhesion and signaling in platelets and smooth muscle cells, and Gal-8 modulates cell adhesion and cell spreading in a number of cell types including neutrophils, fibroblasts, and several tumor cell lines (15–19).

The best-characterized family member, Gal-3, has an important role in a wide variety of physiological and pathological phenomena, which is at least in part mediated through its interactions with integrins. Gal-3 regulates the adhesion of epithelial cells to collagen and laminin, and promotes keratinocyte migration over LN-332 and wound re-epithelialization in mice (14, 20–23). Furthermore, Gal-3 stimulates neutrophil adhesion and migration, as well as eosinophil adhesion and rolling (24, 25). Gal-3 also regulates adhesion and spreading of a number of cancer cell lines (26–28). In addition, Gal-3 promotes FA turnover and α5β1-dependent assembly of fibrillar adhesions and FN fibrillogenesis in tumor cells (29, 30). Finally, Gal-3 increases the adhesion of disseminating cancer cells to endothelium, thereby protecting them from anoikis (12, 31).

In this study, we investigated whether galectins are involved in the β1-induced phenotype in GE11 cells. Intriguingly, we found that β1 but not β3 specifically triggers transcriptional activation of Gal-3, through a mechanism that involves the demethylation of the Lgals3 promoter. In turn, Gal-3 promotes β1-mediated cell adhesion and cell migration. Thus, we identify a functional feedback-loop between β1 integrins and Gal-3.

**EXPERIMENTAL PROCEDURES**

**Antibodies and Other Materials**—Antibodies used in this study were directed against actin (Millipore), Gal-1 (R&D systems), Gal-3 (Abcam), integrin β1 (TS2/16), and paxillin (BD Transduction Labs), RhoA (Santa Cruz), Dnmt3b (Abcam), and tubulin (Sigma). Texas Red-conjugated Phalloidin and DAPI were from Molecular Probes, FITC- or Texas Red-conjugated secondary antibodies were from Jackson ImmunoResearch Laboratories, decitabine (5-aza-deoxycytidine), FN, Y-27632, and PI-103 from Sigma, PI-103 was from Merck, and FITC-conjugated streptavidin was from Pierce Chemical Co. Collagen (Col)-1 was purchased from Vitrogen and LN-511 from BioLamina. Biotinylated-FN was prepared as described previously (8).

**Cells, Plasmids, and Transient Transfections**—The pcDNA3-GFP-Rhoa-Q63L construct was kindly provided by Dr. Sylvio Gutkind (NIH, Bethesda, MD). The β1–3 and β1–3–1 expression plasmids were a kind gift from Dr. Yoshikazu Takada (UC Davis, Sacramento, CA). GE11 cells are epithelial in origin and were obtained by injecting β1-null mouse embryonic stem cells into blastocysts, which were allowed to develop into whole chimeric embryos until day E10.5. Cells were then retrieved from these embryos, immortalized with SV-40, and selected with G418. Polarized cells which had formed small colonies were cloned (7). GE11 cells expressing human β1, human β3, or the chimeric human integrins β1–3 and β1–3–1 were generated by transfectingPhoenix packaging cells with retroviral constructs encoding the indicated integrins, to produce culture supernatants containing virus. GE11 cells were infected with the supernatants, selected with zeocin, and FACS-sorted for expression of the respective integrins at the cell surface (7, 8, 32). All GE-derived cell lines were cultured in DMEM supplemented with 10% FCS and 100 units/ml penicillin/streptomycin (Invitrogen). Mouse keratinocytes MKnA3 were isolated as described previously (33). Briefly, skin was obtained from newborn mice and epidermis and dermis were separated with trypsin. Keratinocytes were retrieved by gentle shaking and centrifugation, and incubation in keratinocyte serum-free medium (Invitrogen) supplemented with 50 μg/ml bovine pituitary extract, 5 ng/ml EGF, and penicillin/streptomycin. Spontaneously immortalized clones were obtained after several weeks. These were named MKnA3 and cultured routinely on Col-1 (3 μg/ml). All cells were maintained at 37 °C in a humidified atmosphere containing 5% CO2. Transient transfections were performed using the Amaxa nucleofector, according to the manufacturers’ instructions.

**Western Blotting**—Cells were washed with ice-cold PBS and lysed in protein loading buffer (62.5 mM Tris-HCl, pH 6.8, 20% glycerol, 2% SDS, 5% mercaptoethanol) at 4 °C. Proteins were separated on SDS-PAGE gels, transferred to polyvinylidene difluoride membranes (Millipore), and analyzed by Western blotting followed by ECL using the Super signal system (Pierce Chemical Co.).

**Q-PCR Analysis**—Q-PCR was performed using the iCycler (Bio-Rad) in a total volume of 25 μl containing 1.5 μl of cDNA, 12.5 μl of iQ SYBR Green Supermix (Bio-Rad), and 400 nM of both the forward and reverse primer. The primers were designed and validated according to a previously described protocol (34), and included primers for Gal-1 (5′-TGTGTGTAACCAAGGAAGAT-3′ forward and 3′-ACCTCTGTGATGCTC-CCG-5′ reverse), Gal-2 (5′-CAATGATTGTGCCCGCTTC-3′ forward and 3′-ACAGACAATGGTGAGTCAT-CC-5′ reverse), Gal-3 (5′-CAGAGTGTTCAGTATGTTCCG-3′ forward and 3′-TGTCTTGCTGTTGTTACAC-5′ reverse, or alternatively 5′-GGCCTACCCCCATGCTCCT-3′ forward and 3′-GGTC-CATAGGGCACCCTGA-5′ reverse), Gal-4 (5′-GAAGAGAGGAAGTTGCGCTTAC-3′ forward and 3′-CATTGGAATGCT-TGGACC-5′ reverse), Gal-5 (5′-ACACCAAGGAAGCGCCCA-3′ forward and 3′-TGGAGTGGAGATATCCTGTA-5′ reverse), Gal-8 (5′-CTGAGGCTGATCATTGAAA-3′ forward and 3′-GATGGGTTGGCTGAAGA-5′ reverse).
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and 3′-AAGCCTCGGGCATTTG-5′ reverse), Gal-9 (5′-CTTTCTAACCCTCCATCCA-3′ forward and 3′-GATA-TGGAGCNCCGTGATCT-5′ reverse), Gal-10 (5′-CTGAG-TGACTTGGTCTAGGTTG-3′ forward and 3′-AGATGCTCAGATTAAATGGTGTTT-5′ reverse), Gal-12 (5′-CCTGAG-GCTGAGAGATG-3′ forward and 3′-GGAGATACA-GCTTCTTTGT-5′ reverse). As a negative control, the cDNA was replaced by milliQ. Cyclophilin A, CAT, CCTTTGAGAGTGGCAAACCAT, CCCAACGCAAA-CATGATTGTT, cloned into pLKO.1, were obtained from the manufacturers’ instructions. Viral supernatant was harvested 48 h later, added to HEK 293FT cells together with the VirapowerTM Packaging Kit, derived from independent experiments, were analyzed. Each sample was analyzed in triplicate, and graphs represent the average of three independent experiments.

