Down-Regulation of Galectin-3 Suppresses Tumorigenicity of Human Breast Carcinoma Cells

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ABSTRACT

Galectin-3 is an endogenous β-galactoside-binding protein with specificity for type I and II ABH blood group epitopes and poly-N-acetyllactosamine glycan-containing cell surface glycoproteins and is the major nonintegrin cell surface glycoprotein with an elevated level in a wide range of neoplasms, and expression was shown to be associated in some tumor cell systems with metastases. Here we determined the functional consequence of blocking galectin-3 expression in highly malignant human breast carcinoma MDA-MB-435 cells. Inhibition of galectin-3 expression led to reversion of the transformed phenotype as determined by altered morphology, loss of serum-independent growth, acquisition of growth inhibition properties by cell contact, and abrogation of anchorage-independent growth. The blockage of galectin-3 expression led to a significant suppression of tumor growth in nude mice. These results provide direct evidence that galectin-3 expression is necessary for the maintenance of the transformed and tumorigenic phenotype of MDA-MB-435 breast carcinoma cells.

INTRODUCTION

Galectin-3 is a member of a growing family of multifunctional galactoside-binding proteins. Members of this gene family share affinity for β-galactoside-containing glycoconjugates. Galectin-3 is a 30,000 protein composed of three distinct structural domains: (a) a short NH2 terminus of 12 amino acids that controls its cellular targeting; (b) a repetitive collagen-like sequence rich in glycine, tyrosine, and proline, which serves as a substrate for matrix metalloproteinases; and (c) the COOH-terminal domain, a globular structure encompassing the carbohydrate-binding site (1–4). In some human tumors, a direct relationship was shown between galectin-3 levels and the stage of tumor progression (5–11). We have recently reported (9) that the expression of galectin-3 is related to neoplastic transformation and progression toward metastasis in colon carcinoma. In gastric carcinoma, it was found that tissue levels of galectin-3 were higher in certain primary tumors and their metastases than in the adjacent normal mucosa (7). In ovarian carcinoma, however, no correlation was observed between galectin-3 expression and clinicopathological features (12). In steroid-sensitive breast carcinoma cells, it was suggested that estradiol and progesterin might act as coordinates regulating specific genes, including up-regulation of galectin-3 expression, leading to the acquisition of metastatic phenotype (13). Previously, we studied galectin-3 expression in cultured human breast carcinoma cell lines characterized as nontumorigenic, poorly metastatic, or metastatic in nude mice, and the expression of galectin-3 correlated with the reported tumorigenicity of the cells (14). The introduction of rgalectin-34 into null-expressing nontumorigenic BT-549 cells resulted in the acquisition of anchorage-independent growth properties and tumorigenicity, suggesting a relationship between galectin-3 expression and malignancy of human breast carcinoma cell lines (14). Down-regulation of galectin-3 expression by colon carcinoma cells resulted in a significant decrease in liver colonization ability, whereas up-regulation of galectin-3 increased metastatic potential (15). These findings imply an involvement of galectin-3 in malignant progression of carcinomas and suggest a possibility that galectin-3 may serve as a potential molecular target for therapy of carcinomas harboring overexpressed galectin-3. Thus, the present study was designed to directly examine and establish the role of galectin-3 in breast cancer. To this end, we blocked galectin-3 expression in the highly tumorigenic MDA-MB-435 human breast carcinoma cell line by antisense transfection and analyzed the effect of down-regulation of galectin-3 expression on cellular phenotypes associated with transformation in vitro and in vivo.

MATERIALS AND METHODS

Cell Culture. The human breast cancer cell line MDA-MB-435 was grown as described previously (14) in monolayer culture in DMEM (Life Technologies, Inc., Grand Island, NY) supplemented with 10% heat-inactivated FBS, essential and nonessential amino acids, vitamins, penicillin, and streptomycin (Life Technologies, Inc.). The culture was maintained at 37°C in a humidified atmosphere of 5% CO2 and 95% air. Derivative
cell lines were also grown under these conditions; however, the medium was supplemented with 1.2 \mu g/ml Genetin (G418).

