Integrin β4 Regulates SPARC Protein to Promote Invasion*

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The αβ4 integrin (referred to as “β4” integrin) is a receptor for laminins that promotes carcinoma invasion through its ability to regulate key signaling pathways and cytoskeletal dynamics. An analysis of published Affymetrix GeneChip data to detect downstream effectors involved in β4-mediated invasion of breast carcinoma cells identified SPARC, or secreted protein acidic and rich in cysteine. This glycoprotein has been shown to play an important role in matrix remodeling and invasion. Our analysis revealed that manipulation of β4 integrin expression and signaling impacted SPARC expression and that SPARC facilitates β4-mediated invasion. Expression of β4 in β4-deficient cells reduced the expression of a specific microRNA (miR-29a) that targets SPARC and impedes invasion. In cells that express endogenous β4, miR-29a expression is low and β4 ligation facilitates the translation of SPARC through a TOR-dependent mechanism. The results obtained in this study demonstrate that β4 can regulate SPARC expression and that SPARC is an effector of β4-mediated invasion. They also highlight a potential role for specific miRNAs in executing the functions of integrins.

Integrins are a family of heterodimeric transmembrane cell surface receptors composed of α and β subunits that collectively link the cytoskeleton to components in the extracellular matrix or to neighboring cells (1, 2). The integrin αβ4, referred to as “β4 integrin,” is an adhesion receptor for the laminins that plays a pivotal role in both normal tissue development and homeostasis as well as in carcinoma progression (3, 4). β4 mediates the formation of hemidesmosomes, inert structures on the basal surface of epithelial cells anchoring the intermediate cytoskeleton to laminins in the basement membrane (5, 6). Factors in the tumor microenvironment of invasive carcinomas liberate β4 from hemidesmosomes and promote its relocation to the leading edge of cells, where it becomes signaling competent and associates with F-actin in lamellae and filopodia to promote migration and invasion (3, 7–11). In the context of breast cancer, this integrin is associated with a “basal-like” subset of tumors, and its expression predicts decreased time to tumor recurrence as well as decreased patient survival (12). The contributions of β4 to carcinoma progression stem in part from its ability to regulate the expression and function of downstream effector molecules (3, 13–18).

We conducted an analysis of published Affymetrix GeneChip data (19) and identified SPARC, or secreted glycoprotein acidic and rich in cysteine, as a potential effector of β4-mediated function. SPARC plays a key role in extracellular matrix remodeling and cell motility (20). The data we obtained demonstrate that β4 expression and ligation can regulate SPARC and that SPARC is an effector of β4-mediated invasion. Interestingly, SPARC was identified as a target of miR-29a in osteoblasts (21), prompting us to examine the role of miRNAs downstream of β4 in the regulation of SPARC. miRNAs are non-coding single-stranded RNAs ~22 base pairs in length that regulate gene expression through mRNA degradation or translational inhibition and have been shown to play an increasingly significant role in tumorigenesis (22, 23). We identify miR-29a as a β4-regulated miRNA that can influence SPARC expression and invasion. The regulation of miR-29a by β4 is seen in cells that exhibit high miR-29a expression; in cells that express endogenous β4, miR-29a expression is low. Finally, we provide evidence that β4 expression and ligation facilitate the translation of SPARC.

**Background:** Integrin αβ4 is an adhesion receptor for the laminins that promotes carcinoma invasion.

**Results:** αβ4 ligation enhances SPARC translation, and its expression can repress a miRNA that inhibits invasion and targets SPARC.

**Conclusion:** The regulation of SPARC by integrin-mediated mechanisms can facilitate invasion.

**Significance:** These data enhance our understanding of how αβ4 contributes to the invasive process and demonstrate integrin regulation of miRNAs.
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EXPERIMENTAL PROCEDURES