Scratch Assays—Cells in 24-well plates were grown to confluence and subsequently serum-starved overnight. Proliferation was inhibited with 10 μg/ml mitomycin C (Nycomed Inc.) 2 h before scratching the monolayers with a yellow pipette tip. Cells were then washed twice in serum-free medium to remove cell debris, stimulated with fresh medium, and incubated at 37°C. Scratched areas were photographed overnight at 10× magnification. To determine the relative migration, the area that was not covered by cells at the end of the assay (end) was calculated over the open area at the beginning of the assay (t0). The resulting ratio in untreated cells was set to 1. Experiments were performed three times in triplicate.

RhoA Assay—Cells were transfected with control or Gal-3 siRNAs, plated in tissue-culture plates, and RhoA activity was assessed 24 h post-transfection using the Rho G-LISA kit (Cytoskeleton), according to the manufacturers’ instructions.

Fibrillogenesis Assay—Cells on coverslips were grown to confluence and then incubated overnight in DMEM containing FN-depleted serum, supplemented with 10 μg/ml biotinylated FN. Thereafter, they were processed for immunofluorescence as described above. FN was visualized with FITC-conjugated streptavidin and nuclei were stained with DAPI. Several fields were photographed, and total fluorescence for each field was measured using ImageJ software and divided by the number of cells. Graphs represent the averages of three independent experiments.

Analysis of Promoter Methylation—For methylation analysis of the Lgals3 promoter, genomic DNA was isolated using the nucleospin tissue DNA isolation kit (Machery Nagel). The purified DNA was bisulfite-converted using the Epitect Bisulfite Kit (Qiagen) according to the manufacturers’ instructions. Next, PCR for regions of the Lgals3 promoter was performed using the following mouse- and bisulfite-specific primers: 5′-CTTTAGTTTATTTTGAAACTT-AA- (forward); 5′-AAAACCCCAAGACCCCTCTTATACACC-3′ (reverse), amplifying a 268-bp product which contained 21 CpGs located adjacent to the start of exon 1. PCR products were isolated from gel using a gel extraction kit (Qiagen), cloned into the pCRII-topo TA cloning mix (Invitrogen), using Lipofectamine 2000 according to the manufacturers’ instructions. Viral supernatant was harvested 48 h later, added to GEβ1 or MKo3 cells, and positive cells were selected with puromycin.

Adhesion Assays—96-well plates were coated at 37 °C with 1 μg/ml Col-1 (5 min), 1 μg/ml FN (1 h), or 8 μg/ml LN-511 (overnight). The plates were washed twice with PBS, blocked in 2% BSA for 1 h at 37 °C, and washed twice with PBS. Cells were seeded at a density of 3 × 10^4 – 1 × 10^5 per well and allowed to adhere at 37 °C for the indicated timepoints. Non-adherent cells were washed away with PBS before the addition of substrate buffer (7.5 mm p-nitrophenyl N-acetyl-β-d-glucosaminide (NPAG) in 0.1 m sodium citrate pH 5, 0.5% Triton X-100).

Plates were then incubated overnight at 37 °C, after which stop buffer (50 mm glycine, pH 10.4, 5 mm EDTA) was added, and the absorbance was measured at 405 nm on a Bio-Rad microplate reader. Alternatively, cells were processed as described previously (33). Each condition was analyzed in triplicate, and graphs represent the average of three independent experiments.

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Plates were then incubated overnight at 37 °C, after which stop buffer (50 mm glycine, pH 10.4, 5 mm EDTA) was added, and the absorbance was measured at 405 nm on a Bio-Rad microplate reader. Alternatively, cells were processed as described previously (33). Each condition was analyzed in triplicate, and graphs represent the average of three independent experiments.
amount of input DNA and analyzed on gel. All experiments were performed in triplicate.

**Analysis of Global Genomic Methylation by HPLC**—To assess global genomic methylation levels, the ratio of 5-methyl-deoxycytosine (5-Me-dC) over total deoxycytosine (dC) was determined using HPLC (36). Cell lysates were depleted of RNA with 1 mg/ml RNase A (Sigma) for 5 min, after which DNA was extracted as described above. Next, 1 μg of DNA was digested to single nucleotides using a combination of DNase I (Sigma), Nuclease P1 (Sigma) and alkaline phosphatase (New England Biolabs), and dC and 5-Me-dC content were quantified with an HPLC-UV system (Shimadzu) equipped with a 125 × 4 mm Nucleosil 100-10 SA column (Macherey-Nagel). The mobile phase consisted of 40 mM acetic acid in 15% acetonitrile (pH 4.8), and the flow rate was 0.6 ml/min. Global 5-Me-dC content was expressed as a percentage of the total dC content. Six replicates were analyzed per condition.