**Plasmid Constructs.** The second intron of the galectin-3 gene containing 649 bp flanked by 28 bp of the second exon on the 5' end and 30 bp of the third exon on the 3' end was amplified by PCR from genomic DNA of MDA-MB-435. The sense primer used was 5'-CGAGCGGAAATGGGAGG3A3', and the antisense primer used was 5'-GTTCGAGCCTCAA-GATAACG3'. Appropriately sized (0.7 kb) PCR product was ligated into pGEM-T vector (Promega, Madison, WI), which was then transformed into JM109 competent cells (Promega). An EcoRI-EcoRI fragment was subcloned into eukaryotic expression vector pCNC10 (a generous gift from Dr. F. G. Kern; Lombardi Cancer Research Center, Washington, D.C.) in the antisense orientation downstream of the cytomegalovirus promoter. A clone was selected for orientation by restriction digestion analysis and direct DNA sequencing and was designated pCNC10-G-AS.

**Transfection.** MDA-MB-435 human breast carcinoma cells were transfected with either the control (pCNC10) or antisense (pCNC10-G-AS) plasmid DNA by LipofectAMINE reagent (Life Technologies, Inc.) according to the supplier’s protocol. Briefly, 2 \mu g of plasmid DNA were incubated with 20 \mu l of LipofectAMINE reagent and 400 \mu l of serum-free DMEM at room temperature for 45 min. The mixture was added to cells grown to 70% confluence in a 35-mm dish in 2 ml of serum-free DMEM and incubated at 37°C for 5 h, following which DMEM containing 20% serum was added to the cells. After incubation for 48 h, G418 was added to the medium to a concentration of 800 \mu g/ml, and the cells were maintained for 2 weeks. The G418-resistant clones were subcloned by cloning cylinders, and two clones, MDA-MB-435-AS1 and MDA-MB-435-AS2, were selected as antisense transfectants for subsequent experiments. MDA-MB-435-CONT, a control transfectant, was also obtained. Stable integration of the plasmid DNA into the transfec-
tants was verified by Southern blot analysis as described below.

**Southern Blot Analysis.** Genomic DNA (15 \mu g) was digested with EcoRI (Promega) for 2 h at 37°C. The resulting fragments were electrophoresed in 0.7% agarose gel, transferred to Magna Charge nylon membrane (Micron Separation, Inc., Westborough, MA), and UV cross-linked. The membrane was prehybridized at 42°C for 4 h with a solution containing 50% formamide, 5% dextran sulfate, 5 \times Denhardt's solution, 50 mM sodium phosphate, 5 \times SSC, and 300 \mu g/ml denatured salmon sperm DNA. Hybridizations were performed with a randomly primed \(^{32}P\)-labeled DNA insert from the original plasmid clone as a probe (Ready-To-Go DNA labeling beads; Pharmacia). The membranes were washed at 55°C with 2 \times SSC and 0.1% SDS for 30 min, followed by a wash with 0.2 \times SSC and 0.1% SDS for 30 min. The extent of hybridization was visualized by exposure to X-ray film (Eastman Kodak, Rochester, NY).

**Northern Blot Analysis.** Total cellular RNA was isolated from cultured cell lines as described previously (14). Twenty \mu g of total RNA were separated on 1% denaturing formaldehyde-agarose gels, transferred to Magna Charge nylon membrane, and UV cross-linked. Prehybridization was performed in the same way as described for the Southern blot. Hybridizations were performed with a randomly primed \(^{32}P\)-labeled probe (Ready-To-Go DNA labeling beads) representing the entire open reading frame of the galectin-3 cDNA. The membrane was washed twice, and the extent of hybridization was visualized as described above. Human \beta-actin was also used as a probe for normalization.

