Published in final edited form as:

Differentiation. 2013 October ; 86(3): 126–132. doi:10.1016/j.diff.2013.03.003.

Extracellular matrix proteins regulate epithelial-mesenchymal transition in mammary epithelial cells

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Abstract

Mouse mammary epithelial cells undergo transdifferentiation via epithelial-mesenchymal transition (EMT) upon treatment with matrix metalloproteinase-3 (MMP3). In rigid microenvironments, MMP3 upregulates expression of Rac1b, which translocates to the cell membrane to promote induction of reactive oxygen species and EMT. Here we examine the role of the extracellular matrix (ECM) in this process. Our data show that the basement membrane protein laminin suppresses the EMT response in MMP3-treated cells, whereas fibronectin promotes EMT. These ECM proteins regulate EMT via interactions with their specific integrin receptors. α 6-integrin sequesters Rac1b from the membrane and is required for inhibition of EMT by laminin. In contrast, α 5-integrin maintains Rac1b at the membrane and is required for the promotion of EMT by fibronectin. Understanding the regulatory role of the ECM will provide insight into mechanisms underlying normal and pathological development of the mammary gland.

Keywords

mechanical stress; lrECM; integrin; cell shape

Introduction

Epithelial-mesenchymal transition (EMT) is characterized by a series of phenotypic changes through which epithelial cells acquire mesenchymal characteristics, including dissolution of cell-cell junctions, change in cell shape, modification of cytoskeletal structure and adhesion molecules, production of stromal extracellular matrix (ECM) proteins, and increased cell motility and invasiveness (Radisky, 2005). EMT is essential during embryogenesis for formation of the mesoderm and migration of cells from the neural crest (Hay, 1995). However, inappropriate activation of EMT can lead to pathological development (Tomasek et al., 2002). In a special type of EMT, epithelial-myofibroblast transition (EMyT) (Radisky et al., 2007), epithelial cells transdifferentiate into myofibroblasts which synthesize excessive amounts of ECM proteins and exert contractile force on surrounding tissues. The

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prolonged activation of myofibroblasts and their failure to undergo apoptosis can lead to a condition known as fibrosis (Kalluri and Neilson, 2003), characterized by collagen deposition and tissue stiffening, which is a significant clinical problem and which also has been found to confer substantially increased risk of subsequent cancer development (Boyd et al., 2005; Levental et al., 2009; Paszek and Weaver, 2004; Paszek et al., 2005).

The basement membrane (BM), a specialized layer of ECM that separates the epithelium from the mesenchyme, is necessary for the maintenance of epithelial cell phenotype. Mammary epithelial cell differentiation requires contact with BM proteins: mammary epithelial cells synthesize the milk protein κ -casein in the presence of lactogenic hormones only when in contact with the BM component laminin-111 (Muschler et al., 1999; Roskelley et al., 1994; Streuli and Bissell, 1990). By contrast, exposure of epithelial cells to ECM components found in the mesenchymal compartment can induce loss of epithelial function: type I collagen stimulates a mesenchymal phenotype including scattering and upregulation of N-cadherin in mammary epithelial cell (Shintani et al., 2006). Additionally, α -smooth muscle actin (α -SMA) expression and myofibroblast activation are suppressed by laminin but promoted by fibronectin (Sohara et al., 2002; Thannickal et al., 2003). Thus, the ECM microenvironment acts as a critical regulator of EMT for mammary epithelial cells.

Matrix metalloproteinase-3 (MMP3), a matrix-degrading enzyme secreted by stromal fibroblasts, induces EMT in mammary epithelial cells both in culture and *in vivo* (Lochter et al., 1997; Sternlicht et al., 1999). Exposure to MMP3 causes cells to upregulate the expression of Rac1b, a splice variant of Rac1, stimulating the production of reactive oxygen species (ROS) and expression of Snail1, an EMT-promoting transcription factor (Radisky et al., 2005). Further studies have shown that cells plated at a high density (Nelson et al., 2008) or cultured on soft matrices (Lee et al., 2012) fail to undergo EMT in response to treatment with MMP3, indicating that specific alterations in cell morphology and interactions with the ECM are required for MMP3-induced EMT in cultured mammary epithelial cells.

Here we investigated how the biochemical composition of the ECM might regulate MMP3-induced EMT. We first compared the response of cells to treatment with MMP3 in the presence of various ECM proteins, and found that EMT was suppressed by laminin-rich ECM (lrECM) but promoted by fibronectin. These ECM proteins appeared to regulate EMT at least partially through the interactions with their corresponding integrin receptors and modulation of cell morphology. We found that the laminin-binding α 6-integrin was required for sequestration of Rac1b from the cell membrane and inhibition of EMT by lrECM, whereas α 5-integrin was required for localization of Rac1b to the cell membrane and the promotion of EMT by fibronectin. These results indicate a specific requirement for integrin switching in MMP3-induced EMT.

Materials and Methods

Cell culture

Functionally normal SCp2 mouse mammary epithelial cells (Desprez et al., 1993; Reichmann et al., 1989) stably expressing MMP3 under the control of the tetracycline (tet) repressor (Lochter et al., 1997) were maintained in DMEM:F12 medium (Hyclone) supplemented with 2% tet-free fetal bovine serum (Atlanta Biologicals), 50 μ g/ml gentamycin (Sigma), 5 μ g/ml insulin (Sigma), 0.4 μ g/ml G418 (A.G. Scientific) and freshly added 5 μ g/ml tetracycline (Sigma); the withdrawal of tetracycline stimulates synthesis of MMP3 and induction of EMT (Radisky et al., 2005). ECM proteins were diluted and coated on bacterial-grade polystyrene dishes for 24 hours, rinsed with PBS, blocked with 1% (w/v) pluronics F108 (BASF) in PBS for 30 min, and rinsed again with PBS before plating cells. The ECM proteins and concentrations used were as follows: collagen I at 100 μ g/ml in

MilliQ H₂O, fibronectin at 25 µg/ml in PBS, Matrigel™ (with 56% laminin and 31% collagen IV) at 100 µg/ml in serum-free medium, laminin at 50 µg/ml in PBS, collagen IV at 28 µg/ml in 0.05M HCl (all from BD Biosciences).