Cell Lines, Antibodies, and Reagents—MDA-MB-435 and MDA-MB-231 cells were obtained from the Lombardi Cancer Center (Georgetown University, Washington, D. C.). SUM-159 and SUM1315 cells were obtained from Dr. Stephen Ethier (Wayne State University School of Medicine, Detroit, MI). MDA-MB-435 and MDA-MB-231 cell lines were maintained in low glucose DMEM medium (Invitrogen) supplemented with 10 mM HEPES, 5% fetal bovine serum, and 1% streptomycin and penicillin. SUM-159 cells were maintained in Ham’s F-12 medium (Invitrogen) supplemented with 5% fetal bovine serum, insulin (5 µg/ml), hydrocortisone (1 µg/ml), and 1% streptomycin and penicillin. SUM1315 cells were maintained in Ham’s F-12 medium (Invitrogen) supplemented with 5% fetal bovine serum, insulin (5 µg/ml), EGF (10 ng/ml), and 1% streptomycin and penicillin. All cell lines were grown at 37 °C in an incubator supplied with 5% CO₂. MDA-MB-435 mock transfectants (6D2 and 6D7 subclones), β4 transfectants (3A7 and 5B3 subclones), and β4ACYT transfectants (5D5) were generated and characterized as previously described (17). Antibodies to SPARC (Hematological Technologies, Essex Junction, VT), pS6K (Cell Signaling, Beverly, MA), p4E-BP (Cell Signaling), tubulin (Sigma), and actin (Sigma) were used for immunoblotting. The same SPARC antibody was used as a functional blocking antibody for invasion assays. The 505 antibody to β4, used for immunoblotting, and the 2B7 antibody to α6, used for clustering, were produced by our laboratory as previously described (9, 24). The AIIIB2 antibody to β1 (Development Studies Hybridoma Bank, University of Iowa, Iowa City, IA) was used for clustering experiments. For inhibitor experiments, rapamycin (Sigma) was used at a concentration of 50 nm.

Immunoblotting—Cells were solubilized on ice for 10 min in Triton X-100 lysis buffer (Boston Bioproducts, Ashland, MA) containing 50 mM Tris buffer, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, and protease inhibitors (Complete mini tab; Roche Applied Science) (Lysis Buffer A). Nuclei were removed by centrifugation at 16,100 × g for 10 min. Culture media was concentrated 8-fold using Ultra-4 Centrifugal Filter Units with a 10-kDa cutoff by spinning at 340 × g for 25 min (Millipore, Indianapolis, IN). Concentrations of total cell lysate and culture media were assayed by the Bradford method. Lysates (50 µg) and concentrated culture media (25 µg) were separated by electrophoresis through 10% SDS-PAGE and transferred to 0.2-µm nitrocellulose membranes (Bio-Rad). Membranes were blocked in 5% nonfat milk in Tris-buffered saline, Tween 20 for 1 h and blotted with the antibodies to SPARC (1:10,000), pS6K (1:500), p4E-BP (1:1,000), β4 (1:4,000), actin (1:5,000), or tubulin (1:10,000) overnight at 4 °C. Proteins were detected by enhanced chemiluminescence (Pierce) after incubation for 1 h with horseradish peroxidase-conjugated secondary antibodies.

miRNA and RNA Isolation and Detection—Total RNA was isolated using the miRVana miRNA Isolation Kit according to manufacturer protocol (Ambion). Quantitative real time PCR (qPCR) detection of mature miRNAs was performed using TaqMan miRNA Reverse Transcription kit and TaqMan human Microarray Assays for miR-29a (Applied Biosystems, Austin, TX) according to manufacturer protocol. U6 small nuclear RNA was used as an internal control. qPCR detection of SPARC mRNA was performed using Superscript II reverse transcriptase (Invitrogen) and Power SYBR Green (Applied Biosystems) according to manufacturer protocol. GAPDH was used as an internal control. miRNA and SPARC expression levels were quantified using the ABI Prism 7900HT Sequence detection system (Applied Biosystems). Primers to SPARC (5′-AGACACCCCATTTGACGGGTA′-3′ and 5′-GGTCACAGGTCTCAGGAAGCG-3′) and GAPDH (5′-ATCATCCCTGCTCCTACTGG-3′ and 5′-GTCAGTCCCCACCTGACGAC-3′) were used for analysis.