**Western Analysis.** Cells were grown to 80% confluence, and whole-cell lysates were prepared in lysis buffer [20 mM Tris-HCl (pH 7.4), 0.1% SDS (Fisher Scientific, Pittsburgh, PA), 1.0% Triton X-100, 1.0% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride, 1 \mu g/ml leupeptin, and 1 \mu g/ml aprotonin (Sigma, St. Louis, MO)]. Equal volumes of each lysate were resolved by 12.5% SDS-PAGE and electroblotted onto polyvinylidene difluoride plus membrane (Micron Separation, Inc.). Membranes were quenched in a solution of PBS containing 5% nonfat dry milk and 0.1% Tween 20 for 30 min on a rotary shaker. Blots were incubated with a 1:500 dilution of rat anti-galectin monoclonal solution for 1 h. After washing three times (for 10 min each) in quench solution, membranes were incubated with a 1:5000 dilution of anti-rat horseradish peroxidase-conjugated secondary antibody (Zymed, South San Francisco, CA) in quench solution for 1 h, washed three times (for 10 min each) in quench solution, and then visualized by exposure for 1 min to a freshly prepared chemiluminescent substrate containing 0.0624 mM luminol (3-aminopthalhydrazide; Sigma), 100 mM p- cupameric acid (Sigma), and 1.4 mM H,O (Fisher Chemical, Fair Lawn, NJ) in 5 mM Tris buffer (Sigma; pH 8.5). Excess liquid was removed by touching the corner of the membrane to Whatman 3 MM Chr paper, (Whatman International Ltd., Maidstone, United Kingdom). The membrane was then wrapped in Saran Wrap and exposed to Kodak Biomax-MJR film (Eastman Kodak) for 1 min. The bands were quantified on Kodak Image Station 440CF using ID Image Software (Eastman Kodak).

rgalectin-3. Human rgalectin-3 was isolated from bacterial cells by affinity chromatography on asialofetuin-Affi-Gel15 (Bio-Rad, Hercules, CA) as described previously (16), followed by extensive dialysis against PBS (pH 7.4). Subsequently, PBS was replaced with DMEM using Ultrafree-MC filters (Millipore, Bedford, MA).

**Assessment of Saturation Density and Cell Morphology.** Logarithmically growing cells were plated at a density of 5 \times 10^3 cells/cm² onto 60-mm culture dishes. The tissue culture medium was replaced daily to ensure that growth arrest would be a function of cell density rather than medium depletion. Cells were observed morphologically before trypsinization. Phase-contrast microscopy was used to obtain photographs. Viable cell counts were determined daily by trypan blue dye exclusion.

**Growth in Monolayer Culture.** Cells (1 \times 10^5) were seeded onto 60-mm culture dishes in the normal medium with four different serum concentrations (10%, 5%, 2.5%, and 1.25%). The tissue culture medium was changed every day to avoid medium depletion. Viable cell counts were determined daily using a hemocytometer and trypan blue dye exclusion. In another set of experiments, rgalectin-3 was added to the medium at a concentration of 10 \mu g/ml. Each experiment was performed in triplicate.

**Immunofluorescence Labeling.** Cells were detached from 100-mm plates with 0.25% trypsin-EDTA (Life Technologies, Inc.) and washed twice with PBS containing Ca²⁺ and Mg²⁺.
Mg^{2+}. Coverslips sterilized with methanol were placed into 6-well plates; 1 × 10^6 cells were seeded into each well, and the cells were grown overnight in 10% DMEM. The cells were rinsed twice with PBS; for cell surface staining, they were fixed with 3.5% paraformaldehyde for 5 min on ice and 5 min at room temperature; for intracellular staining, cells were fixed and permeabilized with 100% methanol for 15 min at room temperature. Cells were then washed twice with PBS and blocked with 1% BSA in PBS. After a 30-min incubation on ice, rabbit galectin-3 antiserum was added at a 1:50 dilution in 1% BSA and incubated for 1 h at room temperature, followed by three washes with PBS. The following steps were performed in the dark. Secondary antibody (FITC goat antirabbit IgG; Zymed; Ref. 14) was added at a 1:200 dilution in 1% BSA, incubated for 1 h, and then washed three times with PBS. The coverslips were transferred upside down onto glass slides with one drop of polyvinyl alcohol in PBS. Slides were then wrapped with aluminum foil and stored at 4°C until visualization.