**RESULTS**

**Gal-3 Expression Is Induced by the Integrin β1, but Not the β3 Subunit**—To determine the expression of galectin family members in GE11 cells and GE11 cells stably expressing the β1-subunit (GEβ1), we performed a quantitative-PCR (Q-PCR) analysis of 9 galectins. The only galectin that was expressed at a detectable level in GE11 cells was Gal-1, and its expression was increased 4-fold in GEβ1 cells. Intriguingly, Gal-3 was hardly expressed in GE11 cells but its expression increased dramatically (28-fold) in GEβ1 cells (Fig. 1A). Next, we determined protein levels of Gal-1 and Gal-3 by Western blotting. To establish if the regulation of these galectins was specific for the β1 subunit, Gal-1 and Gal-3 expression was also analyzed in GE11 cells that overexpress β3 (GEβ3), which leads to elevated cell-surface expression of αvβ3 (8). Whereas Gal-1 protein was detected in GE11 cells and its expression was enhanced in both GEβ1 and GEβ3 cells, high expression of Gal-3 protein was only detected in GEβ1 but not in GE11 or GEβ3 cells (Fig. 1B). Thus, whereas both β1 and β3 enhance Gal-1 expression, high Gal-3 expression is induced by β1 but not by β3.

**The Hypervariable Region of the I-like Domain of β1 Is Required for Gal-3 Expression**—Because β1 but not β3 induces Gal-3 expression, and these integrin subunits trigger very different morphological responses, we investigated whether the induction of Gal-3 expression is correlated with cell morphology. Furthermore, we addressed the importance of the β1-cytoplasmic tail in the regulation of Gal-3 expression. We therefore used the β1–3 chimeric integrin, which consists of the extracellular and transmembrane regions of β1, fused to the cytoplasmic domain of β3 (Fig. 2A). Expression of this chimera in GE11 cells induces a phenotype similar to that induced by wild-type β1, including the loss of cell-cell contacts, cell scattering, and a fibroblast-like morphology with multiple protrusions and peripheral FAs (Fig. 2A; Ref. 8). Thus, the extracellular but not the cytoplasmic domain of β1 is required for these effects, and the extracellular domain of β3 cannot substitute for it. The extracellular domains of integrin β-subunits vary widely in a region contained within the I-like domain, called the hypervariable region. This region affects ligand specificity and regulates the activity of Rho GT-Pases (37, 38). Replacing the hypervariable region in β1 with that of β3 (creating β1–3-1) prevents RhoA-mediated contractility and FN fibrillogenesis, and induces a GEβ3-like morphology (Fig. 2A; Ref. 32). Intriguingly, we detected high levels of Gal-3 expression in GEβ1 and GEβ1–3 cells, but not in GEβ3 or GEβ1–3-1 cells. In contrast, the level of Gal-1 expression was similar in GEβ1, GEβ1–3, and GEβ1–3-1 cells and was only marginally lower in GEβ3 cells (Fig. 2B). Thus, these findings show that Gal-3 expression is specifically correlated with the contractile, mesenchymal-like phenotype, and does not depend on the cytoplasmic tail of β1. Rather, the hypervariable region in the extracellular domain of

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**Epigenetic Regulation of Galectin-3 by β1 Integrins**

![Diagram](image)

**FIGURE 2.** Gal-3 expression correlates with mesenchymal morphology and depends on the hypervariable region in the I-like domain of β1. A, top: schematic presentation of the β1- and the β3-subunit, as well as the β1–3 and β1–3-1 swap mutants. Indicated are the extracellular (ex), transmembrane (TM), and intracellular (in) domains, as well as the I-like domain. The box represents the amino acid sequence of the hypervariable regions of β1 and β3. Bottom: GEβ1, GEβ3, GEβ1–3, and GEβ1–3-1 cells were cultured on coverslips and processed for immunofluorescence. FAs were visualized using an antibody against paxillin (green), and the actin cytoskeleton was stained with phalloidin (red). Scalebar, 10 μm. B, Gal-1 and Gal-3 expression in GEβ1, GEβ3, GEβ1–3, and GEβ1–3-1 cells was investigated by Western blotting.
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β1 is required for both the β1-induced cell morphology, and the induction of Gal-3 expression.

β1-induced Gal-3 Expression Is Not Regulated by RhoA Signaling or Kinase-regulated Signal Transduction Pathways—As the hypervariable region of β1 is required for both Gal-3 expression and RhoA activity, we reasoned that RhoA signaling might regulate Gal-3 expression downstream of β1. A major effector of RhoA is Rho kinase (ROCK). To test if ROCK is involved in Gal-3 expression, GEβ1 cells were incubated for 48 h with the ROCK-inhibitor Y-27632. This treatment efficiently inhibited ROCK activity, as judged by the dissolution of actin stress fibers and the induction of long, thin protrusions (supplemental Fig. S1A). However, Gal-3 expression was not affected by Y-27632 at any concentration tested (supplemental Fig. S1B). To assess whether RhoA regulates Gal-3 expression through another pathway, we depleted RhoA using RNAi. Whereas RhoA was completely absent 48 h after transfection, Gal-3 levels remained unchanged (supplemental Fig. S1C). We also investigated if active RhoA can induce Gal-3 expression independently of β1, by introducing a constitutively active RhoA mutant fused to GFP (GFP-RhoA-Q63L; Ref. 39) into GEβ3 cells. Transfected cells were FACS-sorted for GFP and subsequently grown overnight, after which Gal-3 protein levels were determined by Western blotting. Expression of GFP-RhoA-Q63L did not induce a GEβ1-like phenotype in GEβ3 cells, indicating that the activation of Rho signaling alone is not sufficient for the induction of EMT-like changes, and that β1 is absolutely required for this effect (data not shown). In line with the previous results, expression of GFP-RhoA-Q63L did not induce Gal-3 expression in the absence of β1 (supplemental Fig. S1D). Taken together, these results suggest that RhoA signaling is not involved in the induction of Gal-3 expression by β1.