Gene Set Enrichment Analysis—For miR target enrichment analysis, mRNA expression data generated by Chen et al. (19) were downloaded from the NCBI Gene Expression Omnibus (GEO), series number GSE11466. Affymetrix CEL files were processed with the robust multi-chip average (RMA) algorithm (25) using BRB-ArrayTools. TargetScanHuman Release 5.1 (26, 27) was used to predict conserved miRNA targets. Using total context score, the top 500 targets for miR-29 or miR-93 were compiled into gene set lists. miR-93 targets were used as a negative control gene set because miR-93 is highly abundant, yet it did not change expression in the β4 versus mock miRNA array analysis. Log base 2 mRNA data were loaded into the Broad Institute’s Gene Set Enrichment Analysis (GSEA) software v2.06 (28, 29). β4 phenotype was compared with mock phenotype by first collapsing the dataset to gene symbols and then using a weighted, difference of classes metric for ranking genes. Gene set permutations were performed to generate nominal p values for each miRNA target gene set list.

Oligonucleotide Transfection—miRIDIAN-microRNA Mics are synthetic chemically modified mature miRNAs (Dharmacon, Lafayette, CO). MDA-MB-435 β4 transfectants were transfected with 20 nM hsa-miR-29a mimic or a miRNA mimic negative control at 50% confluency using DharmaFECT 4 Transfection Reagent (Dharmacon). At 72 h post-transfection, cells were plated for invasion assays or harvested for total cell lysate. A miRIDIAN microRNA Hairpin Inhibitor to mature miR-29a was used for loss-of-function analyses along with a hairpin inhibitor negative control (Dharmacon). MDA-MB-435 mock transfectants were transfected with 20 nM miR-29a inhibitor or negative control inhibitor as described above. At 72 h post-transfection, cells were harvested for protein or total RNA as described above.

Invasion Assays—The upper surfaces of the transwells were coated with 0.5 µg of Matrigel (BD Biosciences) and allowed to dry overnight at room temperature. Cells were harvested at 80% confluence by trypsinization and resuspended low glucose DMEM containing 0.25% heat-inactivated fatty acid-free bovine serum albumin. The coated surfaces of the transwells were blocked with media containing bovine serum albumin for 60 min at 37 °C. For SPARC blocking antibody experiments, cells were incubated with 16 µg/ml of SPARC antibody (Hematological Technologies) or normal mouse IgG for 30 min at room temperature with intermittent agitation. 10⁵ cells in a total volume of 100 µl were loaded into the upper chamber, and NIH-3T3 conditioned media was added to the lower chamber. Assays proceeded for 4 h at 37 °C. At the completion of the assays, the upper chamber was swabbed to remove residual cells.
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**RESULTS**

**β4 Integrin Regulates Expression of SPARC**—MDA-MB-435 breast carcinoma cells were utilized initially as a model system to identify β4-regulated genes that facilitate invasion. Despite some reports claiming that these cells are of melanocytic origin (30–32), several reports have refuted this claim and have provided convincing data that this is a poorly differentiated cell line of breast cancer origin (33–37). These cells express α6β1 endogenously but lack α6β4. Introduction of the β4 subunit leads to preferential heterodimerization of the α6 subunit with the β4 subunit (38, 39). Stable subclones were generated expressing wild-type β4 (referred to as β4 transfectants) or a β4 deletion mutant (referred to as βΔCYT transfectants) that lacks the cytoplasmic domain of the β4 subunit. This deletion impedes the signaling capacity of the integrin, and it eliminates the formation of the α6β1 heterodimer (17, 40). Mock transfectants were also generated. The β4 transfectants are significantly more invasive than either the mock or βΔCYT transfectants (17).

To identify potential regulators of β4-mediated invasion, we conducted an analysis of published Affymetrix GeneChip data that were obtained using the MDA-MB-435/β4 transfectants (19). SPARC was identified using this approach. This secreted glycoprotein is involved in extracellular matrix remodeling and invasion (20). SPARC mRNA and protein expression was examined to determine whether β4 differentially regulates its expression in this system. Quantitative real time PCR (qPCR) confirmed that SPARC message levels are elevated over 3-fold in the β4 transfectants compared with controls (Fig. 1A). Further, SPARC protein expression is elevated significantly in the total cell extract and culture media of the β4 transfectants compared with either the mock or βΔCYT transfectants. *, p < 0.04. β, shown is expression of SPARC in total cell extract (50 μg) and culture medium (25 μg) across MDA-MB-435 subclones.