**Anchorage-independent Growth and Tumorigenicity.** Assays were performed in 6-well dishes coated with 1% agarose dissolved in DMEM supplemented with 10% FBS. One thousand cells uniformly suspended in 0.5% agarose/DMEM were overlaid on the bottom layer. The dishes were kept at 4°C for 2 h to solidify the agarose and incubated at 37°C. After 24 h, 2 ml of fresh medium were placed on agar. The medium was replaced every 2 days. After 14 days, the number of colonies was counted and photographed using phase-contrast photomicrography. Colonies measuring 0.1 mm in diameter or greater were scored. Results were expressed as the percentage of colonies formed per total number of seeded cells. In another set of experiments, galectin-3 (10 μg/ml) was added to the medium. Each experiment was performed in triplicate.

To determine in vivo tumorigenicity, 5 × 10^5 or 1 × 10^5 cells in a total volume of 0.1 ml of PBS were injected into the MFP region of 6-week-old athymic female nude mice. The growth of MFP tumors was monitored by weekly examination, and growth rates were determined by caliper measurements of width and length.

**Statistics.** All data points represent the mean ± SE. Statistical analysis was performed by using the Mann-Whitney test.

**RESULTS**

**Inhibition of Galectin-3 Expression in MDA-MB-435 Antisense Transfectants.** To better understand whether galectin-3 plays a functional role in breast cancer, human breast carcinoma MDA-MB-435 cells were transfected with antisense galectin-3 and control vectors, and the biological consequence of down-regulation of galectin-3 was evaluated. Individual G418-resistant cell clones were isolated, and stable integration of expression constructs was verified by Southern blot analysis. The expression levels of galectin-3 mRNA were determined by Northern blot analysis using full-length galectin-3 cDNA as a probe, and because the antisense galectin-3 DNA construct was transcribed only from the second intron together with the flanking regions, this analysis would detect only endogenous galectin-3 mRNA, and not antisense RNA. Fig. 1A reveals that the control transfection did not alter galectin-3 expression, whereas introduction of galectin-3 antisense into MDA-MB-435 resulted in a marked reduction in the expression of galectin-3-specific mRNA, ranging from >90 to 50% inhibition. Two representatives clones are depicted in Fig. 1A, Lanes 3 and 4, respectively. These changes in mRNA levels were accompanied by corresponding decreases in galectin-3 protein levels (Fig. 1B). Cells of representative clones MDA-MB-435-AS1 and MDA-MB-435-AS2 showed a reduction of galectin-3 expression by 91.9% and 61.8%, respectively, as compared with parental MDA-MB-435 cells (Fig. 1B, bottom panel).

**In Vitro Growth Properties and Tumorigenicity Associated with Galectin-3 Down-Regulation.** Inhibition of galectin-3 expression led to a marked alteration in cell morphology. Fig. 2 depicts the morphology of the cells after 2 (Fig. 2, left panels) and 7 days (Fig. 2, right panels) in culture. At low cell density, parental MDA-MB-435 (Fig. 2A) and MDA-MB-435-CONT cells (Fig. 2C) showed a similar retractile,
plated onto 60-mm tissue culture dishes in media supplemented with 50 and H low as 3.0 and MDA-MB-435-AS2 cells reached saturation at a density as A, C, E, described in "Materials and Methods." Cells were photographed by E 2 G (4) cells exhibited a highly spindle-shaped morphology, whereas MDA-MB-435-A