Besides Rho GTPases, β1-integrins activate a variety of signal transduction pathways (4, 5). In an attempt to identify a β1-induced signaling pathway that regulates Gal-3 expression, we treated GEβ1 cells with standard concentrations of a variety of compounds to inhibit signal transduction pathways that are commonly activated by β1-integrins, including PD98059 and UO126 to inhibit the extracellular signal-regulated kinase-1/2 (ERK-1/2) cascade, SB203580 to inhibit p38 mitogen-activated protein kinase (p38MAPK), PI-103, and LY-294002 to inhibit phosphatidylinositol-3-OH kinase (PI3-K), PP1 to inhibit the Fyn/Src/Yes family of kinases, and G60983 to inhibit all isoforms of protein kinase-C (PKC). In addition, we used the broad-spectrum tyrosine kinase inhibitor genistein, and the broad-spectrum serine/threonine kinase inhibitor staurosporine. None of the used compounds prevented Gal-3 expression in GEβ1 cells although genistein did reduce Gal-3 expression to about 70% of control levels, which is possibly due to cytotoxic side-effects (supplemental Fig. S2A). Notably, whereas some inhibitors seemed to enhance Gal-3 expression in GEβ1 cells, they failed to do so in GE11 cells, further underlining that expression of Gal-3 cannot be activated in the absence of β1. We then analyzed whether Gal-3 expression in GEβ1 cells depends on soluble factors that can trigger signaling pathways at the cell-surface, by culturing GE11 and GEβ1 cells in the absence or the presence of serum for 24 to 48 h. Gal-3 expression was only slightly elevated by serum but was not prevented in its absence, suggesting that although serum-components may potentiate β1-induced Gal-3 expression, they are not absolutely required for it (supplemental Fig. S2B).

β1 Integrins Induce Gal-3 Expression by Demethylation of the Lgals3 Promoter—The results reported in the previous sections indicate that Gal-3 expression in GEβ1 cells is not maintained by RhoA signaling, serum factors, or kinase-regulated signaling. In addition, the β1-cytoplasmic tail, which contains docking sites for numerous signaling proteins, is dispensable for Gal-3 expression. It therefore seems unlikely that Gal-3 expression in GEβ1 cells depends on a classic integrin signaling event. The expression of Gal-3 and other galectins is often silenced epigenetically by DNA methylation, which can be reversed to induce de novo expression, for example during tumor progression (40–42). We therefore investigated the DNA methylation status of the promoter region of Lgals3, the gene encoding Gal-3, by bisulfite sequencing. The murine Lgals3 gene contains a CpG island in the promoter region, which overlaps the start of exon 1 (Fig. 3A). Following bisulfite-conversion of genomic DNA, a 268 bp Lgals3 promoter fragment containing 21 CpG sites was amplified using a bisulfite treatment-specific primer set (Fig. 3A). The purified products were cloned into a TOPO vector, and sequencing of multiple individual clones (n > 9) revealed that virtually all the CpG sites were methylated in GE11 and GEβ3 cells, corresponding with the lack of Gal-3 expression (Fig. 3B). In contrast, the Lgals3 promoter was predominantly demethylated in GEβ1 cells (Fig. 3B).

We subsequently employed an alternative strategy to confirm the differences in methylation status of the Lgals3 promoter, making use of the selective digestive properties of the HpaII/MspI restriction enzyme pair. Whereas both enzymes recognize the CCGG sequence, digestion by HpaII but not MspI is prevented by DNA methylation. The Lgals3 promoter contains 3 HpaII/MspI sites. When fully methylated, digestion of genomic DNA with HpaII, but not MspI, should generate a PCR fragment of 466 bp with primers overlapping the restriction sites (Fig. 3C). Therefore, genomic DNA was treated with HpaII or MspI, and the 466 bp fragment was amplified by PCR and analyzed on gel. The expected fragment was generated from HpaII-digested genomic DNA isolated from GE11 and GEβ3, but not from GEβ1 cells (Fig. 3C). In contrast, digestion of genomic DNA with MspI never resulted in an amplified product, thus confirming that the Lgals3 promoter is indeed methylated in GE11 and GEβ3 cells, but not in GEβ1. These findings strongly suggest that the induction of Gal-3 expression in GEβ1 cells is caused by demethylation of the Lgals3 promoter. DNA methylation is maintained by DNA methyltransferases (Dnmts). To investigate whether the demethylation of the Lgals3 promoter in GEβ1 cells was the result of decreased overall expression or decreased activity of Dnmts, we tested Dnmt expression by Western blotting. Whereas Dnmt1 and Dnmt3a were not detected, the expression of Dnmt3b was not decreased in GEβ1 cells as compared with GE11 cells and perhaps even slightly elevated (Fig. 3D). As a readout for general Dnmt activity, we then tested the overall content of methylated cytosines in GEβ1 cells by HPLC (36). The ratio of methylated/unmethylated cytosines was not decreased in GEβ1 cells, suggesting that demethylation of the Lgals3 promoter is a specific
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FIGURE 3. Transcriptional activation of Gal-3 by β1-integrins is caused by demethylation of the Lgals3 promoter. A, relative distribution of 21 CpG sites located in the promoter region of the Lgals3 gene. Shown is the region from −164 bp upstream to +125 bp downstream of the start of exon 1. Arrows indicate start positions of amplification. B, methylation status of CpG sites in the Lgals3 promoter was determined in GE11, GEβ1, and GEβ3 cells by bisulfite modification followed by methylation-specific PCR and sequencing. Depicted is the representative methylation status of 21 CpG sites for each cell line, determined from at least 9 individual clones. Closed circles represent methylated CpG sites, whereas open circles represent unmethylated CpG sites. The start of exon 1 is indicated by (0). C, top: schematic representation of the Lgals3 promoter, showing the location of 3 HpaII/MspI sites. Bottom: genomic DNA of GE11, GEβ1, and GEβ3 cells was treated with HpaII or MspI, subjected to PCR, and reaction products were analyzed on gel. Shown is a representative experiment (n = 3). D, expression of Dnmt3b in GE11 and GEβ1 cells was investigated by Western blotting (left). Content of 5-Me-dC, expressed as a percentage of total dC content, in GE11 and GEβ1 cells was determined by HPLC (right). E, GE11 cells were incubated for 48 h with 1 μM decitabine, and the methylation status of the Lgals3 promoter was investigated using digestion with HpaII or MspI, followed by PCR. F, GE11 cells were incubated for 48 h with the indicated concentrations of decitabine, and expression of Gal-3 was investigated by Q-PCR (left) and Western blotting (right). G, GEβ3 cells were incubated for 48 h with the indicated concentrations of decitabine, and expression of Gal-3 was investigated by Q-PCR (left; expressed relative to GE11 without decitabine) and Western blotting (right).