**β4 Expression Inversely Correlates with miR-29a Expression**—SPARC was recently identified as a target of miR-29a in osteoblasts (21), prompting us to examine the role of miRNAs downstream of β4 in the regulation of SPARC. SPARC contains two conserved miR-29-predicted miRNA binding sites and one conserved miR-203-predicted binding site in its 3’-UTR. These observations are relevant because of results from a miRNA microarray conducted by our laboratory to assess global

and fixed with methanol. Cells on the lower surface of the membrane were mounted in 4',6-diamidino-2-phenylindole mounting media (Vector Laboratories, Burlingame, CA), and the number of cells was determined for five independent fields in triplicate with a 10× objective and fluorescence.

**siRNA Experiments**—SUM-159 cells were transfected with 20 nM On-TARGETplus SMARTpool siRNA targeting β4 (Dharmacon) at 50% confluency using DharmaFECT 4 transfection reagent (Dharmacon). A non-targeting siRNA pool (Dharmacon) was used as a control for these experiments. At 72 h post-transfection, cells were harvested for protein or total RNA as described above.

**Integrin Clustering**—MDA-MB-435/β4 and SUM-159 cells were serum-starved overnight in DMEM containing 0.1% BSA and F-12 containing 0.1% BSA, respectively. Cells were trypsinized, washed, and resuspended at a concentration of 10⁶ cell/ml. For laminin experiments, cells were plated on laminin (100 μg/10 cm plate) or maintained in suspension. For antibody-mediated clustering experiments, cell suspensions were incubated for 30 min with integrin-specific antibodies (2 μg/ml) in DMEM containing 0.1% BSA. The cells were washed and added to plates that had been coated overnight with anti-mouse or anti-rat IgG (33 μg/6-cm plate). For both laminin and antibody-mediated clustering experiments, cells were treated with 50 nM rapamycin or DMSO for 10 min before plating cells on coated plates. After incubation at 37 °C for 45 min, the cells were washed twice with PBS and lysed for protein in a 20 mM Tris buffer, pH 7.4, containing 10% glycerol, 136 mM NaCl, 10% Nonidet P-40, 1 mM sodium orthovanadate (Na₃VO₄), 1 mM sodium fluoride (NaF), 2 mM phenylmethanesulfonyl fluoride, and complete protease inhibitor mixture (Roche Applied Science) (Lysis Buffer B) or for total RNA as described above.

**Rapamycin Experiments**—SUM-159 parental cells were treated with 50 nM rapamycin or DMSO in serum-containing medium for 4 or 6 h. Cells were lysed using Lysis Buffer B, and samples were prepared for analysis as described above.

**Statistical Analysis**—Data are presented as the mean ± S.E. Student’s t test was used to assess the significance of independent experiments. The criterion p < 0.05 was used to determine statistical significance.
miRNA expression in the MDA-MB-435/β4 system. Specifically, two subclones of the β4 transfectants (3A7 and 5B3) and two subclones of the mock transfectants (6D2 and 6D7) as well as the MDA-MB-435 parental cells were examined using a novel microarray technology termed qNPA (supplemental Experimental Procedures). The results of the array demonstrated that β4 expression repressed the expression of miR-29a and miR-29b (Fig. 2A, supplemental Fig. 1 and Table 1). miR-29c and miR-203 levels, however, were unchanged (supplemental Table 1). We focused on miR-29a because it has been shown to target SPARC and because miR-29b undergoes rapid decay after nuclear import in cycling cells (41). The microarray data were confirmed using qPCR. The expression of β4 in MDA-MB-435 cells resulted in an approximate 4-fold decrease in miR-29a compared with the mock transfectants. Furthermore, a subclone of the β4ΔCYT transfectants (5D5) was also examined and found to express levels of miR-29a similar to those detected in the mock transfectants (Fig. 2B), indicating that the cytoplasmic tail of β4 is required for repression of miR-29a.