Fig. 2 Morphology of MDA-MB-435 cells and transfectants. MDA-

MB-435, A and B; MDA-MB-435-CONT, C and D; MDA-MB-435-

AS1, E and F; MDA-MB-435-AS2, G and H. Cells (1 × 10^6) were

plated onto 60-mm tissue culture dishes in media supplemented with 10% FBS and incubated in the CO2 incubator under the conditions described in "Materials and Methods." Cells were photographed by phase-contrast microscopy after 2 (A, C, E, and G) or 7 days (B, D, F, and H) of growth. All fields were examined at the same magnification. Bar, 50 μm.

spindle-shaped morphology, whereas MDA-MB-435-AS1 (Fig. 2E) and MDA-MB-435-AS2 (Fig. 2G) cells exhibited a highly spread flat morphology with an increased cytoplasm:nuclear volume ratio. At high density, MDA-MB-435 (Fig. 2B) and MDA-MB-435-CONT cells (Fig. 2D) grew as multilayered sheets, forming a three-dimensional foci. In contrast, MDA-

MB-435-AS1 (Fig. 2F) and MDA-MB-435-AS2 cells (Fig. 2H) remained as a uniform monolayer with a cobblestone-like pattern. Furthermore, the highest cell densities reached by MDA-

MB-435 parental and MDA-MB-435-CONT cells were 4.2 × 10^5 and 4.1 × 10^5 cells/cm^2, respectively. MDA-MB-435-AS1 and MDA-MB-435-AS2 cells reached saturation at a density as low as 3.0 × 10^5 and 3.3 × 10^5 cells/cm^2, respectively. Next we tested the response of the cells to a reduced serum concentration in the growing medium (Fig. 3). At relatively high serum concentrations of 10% and 5%, the proliferation rate of all four cell lines tested was similar (Fig. 3, A and B). However, when the serum concentration was reduced to 2.5% or 1.25% (Fig. 3, C and D), MDA-MB-435 and MDA-MB-435-CONT cells maintained their normal growth rate, whereas the reduced level of galectin-3 expression was associated with a significant reduction in the growth rate of the cells in antisense clones (Fig. 3, C and D). The addition of exogenous galectin-3 (10 μg/ml) to the culture medium did not affect either the cell morphology or growth rate of any of the cell lines (data not shown), suggesting that the observed effects of blockage of galectin-3 are intracellular.

Next we analyzed the clonogenic and growth properties in semisolid medium. From the data summarized in Fig. 4, it is apparent that colony-forming efficiency was markedly reduced by antisense inhibition by galectin-3 expression. The number of colonies developed by MDA-MB-435-AS1 or MDA-MB-435-AS2 cells was much lower than that of MDA-MB-435 or MDA-MB-435-CONT cells (Fig. 4, compare C and D to A and B). The reduction in colony formation was not restricted to cell number but also applied to size. As shown in Fig. 4, MDA-MB-435 and MDA-MB-435-CONT cells grew to form similarly large-size colonies (Fig. 4, A and B), whereas the colony size of MDA-MB-435-AS1 was markedly smaller (Fig. 4, C and D) and that of MDA-MB-435-AS2 seemed intermediate (Fig. 4D). Anchorage-independent growth in vitro serves as a hallmark for cell transformation and may be correlated with tumorigenicity in vivo (17, 18). Thus, we questioned whether the reduction in galectin-3 expression might also be reflected in altered tumorigenicity in nude mice. To address this, we injected equal amounts of each cell line into the MFP of female nude mice, the preferred site of growth for MDA-MB-435 cells (19). Injection of 1 × 10^5 MDA-MB-435 and MDA-MB-435-CONT cells led to the formation of visible tumors within 3–4 weeks in all mice, whereas no tumor developed for up to 10 weeks in mice that received injection of an equal number of MDA-MB-435-AS1 or MDA-MB-435-AS2 cells (Table 1; Fig. 5A). When the inoculum was increased to 5 × 10^5 cells, again all of the mice injected with MDA-MB-435 or MDA-MB-435-CONT cells rapidly developed tumors. In contrast, only 3 of 10 mice injected with MDA-MB-435-AS1 or MDA-MB-435-AS2 cells developed slow-growing tumors (Fig. 5B). The rest of the mice remained tumor free for the 10-week duration of the experiments (Table 1; Fig. 5B). At the termination of experiments, the tumors developed by each cell line were removed and dissociated, and cells were regrown in culture to determine whether any change in galectin-3 expression might occur during tumor development in mice, and whether the cells have their original phenotype (data not shown).