event, and is not the result of decreased overall Dnmt activity (Fig. 3D). To confirm that DNA methylation is the cause of Gal-3 silencing in GE11 cells, we incubated GE11 cells for 48 h with the Dnmt inhibitor decitabine, and performed digestion of genomic DNA with HpaII/MspI as described above. The 466 bp fragment was hardly amplified from GE11 cells after treatment with decitabine, indicating that the Lgals3 promoter was demethylated by this treatment (Fig. 3E). In addition, decitabine caused mRNA and protein expression of Gal-3 in GE11 cells in a dose-dependent manner, confirming that demethylation of the Lgals3 promoter leads to transcriptional activation of the Lgals3 gene (Fig. 3F). Finally, decitabine treatment also increased Gal-3 mRNA and protein in GEβ3 cells (Fig. 3G). These findings unambiguously demonstrate that 1) DNA methylation maintains Gal-3 silencing in GE11 and GEβ3 cells, and 2) demethylation is sufficient for activating Gal-3 transcription, even in the absence of β1.

Gal-3 Promotes β1 Integrin-mediated Cell Adhesion—We next investigated whether Gal-3 contributes to β1-mediated adhesion of GEβ1 cells. The β1-integrin repertoire in GEβ1 cells consists primarily of the FN-binding integrin α5β1, whereas FN-binding αvβ1, as well as the LN-binding integrins αβ1 and α6β1 are also expressed at low levels (7, 8). We first tested whether adhesion to FN was affected by the knockdown of Gal-3 expression with siRNA. Because β1 also enhanced Gal-1 levels, Gal-1 was depleted in parallel. Both siRNAs caused a strong and specific reduction of their respective targets within 24 h after transfection, while a scrambled siRNA-control had no effect (Fig. 4A). Interestingly, cell adhesion to FN was reduced to 50% of that of control cells by Gal-3 depletion, but was not affected by the loss of Gal-1 (Fig. 4, B and C). Thus, Gal-3 but not Gal-1 contributes to adhesion of GEβ1 cells to FN. Similar results were obtained using stable cell lines expressing shRNAs directed against Gal-3, and subsequently selected with puromycin (data not shown). Because the small amounts of α3β1 and α6β1 on GEβ1 cells allow weak adhesion to laminins, we next tested whether Gal-3 also promotes cell adhesion to LN-511, a ligand for both α3β1 and α6β1. In line with the results obtained on FN, Gal-3 depletion reduced cell adhesion to LN-511, albeit to a lesser extent (Fig. 4D). Thus, Gal-3 promotes β1-integrin-mediated cell adhesion to FN and LN-511 in GEβ1 cells.

We then determined whether Gal-3 affects β1-mediated adhesion in epithelial cells that endogenously express Gal-3 and β1-integrins. For this purpose we used a keratinocyte cell line designated MKα3, which we have described previously (33). MKα3 cells are non-transformed keratinocytes isolated from mouse epidermis, and their predominant β1-integrins are α2β1 and α3β1, whereas α5β1 is not expressed (33). Knockdown of Gal-3 expression in MKα3 cells reduced α2β1-medi-
ated cell adhesion to Col-1 (Fig. 4E). In addition, MKα3 adhe-
sion to LN-511, which is primarily mediated by α3β1, was also
decreased though less prominently than on Col-1 (Fig. 4F).
Together, these results show that Gal-3 promotes β1-integrin-
mediated cell adhesion to FN and LN-511 in GEβ1 cells, and to
Col-1 and LN-511 in MKα3 cells.

β1-induced Gal-3 Expression Promotes Cell Migration, but
Not FN Fibrillogenesis or RhoA-mediated Contractility—Next,
we investigated whether Gal-3 is involved in other β1-induced
phenomena in GEβ1 cells such as cell migration, FN fibrillo-
genesis, and RhoA activity. To study cell migration, GEβ1 cells
were transfected with control or Gal-3 siRNAs and grown to
confluence, and 24 h after transfection the monolayers were
scratched with a pipette tip. Migration into the wound was then
monitored by time-lapse microscopy, and migration speed was
calculated from the movies. The knockdown of Gal-3 expres-
sion diminished migration speed by 30%, compared with that of
cells transfected with control siRNAs (Fig. 5A). Thus, in addi-
tion to cell adhesion, Gal-3 expression in GEβ1 cells also pro-
motes cell migration. We then investigated FN fibrillogenesis in
cell monolayers 24 h after siRNA transfection by confocal
microscopy. Whereas FN fibrils in GE11 cells were not
detected, GEβ1 cells transfected with control or Gal-3 siRNAs
both demonstrated abundant FN fibrillogenesis (Fig. 5B), and
quantification revealed that the extent of fibril formation was
not significantly decreased upon Gal-3 depletion (Fig. 5C). Fur-
thermore, Gal-3 knockdown in GEβ1 cells did not affect RhoA
activity, as determined by a RhoA G-LISA (Fig. 5D). Consistent
with this finding, Gal-3 knockdown did also not disrupt the
RhoA-mediated contractile phenotype of GEβ1 cells, charac-
terized by stress fibers and FAs located in the periphery (Fig.
5E). Summarizing, these data indicate that Gal-3 expression in
GEβ1 cells promotes β1-mediated cell adhesion and migration,
but not RhoA-mediated contractility or FN fibrillogenesis.