To assess the relationship between β4 and miR-29a expression further, we examined a series of breast carcinoma cell lines with differential β4 expression. The β4-null MDA-MB-435 parental cells were compared with the β4-null SUM1315 breast carcinoma cell line and to the β4-expressing MDA-MB-231 and SUM-159 breast carcinoma cell lines (Fig. 2C). Levels of miR-29a were markedly lower in cell lines expressing β4 compared with those not expressing the integrin (Fig. 2D), supporting a relationship between β4 expression and the regulation of miR-29a.

Gene set enrichment analysis of the published Affymetrix GeneChip data (19) was conducted to substantiate the role of miR-29a in the regulation of β4-mediated targets. This analysis examines the population of β4-regulated mRNAs for an overrepresentation of genes predicted to be targeted by our miRNA of interest. Our analysis revealed a significant enrichment \( p < 0.001 \) for miR-29-predicted targets in mRNAs up-regulated by β4 (Fig. 3). In contrast, no enrichment was detected for miR-93, a miRNA selected as a negative control on the basis that it was expressed at robust levels in all samples (data not shown). As part of this analysis, a list of leading edge genes was generated consisting of a group of mRNAs that are the important contributors to the detected enrichment. The list of leading edge genes contained 116 mRNAs (supplemental Table 2), the top 25 of which are listed in Table 1 ranked in order of contribution to the detected enrichment. As anticipated, SPARC appears on this list. Of interest, other genes in this table have also been implicated in the invasive process in breast carcinoma and
null
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**FIGURE 4.** β4-Mediated repression of miR-29a can promote SPARC-dependent invasion. A. β4 transfectants and SUM-159 cells were subjected to Matrigel invasion assays after transfection with a miR-29a mimic. *, $p < 0.02$. B, mock transfectants were subjected to Matrigel invasion assays after transfection with a miR-29a hairpin inhibitor. Data for invasion assays represents the means ± S.E. from a representative experiment. C, shown is expression of SPARC in total cell lysate (50 μg) after expression of miR-29a mimic in β4 transfectants 72 h post-transfection or expression of miR-29a hairpin inhibitor in mock transfectants 72 h post-transfection. D, β4 transfectants were subjected to Matrigel invasion assays after 30 min of preincubation with normal mouse IgG or a SPARC function blocking antibody. *, $p < 0.001$. Data for invasion assay represents the means ± S.E. from three independent experiments.

ics β4-induced miR-29a repression. The results from this experiment demonstrate that inhibition of miR-29a is not sufficient to induce the invasive phenotype of cells in the absence of β4 (Fig. 4B), consistent with our observation that overexpression of SPARC in the mock transfectants resulted in no change in invasion (data not shown).

To establish that miR-29a represses SPARC as a function of β4 expression, SPARC expression was examined after manipulation of miR-29a levels in both the MDA-MB-435/β4 and mock transfectants. Transfection of the β4 transfectants with the miR-29a mimic produced a significant decrease in SPARC expression compared with mock-transfected cells and cells transfected with a nonspecific negative control mimic (Fig. 4C). Conversely, transfection of the mock transfectants with a miR-29a inhibitor substantially increased SPARC expression compared with mock-transfected cells and cells transfected with a nonspecific negative control mimic (Fig. 4C). Importantly, these data substantiate the invasion assays described above by confirming that the mimic and hairpin inhibitor are functional, as functionality is established by their ability to regulate target gene expression. Furthermore, the protein data from the inhibitor studies provide a control for the invasion assay presented in Fig. 4B, insuring that the poorly invasive phenotype of the cells transfected with the miR-29a inhibitor is not due to a technical problem with the inhibitor.

To determine whether SPARC is necessary for β4-mediated invasion, β4 transfectants were subjected to an invasion assay after incubation with a functional blocking antibody to SPARC. The ability of these cells to invade Matrigel was decreased 2.5-fold compared with cells receiving no treatment and cells preincubated with normal mouse IgG (Fig. 4D), establishing a role for this β4 target in mediating invasion downstream of the integrin.