Cellular Localization of Galectin-3. The spatial cellular localization of galectin-3 was established by immunostaining of both controls and antisense-transfected cells using anti-galectin-3 antibodies (Fig. 6). Cytoplasmic and nuclear localization of galectin-3 was readily visualized in the parental (Fig. 6, A) and control-transfected cells (Fig. 6B). The galectin-3 staining was greatly diminished in MDA-MB-435-AS1 and MDA-MB-435-AS2 cells, and no nuclear deposition could be detected in cells containing the galectin-3 antisense sequence (Fig. 6, C and D). Control labeling of the cells with only the secondary antibody was negative (data not shown).
DISCUSSION

The data presented herein suggest that blockage of galectin-3 expression in MDA-MB-435 breast carcinoma cells leads to partial reversion of the transformed phenotype in vitro and to a significant reduction in tumorigenicity in vivo.

Galectin-3 is a member of the galectin gene family that is expressed at elevated levels in a variety of neoplastic cell types (4–11), and it has been associated with alterations in cell growth, transformation, and metastasis (16, 20–22). Although the precise biological function of galectin-3 is unknown, its expression has been associated with events that may promote tumor progression and metastasis, such as carbohydrate-mediated homotypic aggregation, angiogenesis, and inhibition of apoptosis.

We have previously analyzed galectin-3 expression in relation to the malignant phenotypes of five established and well-
characterized human breast carcinoma cell lines, namely T47D, MDA-MB-231, MDA-MB-435, BT-549, and SK-BR-3. Of the five, the two cell lines (BT-549 and SK-BR-3), that are nontumorigenic in nude mice do not express galectin-3 (14). The introduction of human rgalectin-3 into the null BT-549 cells resulted in the acquisition of anchorage-independent growth properties and tumorigenicity. The current study was designed to provide more direct evidence that galectin-3 plays a role in the tumorigenicity of breast cancer cells.

As target cells, we chose the well-characterized human breast cancer MDA-MB-435 cells, which are highly tumorigenic and metastatic and overexpress galectin-3 (14, 23) and thus provide an excellent experimental model to study the functional role of galectin-3. Introduction of antisense DNA by transfection of target cells with appropriate expression vectors is a potent approach to inhibit the synthesis of endogenous cellular proteins (24, 25). For this purpose, we constructed an expression vector containing a 707-bp fragment of human galectin-3 genomic DNA in the antisense orientation under the transcriptional control of the cytomegalovirus promoter and the neomycin resistance gene. This antisense DNA was generated from the second intron together with flanking exon sequences of galectin-3 genomic DNA. Therefore, it may hybridize with the primary transcript and/or disrupt transcription. The results presented above indicate that this approach yielded an effective blockade in the synthesis of mature endogenous galectin-3 mRNA and protein (Fig. 1). Using these transfectant cells together with parental MDA-MB-435 cells and a vector control transfectant, we demonstrated the following key findings: (a) a decrease of galectin-3 synthesis is associated with change of morphology, acquisition of contact inhibition, reduction of serum independence, and abrogation of anchorage-independent cell growth; (b) these effects are not reversed by adding exogenous galectin-3; and (c) the inhibition of galectin-3 expression was associated with reduced tumorigenicity in nude mice.