DISCUSSION

In this study, we have identified a functional synergistic loop
between β1-integrins and Gal-3, i.e. the epigenetic activation of
Gal-3 expression during β1-induced EMT, which enhances
β1-mediated cell adhesion and migration.

A number of reports demonstrate that galectins can regulate
integrin-mediated cell adhesion. However, the exact mecha-
nism underlying this phenomenon is poorly understood and
galectins seem to have both promoting and inhibiting effects.
For example, whereas overexpression of Gal-3 promotes adhe-
sion and invasion of tumor cells, their adhesion is inhibited by
addition of exogenous Gal-3 (27, 28). Similarly, soluble Gal-8
has been shown to inhibit cell adhesion, whereas Gal-8-coated
surfaces support cell adhesion, cell spreading, and integrin sig-
naling in the same cells (15, 16). It is conceivable that soluble
galectins perform different functions than endogenously
expressed galectins that are associated with membrane pro-
Epigenetic Regulation of Galectin-3 by β1 Integrins

Galectin-3 is a β-galactoside binding lectin that regulates cell behavior by interacting with a variety of ligands, including integrins, glycoproteins, and other glycoconjugates. Galectin-3 can affect cell migration, adhesion, and proliferation by modulating integrin function and signaling pathways.

We find that Gal-3 promotes β1-mediated cell adhesion to FN and LN in GEβ1 cells, and to Col-1 and LN in mouse keratinocytes. Earlier reports have documented that Gal-3 regulates surface expression of αβ1 and α4β7 on cancer cells (26, 28, 31), but we did not detect differences in β1 expression by flow cytometry upon knockdown of Gal-3 expression (data not shown). Moreover, we found that FN fibrillogenesis was not affected upon Gal-3 depletion, while a previous study has shown that soluble Gal-3 can promote this process in mammary tumor cells (30). The discrepancy between the results of the latter study and ours probably reflects differences in the used cell system, as well as differences in the effects of soluble Gal-3 versus endogenous Gal-3.

It is clear that the effects of galectins on integrin function are complex and depend on the integrin repertoire, the cell type, and the particular galectin involved. Accumulating evidence suggests that the pattern of glycosylation is of key importance to the interactions of galectins with their ligands, and thus to the effects of galectins on cell behavior. Galectins bind to N-acetyllactosamine (typically Galβ1,4GlcNAc), which is a recurring sequence in branched N-glycans, and the affinity of galectins increases with the number of branches. Affinity is also regulated by adjacent saccharides, as different galectins show specificity for different oligosaccharides (11, 43). Many galectins exist as dimers or oligomers, and Gal-3 can form up to pentamers, which are able to cross-link multiple ligands at the cell surface to form lattices of glycoproteins (44, 45). In this way, galectins can cluster integrins but they can also form heterogeneous lattices consisting of integrins, non-integrin proteins that regulate adhesion such as MUC1, and other glycoproteins including cytokine- and growth factor receptors (46, 47). Thus, it is increasingly recognized that the effects of galectins can only be understood completely with knowledge of the molecular composition of the glycoproteome at the cell surface, and its pattern and degree of glycosylation (43).

Irrespective of the mechanism by which Gal-3 enhances β1 function, our data reveal a new aspect of galectin-integrin biology, as we document the transcriptional activation of Lgals3 by an integrin, which clearly has significance for integrin function. Gal-3 was specifically induced by β1, whereas neither endogenous αvβ3 in GE11 cells, nor overexpression of the β3-subunit in GEβ3 cells was able to induce Gal-3 expression. Moreover, introduction of the β4-subunit in GE11 also failed to induce Gal-3 (data not shown). Neither of these integrins supports the EMT-like phenotypic changes induced by β1 integrins, and the correlation between this phenotype and Gal-3 expression is further strengthened by our observations using chimeric integrins. Surprisingly, the induction of Gal-3 expression was independent of the β1 cytoplasmic tail, RhoA signaling, or common integrin-induced signal transduction pathways. Rather, its expression in GEβ1 cells is regulated in an epigenetic fashion by the demethylation of DNA sequences around the transcription-initiation site of the Lgals3 gene, which are methylated in GE11 and GEβ3. Demethylation of the Lgals3 promoter is a specific
Epigenetic Regulation of Galectin-3 by β1 Integrins

event, and is not the result of a loss or reduction of global methylation in GEβ1 cells. Our data emphasize the importance of DNA methylation in the regulation of the expression of various galectins, in line with previous reports (40–42).

In the light of our data, two previous studies are particularly interesting. First, it has been shown that the cell-surface glycoprotein MUC1 controls Gal-3 expression in an epigenetic manner in cancer cells, be it through a miRNA-dependent mechanism rather than DNA-methylation. In turn, Gal-3 binds MUC1 at the cell surface and bridges it with the epidermal growth factor receptor, thereby establishing a functional feedback-loop (47).

Second, integrin growth factor receptor, thereby establishing a functional feedback-loop that involves the induction of the expression of accessory proteins via epigenetic mechanisms rather than through a conventional signal transduction cascade.

Future research should aim at the identification of factors that regulate the integrin β1-mediated demethylation of the Lgals3 promoter, and the link between Gal-3 expression and the acquisition of a mesenchymal phenotype. As Gal-3 is considered to be an important player in cancer progression and metastasis, and its expression is frequently regulated by DNA methylation in tumor cells, it will be interesting to determine whether integrins are responsible for de novo expression of Gal-3 during tumor progression.