β4 Can Regulate SPARC Independently of miR-29a—Although the β4 transfectants possess some constitutive activity and can mediate β4-function in a ligand-independent manner (16, 50), ligation of β4 either by adhesion to laminin or antibody-mediated clustering should in principle further repress miR-29a and up-regulate SPARC expression. Interestingly, our data indicate that β4 signaling can up-regulate SPARC expression independently of the miRNA. As depicted in Fig. 5A, ligation of β4 in the β4 transfectants by adhesion to laminin induces SPARC protein expression compared with suspension control. Given that the β4 transfectants retain expression of the β1 integrin subunit (17), antibody-mediated clustering experiments were conducted to substantiate these data and further implicate β4 signaling in the regulation of this effector molecule. Specifically, clustering with an antibody to the α6 subunit of the integrin (mAb 2B7) up-regulates SPARC protein compared with cells clustered with an antibody to β1 (mAb AIIB2), confirming that this regulation is specific to integrin α6β4 (Fig. 5A).

Our observation that SPARC induction occurs in the absence of further miR-29a repression (data not shown) prompted us to examine the expression of SPARC message under these conditions. SPARC mRNA levels are unchanged in cells clustered with the α6 antibody compared with the β1 control (Fig. 5B), suggesting that β4 plays a role in regulating SPARC protein stability or translation. Considering that ligation of this integrin is known to up-regulate mTOR (mammalian target of rapamycin) signaling and VEGF (vascular endothelial growth factor) translation (51), we treated cells with rapamycin, an inhibitor of
TOR cap-dependent translation. As depicted in Figs. 5, A and B, rapamycin blocked β4-mediated induction of SPARC protein as well as pS6K and p4E-BP1 signaling intermediates. Our data suggest that although steady-state levels of SPARC can be regulated by miR-29a in this system, rapid changes in SPARC expression occurring in response to β4 ligation arise through a TOR-dependent translational mechanism.

We next assessed the relationship between β4 and SPARC in breast carcinoma cells that express endogenous β4. For this purpose, the SUM-159 cell line was selected because it is an invasive breast carcinoma cell line in which SPARC is robustly expressed (Fig. 5D). Interestingly, transient depletion of β4 using siRNA diminished SPARC protein expression, but it had no effect on SPARC mRNA levels (Figs. 5, C and D). These data support the hypothesis that β4 can regulate SPARC expression. Depletion of β4 expression, however, did not increase miR-29a (data not shown). Based on our observation that β4 can regulate SPARC independently of the miRNA in the MDA-MB-435 system, we examined the possibility that this translational mechanism was also at play in the SUM-159 cells. As depicted in Fig. 5D, levels of pS6K and p4E-BP1 signaling intermediates were diminished upon loss of β4. To establish that this pathway is required for maintenance of SPARC expression, SUM-159 parental cells were treated with rapamycin. After 6 h a detectable decrease in SPARC protein levels was observed (Fig. 5E), suggesting that β4 regulates SPARC expression in this system through a TOR-dependent translational mechanism.

To assess the role of β4 ligation and signaling in regulating SPARC translation in SUM-159 cells, these cells were plated on laminin in the presence or absence of rapamycin. Work from our laboratory has established that α6β4 is the predominant laminin binding integrin in these cells (52). Laminin-mediated clustering of β4 induces SPARC expression at the protein level compared with suspension control (Fig. 5F), whereas SPARC mRNA levels remain unaffected (Fig. 5G). As anticipated, this induction is abrogated upon treatment with rapamycin (Fig. 5F).

**DISCUSSION**

The major conclusion of this study is that the β4 integrin can regulate the expression of SPARC in breast carcinoma cells. This finding is significant because this integrin is known to facilitate the invasion of carcinoma cells, and its regulation of SPARC adds to our understanding of how β4 can contribute to
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the invasive process. In addition, our data reveal a novel function for the β4 integrin in repressing the expression of a specific miRNA, miR-29a, that can impede invasion. To our knowledge this is the first report that integrins can regulate the expression of miRNAs. One mechanism by which miR-29a impedes invasion is to target SPARC. This mode of miR-29a regulation by β4 is manifested in cells that express high levels of miR-29a. In other cells that express endogenous β4 and low levels of miR-29a, we provide evidence that β4 expression and signaling can enhance SPARC translation. These findings indicate that β4 has the ability to regulate SPARC expression by distinct mechanisms.