Previously, we have shown that transfection of 3T3 fibroblasts with galectin-3 cDNA results in a morphological change, i.e., loss of contact inhibition, anchorage-independent growth in soft agar, and disruption of structural microfilament organization (26), and that proliferative stimulation of 3T3 fibroblast and infection of normal T lymphocytes that scantily express galectin-3 with T lymphotrophic virus I are associated with increased galectin-3 expression in general and in the nuclei in particular (27, 28). These cumulative data strongly suggested that intracellular galectin-3 may directly contribute to the transformation phenotype. However, because we already have shown that galectin-3 is not an oncogene but rather is associated with some aspects of transformation and metastasis (26), it is still unclear in which tumor progression pathway galectin-3 is involved. It is possible that the nuclear exclusion of galectin-3 plays a role in the observed phenomena. Recently, we found that a dissociated expression of cytoplasmic and nuclear galectin-3 during neoplastic progression of the tongue and a reduced expression of galectin-3 from the nucleus were associated with reduced disease-free survival of tongue cancer patients (29). In the prostate, it was suggested that galectin-3 might have antitumor activities when present in the nucleus because cytoplasmic expression is associated with disease progression in a subset of lesions (30). Nuclear and cytoplasmic galectin-3 are likely to be linked with proliferation and differentiation, respectively, in normal epithelium. This assumption may be supported by previous findings that mitogenic stimulation of quiescent fibroblasts results in a prompt increase of nuclear galectin-3 expression (2, 31); nuclear galectin-3 is involved in ribonuclear complexes (32) and has been identified as a factor in pre-mRNA splicing (33). In normal colonic mucosa, cytoplasmic galectin-3 is predominantly observed in the upper areas of the crypt and in the surface epithelium, i.e., terminally differentiated cells (34), and in normal squamous epithelium of the head and neck, distribution of cytoplasmic galectin-3 is confined to the superficial and inter-

### Table 1 Tumorigenicity in nude mice

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tumor formation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Cell no.</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1 × 10⁵</td>
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<tr>
<td>MDA-MB-435</td>
<td>5/5</td>
<td>10/10</td>
</tr>
<tr>
<td>MDA-MB-435-CONT</td>
<td>5/5</td>
<td>10/10</td>
</tr>
<tr>
<td>MDA-MB-435-AS1</td>
<td>0/5</td>
<td>3/10</td>
</tr>
<tr>
<td>MDA-MB-435-AS2</td>
<td>0/5</td>
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<sup>a</sup> Cells were injected into the MFP region of nude mice as described in “Materials and Methods.”

<sup>b</sup> Number of tumors/number of animals.

![Fig. 5 Tumor growth of MDA-MB-435 (○), MDA-MB-435-CONT (□), MDA-MB-435-AS1 (△), and MDA-MB-435-AS2 (■) in nude mice. Either 1 × 10⁵ (A) or 5 × 10⁵ (B) cells were injected into the MFP region of nude mice (see Table 1 for number of animals per group). Cross-sectional tumor diameters were measured externally, and the approximate tumor volume was calculated as described in “Materials and Methods.” * P < 0.05 as compared with parental MDA-MB-435 cells by Mann-Whitney test.](image-url)
mediate layers (35). It is also possible that the observed phenomenon is related to the status of the activation of the L1 retrotransposon gene product, which was suggested to contribute together with galectin-3 to the progression of some breast cancers (36). Recently, we have studied the functional role of the unusual leader sequence of galectin-3 and reported that it determines its nuclear localization (37). Transfection of leader sequence galectin-3 deletion mutant into galectin-3-null BT-549 breast cell species failed to convert the cells into tumorigenic ones unlike the wild type, further attesting to the role of galectin-3 nuclear localization in cancer progression.

In conclusion, we have shown that inhibition of galectin-3 in cultured breast cancer cell line is associated with a "normalization" of the cellular phenotype both in vivo and in vitro and provide direct evidence that galectin-3 plays an important role in breast cancer and may be a target for therapeutic modalities.

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