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REFERENCES

1. Campbell, I. D., and Humphries, M. J. (2011) Cold Spring Harb. Perspect. Biol. 3(3) doi:10.1101/cshperspect.a004994
2. Geiger, B., and Yamada, K. M. (2011) Cold Spring Harb. Perspect. Biol. 3(5) doi:10.1101/cshperspect.a005033
3. Huveneers, S., and Danen, E. H. (2009) Adhesion signaling-crosstalk between integrins, Src and Rho. J. Cell Sci. 122, 1059–1069
4. Legate, K. R., Wickström, S. A., and Fässler, R. (2009) Genetic and cellular biological analysis of integrin outside-in signaling. Genes Dev. 23, 397–418
5. Margadant, C., Charafeddine, R. A., and Sonnenberg, A. (2010) Unique and redundant functions of integrins in the epidermis. FASEB J. 24, 4133–4152
6. Margadant, C., Monsuur, H. N., Norman, I. C., and Sonnenberg, A. (2011) Mechanisms of integrin activation and triggering. Curr. Opin. Cell Biol. 23, 607–614
7. Gimond, C., van Der Flier, A., van Delft, S., Brakebusch, C., Kuikman, I., Collard, J. G., Fässler, R., and Sonnenberg, A. (1999) Induction of cell scattering by expression of β1 integrins in β1-deficient epithelial cells requires activation of members of the rho family of GTPases and down-regulation of cadherin and catenin function. J. Cell Biol. 147, 1325–1340
8. Danen, E. H., Sonneveld, P., Brakebusch, C., Fassler, R., and Sonnenberg, A. (2002) The fibronectin-binding integrins αβ1 and αvβ3 differentially modulate RhoA-GTP loading, organization of cell matrix adhesions, and fibronectin fibrillogenesis. J. Cell Biol. 159, 1071–1086
9. Danen, E. H., van Rheenen, J., Franken, W., Huveneers, S., Sonneveld, P., Jalink, K., and Sonnenberg, A. (2005) Integrins control motile strategy through a Rho-cofilin pathway. J. Cell Biol. 169, 515–526
10. Woo, H. I., Shaw, L. M., Messier, J. M., and Mercurio, A. M. (1990) The major non-integrin laminin binding protein of macrophages is identical to carbohydrate binding protein 35 (Mac-2). J. Biol. Chem. 265, 7097–7099
11. Hughes, R. C. (2001) Galectins as modulators of tumour progression. Nat. Rev. Cancer 5, 29–41
12. Dumic, J., Dabic, S., and Flogel, M. (2006) Galectin-3: an opened-ended story. Biochim. Biophys. Acta 1760, 616–635
13. Kariya, Y., Kawamura, C., Tabei, T., and Gu, J. (2010) Bisecting GlcNAc residues on laminin-332 down-regulate galectin-3-dependent keratinocyte motility. J. Biol. Chem. 285, 3330–3340
14. Levy, Y., Arbel-Goren, R., Hadari, Y. R., Eshhar, S., Ronen, D., Elhanany, E., Geiger, B., and Zick, Y. (2001) Galectin-8 functions as a matricellular modulator of cell adhesion. J. Biol. Chem. 276, 31285–31295
15. Levy, Y., Ronen, D., Bershadsky, A. D., and Zick, Y. (2003) Sustained induction of ERK, protein kinase B and p70 S6 kinase regulates cell spreading and formation of F-actin microspikes upon ligation of integrins by galectin-8, a mammalian lectin. J. Biol. Chem. 278, 14533–14542
16. Nishi, N., Shoji, H., Seki, M., Itoh, A., Miyakawa, H., Yuube, K., Hirashima, M., and Nakamura, T. (2003) Galectin-8 modulates neutrophil function via interaction with integrin αM. Glycobiology 13, 755–763
17. Pacienza, N., Pozner, R. G., Bianco, G. A., D’Atri, L. P., Croci, D. O., Negrotto, S., Malaver, E., Gómez, R. M., Rabinovich, G. A., and Schattner, M. (2008) The immunoregulatory glycan-binding protein galectin-1 triggers human platelet activation. FASEB J. 22, 1113–1123
18. Moiseeva, E. P., Williams, B., Goodall, A. H., Samani, N. J. (2003) Galectin-1 interacts with β1 subunit of integrin. Biochem. Biophys. Res. Commun. 310, 1010–1016
19. Cao, Z., Said, N., Amin, S., Wu, H. K., Bruce, A., Garate, M., Hus, D. K., Kuwabara, I., Liu, F. T., and Panjwani, N. (2002) Galectins-3 and -7, but not galectin-1, play a role in re-epithelialization of wounds. J. Biol. Chem. 277, 42299–42305
20. Friedrichs, J., Torkko, J. M., Helenius, J., Teräväinen, T. P., Füllekrug, J., Geiger, B., and Zick, Y. (2001) Galectin-8 functions as a matricellular molecule to support eosinophil rolling and adhesion under conditions of flow. J. Biol. Chem. 276, 39391–39397
21. Kuwabara, I., Liu, F. T., and Panjwani, N. (1996) Galectin-3 promotes lamellipodia formation in epithelial cells by interacting with complex N-glycans on α3β1 integrin. J. Cell Sci. 112, 3684–3693
22. Ochieng, J., Leite-Browning, M. L., and Warfield, P. (1998) Regulation of cellular adhesion to extracellular matrix proteins by galectin-3. Biochem. Biophys. Res. Commun. 246, 788–791
23. Saravanan, C., Liu, F. T., Gipson, I. K., and Panjwani, N. (2009) Galectin-3 promotes lamellipodia formation in epithelial cells by interacting with complex N-glycans on α3β1 integrin. J. Cell Biol. 179, 7800–7807
24. Rao, S. P., Wang, Z., Zuberi, R. I., Sikora, L., Bahaie, N. S., Zaur, B. L., Liu, F. T., and Shiramarao, P. (2007) Galectin-3 functions as an adhesion molecule to support eosinophil rolling and adhesion under conditions of flow. J. Immunol. 179, 7800–7807
25. Furtak, V., Hatcher, F., and Ochieng, J. (2001) Galectin-3 mediates the endocytosis of β1 integrins by breast carcinoma cells. Biochem. Biophys. Res. Commun. 289, 845–850
26. Ochieng, J., Leite-Browning, M. L., and Warfield, P. (1998) Regulation of cellular adhesion to extracellular matrix proteins by galectin-3. Biochem. Biophys. Res. Commun. 246, 788–791
27. Warfield, P. R., Makker, P. N., Raz, A., and Ochieng, J. (1997) Adhesion of human breast carcinoma to extracellular matrix proteins is modulated by galectin-3. Invasion Metastasis 17, 101–112
28. Goetz, J. G., Bajoie, P. S., Strugnell, S. S., Scudamore, T., Kojic, L. D., and Nabi, I. R. (2008) Concerted regulation of focal adhesion dynamics by galectin-3 and tyrosine-phosphorylated caveolin-1. J. Cell Biol. 180, 1261–1275
29. Lagana, A., Goetz, J. G., Cheung, P., Raz, A., Dennis, J. W., and Nabi, I. R.
Galectin binding to Mgat5-modified N-glycans regulates fibronectin matrix remodeling in tumor cells. Mol. Cell Biol. 26, 3181–3193