Our data support the notion that SPARC, a secreted extracellular matrix glycoprotein with counter-adhesive properties, functions to promote invasion. This role for SPARC is supported by the findings that SPARC can promote cell motility and invasion in various carcinoma cells, including breast (53–60). Moreover, SPARC expression has been associated with basal-like breast cancers (61). This observation is relevant to our findings because we correlated β4 integrin expression with basal-like breast cancers in a previous study (12), and the cell lines used in the current study exhibit a basal phenotype. Some reports, however, have questioned the role of SPARC in breast cancer invasion and progression (62, 63). SPARC has also been shown to decrease the mitogenic potency of various growth factors including VEGF and PDGF by antagonizing their ability to bind to their cognate receptors (64, 65). In contrast, there is evidence that SPARC can enhance integrin and growth factor receptor-regulated kinases, thereby up-regulating key signaling pathways involved in cell motility (59, 66–70), observations that are consistent with our data. This dichotomy of SPARC function may be explained by the hypothesis that SPARC inhibits its early stages of tumorigenesis but potentiates later stages of progression, analogous to the TGF-β pathway (71), a growth factor signaling pathway that SPARC has been shown to regulate (70, 72–76).

Our data provide the first indication that β4 has the ability to regulate the expression of specific miRNAs and that such miRNAs can influence β4-mediated migration and invasion. Because the initial reports that the β4 integrin has the ability to promote the migration and invasion of epithelial and carcinoma cells, numerous mechanisms have been reported to account for this phenomenon. These mechanisms include activation of signaling pathways, especially the PI3K pathway and Rho GTPases, transcription factors (NFAT [nuclear factor of activated T cells]), and cap-dependent translation of key effector molecules (15–17, 51, 77–79). The ability of β4 to regulate the expression of miRNAs adds a new dimension to our understanding of how β4 mediates invasion and other functions. The repression of miR-29a that occurs in response to exogenous expression of β4 is significant in this context because miR-29a represses invasion and targets SPARC. Although our data indicate that β4-mediated repression of miR-29a is required for invasion, our observation that functional inhibition of this miRNA did not induce an invasive phenotype in the poorly invasive, mock transfectants suggests that a single miRNA is unlikely to be solely responsible for a cellular process. Although we observed that this regulation of miR-29a by β4 is manifested in specific cell types, especially those that express high levels of miR-29a, the paradigm that miRNAs contribute to the execution of integrin-mediated functions may be widespread.

The half-life of specific miRNAs could be a significant factor in their potential repression by integrin signaling. Given that the reported half-life of miR-29a is greater than 12 h (41), a detectable decrease in miR-29a after the transient signaling events induced by integrin ligation would require degradation of the pre-existing miRNA. This assumption is supported by our result that antibody-mediated clustering of β4 on MDA-MB-435 cells for times up to 4 h had no significant effect on miR-29a expression. We surmise from these data that exogenous expression of β4 in β4-deficient cells results in a long term and sustained repression of miR-29a expression. This possibility is supported tangentially by our finding that the expression pattern of β4 in breast carcinoma cell lines correlates inversely with miR-29a expression and previous reports that β4-mediated signaling and function can occur independently of its ligation (16, 50). It is also worth noting in this regard our analysis of published microarray data that revealed a significant enrichment in miR-29a predicted targets in miRNAs up-regulated by expression of β4. This finding suggests that a miRNA can broadly affect gene expression downstream of an integrin and corroborate the importance of miR-29a in the regulation of genes whose expression is mediated by β4.

We also provide evidence that SPARC can be regulated at the level of protein translation by β4, particularly in cells that express endogenous β4 and low levels of miR-29a. Ligation of β4 by adhesion to laminin or antibody-mediated clustering up-regulates SPARC protein expression in both MDA-MB-435/β4 transfectants as well as SUM-159 cells. This finding is consistent with a previous report demonstrating that β4 can facilitate the cap-dependent translation of VEGF in breast carcinoma cells (51). In principle, this mode of regulation would enable SPARC expression to be altered rapidly in response to microenvironmental cues that impact β4. Moreover, the β4-mediated regulation of SPARC by miRNA repression and cap-dependent translation mechanisms need not be mutually exclusive.

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