31. Matarrese, P., Fusco, O., Tinari, N., Natoli, C., Liu, F. T., Semeraro, M. L., Malorni, W., and Iacobelli, S. (2000) Galectin-3 overexpression protects from apoptosis by improving cell adhesion properties. Int. J. Cancer 85, 545–554

32. Huveneers, S., Truong, H., Fässler, R., Sonnenberg, A., and Danen, E. H. (2008) Binding of soluble fibronectin to integrin α5β1-link to focal adhesion redistribution and contractile shape. J. Cell Sci. 121, 2452–2462

33. Margadant, C., Raymond, K., Kreft, M., Sachs, N., Janssen, H., and Sonnenberg, A. (2009) Integrin α3β1 inhibits directional migration and wound re-epithelialization in the skin. J. Cell Sci. 122, 278–288

34. Thijsen, V. L., Brandwijk, R. J., Dings, R. P., and Griffioen, A. W. (2004) Angiogenesis gene expression profiling in xenograft models to study cellular interactions. Exp. Cell Res. 299, 286–293

35. Li, L. C., and Dahiya, R. (2002) MethPrimer: designing primers for methylation PCRs. Bioinformatics 18, 1427–1431

36. Rozhon, W., Baubec, T., Mayerhofer, J., Mittelsten Scheid, O., Jonak, C. (2008) Rapid quantification of global DNA methylation by isocratic cation exchange high-performance liquid chromatography. Anal. Biochem. 375, 354–360

37. Takagi, J., Kamata, T., Meredith, J., Puzon-McLaughlin, W., and Takada, Y. (1997) Changing ligand specificities of αvβ1 and αvβ3 integrins by swapping a short diverse sequence of the β subunit. J. Biol. Chem. 272, 19794–19800

38. Miao, H., Li, S., Hu, Y. L., Yuan, S., Zhao, Y., Chen, B. P., Puzon-McLaughlin, W., Tarui, T., Shy, J. Y., Takada, Y., Usami, S., and Chien, S. (2002) Differential regulation of Rho GTPases by β1 and β3 integrins: the role of an extracellular domain of integrin in intracellular signaling. J. Cell Sci. 115, 2199–2206

39. Teramoto, H., Coso, O. A., Miyata, H., Igishi, T., Miki, T., and Gutkind, J. S. (1996) Signaling from the small GTP-binding proteins Rac1 and Cdc42 to the c-Jun N-terminal kinase/stress-activated protein kinase pathway. A role for mixed lineage kinase 3/protein-tyrosine kinase 1, a novel member of the mixed lineage kinase family. J. Biol. Chem. 271, 27225–27228

40. Benvenuto, G., Carpentieri, M. L., Salvatore, P., Cindolo, L., Bruni, C. B., and Chiarotti, L. (1996) Cell-specific transcriptional regulation and reactivation of galectin-1 gene expression are controlled by DNA methylation of the promoter region. Mol. Cell Biol. 16, 2736–2743

41. Chiarotti, L., Salvatore, P., Frunzio, R., and Bruni, C. B. (2004) Galectin genes: regulation of expression. Glycoconj. J. 19, 441–449

42. Ruebel, K. H., Jin, L., Qian, X., Scheithauer, B. W., Kovacs, K., Nakamura, N., Zhang, H., Raz, A., and Lloyd, R. V. (2005) Effects of DNA methylation on galectin-3 expression in pituitary tumors. Cancer Res. 65, 1136–1140

43. Dennis, J. W., Lau, K. S., Demetriou, M., and Nabi, I. R. (2009) Adaptive regulation at the cell surface by N-glycosylation. Traffic 10, 1569–1578

44. Ahmad, N., Gabius, H. J., André, S., Kalmer, H., Sabesan, S., Roy, R., Liu, B., Macaluso, F., and Brewer, C. F. (2004) Galectin-3 precipitates as a pentamer with synthetic multivalent carbohydrates and forms homogeneous cross-linked complexes. J. Biol. Chem. 279, 10841–10847

45. Nieminen, J., Kuno, A., Hirabayashi, J., and Sato, S. (2007) Visualization of galectin-3 oligomerization on the surface of neutrophils and endothelial cells using fluorescence resonance energy transfer. J. Biol. Chem. 282, 1374–1383

46. Partridge, E. A., Le Roy, C., Di Guglielmo, G. M., Pawling, J., Cheung, P., Granovsky, M., Nabi, I. R., Wrana, J. L., and Dennis, J. W. (2004) Regulation of cytokine receptors by Golgi N-glycan processing and endocytosis. Science 306, 120–124

47. Ramasamy, S., Duraisamy, S., Barbashov, S., Kawano, T., Kharbanda, S., and Kufe, D. (2007) The MUC1 and galectin-3 oncoproteins function in a microRNA-dependent regulatory loop. Mol. Cell 27, 992–1004

48. Chen, M., Sinha, M., Luxon, B. A., Bresnick, A. R., and O’Connor, K. L. (2009) Integrin α6β4 controls the expression of genes associated with cell motility, invasion, and metastasis, including S100A4/metastasin. J. Biol. Chem. 284, 1484–1494