Immunofluorescence staining

Cells were plated at 5,000 cells/cm², treated under different conditions for 4 days, and fixed with 4% paraformaldehyde. Samples were permeabilized and blocked with 0.5% Triton-X-100 (Sigma) and 10% calf serum (Atlanta Biologicals) in PBS, treated at room temperature with polyclonal rabbit anti-cytokeratin (Dako) at 1:1000 dilution and with monoclonal mouse anti-SMA (Sigma-Aldrich) at 1:200 dilution for 1 hour, Alexa-fluor 594 goat anti-rabbit IgG and Alexa-fluor 488 goat anti-mouse IgG (Molecular Probes) at 1:500 dilution for 1 hour and Hoechst 33342 (Invitrogen) at 1:10000 dilution for 5 min before imaging.

Imaging and analysis

Phase contrast and fluorescence images were obtained using a Hamamatsu ORCA high resolution charge-coupled device (CCD) camera mounted on a Nikon Eclipse Ti inverted microscope with a 20× or 40× objective. Fluorescence images were pseudocolored, overlaid and merged using IPLab, and analyzed using ImageJ.

Quantitative real time PCR

RNA was extracted from cells using either Trizol or the RNeasy mini kit (Qiagen) and cDNA was synthesized using the Superscript first strand synthesis kit (Invitrogen). Transcript levels were determined by qRT-PCR with the MiniOpticon system (BioRad) using real time SYBR Green/Rox PCR master mix (SA Biosciences) and specific primers (Supplemental Table 1). Amplification was followed by melting curve analysis to verify the presence of a single PCR product.

Constructs and transfections

shRNAs targeting the *Mus musculus* sequences of 5-integrin (NM_008397) and 6-integrin (NM_010577) were obtained from Open Biosystems (Supplemental Table 2). Control scrambled shRNA was obtained from AddGene (plasmid 1864) or Open Biosystems (RHS4346). Expression constructs for 5-integrin and 6-integrin were obtained from AddGene (plasmids 27301 and 13596). Control vectors were obtained from Invitrogen (V920–20) and from AddGene (plasmid 1764). The YFP-Rac1b plasmid and its control vector were used as previously described (Lee et al., 2012). Transfection was performed using Fugene HD (Roche) as per the manufacturer's instructions.

Immunoblotting

Samples were lysed using RIPA buffer, mixed with SDS sample buffer and reducing agent, boiled at 85°C for 2 minutes, resolved by SDS-PAGE using 4–12% Tris-Glycine gels (Invitrogen), and transferred to nitrocellulose membranes (Invitrogen). Membranes were blocked in 5% milk and incubated overnight at 4°C in blocking buffer containing antibodies specific for 5-integrin (Millipore), 6-integrin (Abcam), or -actin (Cell Signaling). Antibodies were visualized using the ECL Plus Western Blotting Detection System (GE Healthcare).

Results

Laminin-rich ECM (lrECM) inhibits MMP3-induced EMT

MMP3 induces EMT in mouse mammary epithelium both in culture and *in vivo* (Lochter et al., 1997; Radisky et al., 2005; Sternlicht et al., 1999). When grown on tissue culture-grade polystyrene (TCPS), MMP3-treated mammary epithelial cells undergo phenotypic changes including increased cell spreading and scattering (Figure 1a, b), downregulation of epithelial keratins, and upregulation of the EMT-promoting transcription factor Snail1 and the mesenchymal genes vimentin and SMA (Figure 1e–j).

To assess how MMP3-mediated EMT is affected by signals from the ECM microenvironment, we cultured cells on substrata coated with collagen I, fibronectin, laminin-rich ECM (lrECM) known as Matrigel™, or the BM components laminin-111 and collagen IV. When cultured on fibronectin, collagen I, or collagen IV, the cells formed typical epithelial clusters, similar to those cultured on uncoated TCPS, but following treatment with MMP3, cells on these substrata acquired a mesenchymal morphology and pattern of gene expression, including enhanced expression of Snail1, SMA, and vimentin, and decreased expression of keratin-14 (Supplemental Figure 1). In contrast, those cultured on lrECM or laminin-111 retained a rounded morphology, as previously described (Muschler et al., 1999; Stahl et al., 1997). Furthermore, treatment of cells cultured on laminin-111 or lrECM-coated substrata with MMP3 did not induce downregulation of cytokeratins or upregulation of mesenchymal markers, as determined by immunofluorescence staining (Figure 1c–f) or by quantitative real-time PCR (RT-PCR) analysis (Figure 1g–I; Supplemental Figure 1k–n). The expression of epithelial marker keratin-14 was elevated in cells cultured on lrECM, and even though the expression of this marker was reduced by treatment with MMP3, it was still significantly higher than in cells cultured on TCPS in the absence of MMP3 (Figure 1j), suggesting that lrECM promotes the epithelial phenotype. Taken together, these data indicate that culture on lrECM or laminin-111 blocks MMP3-induced EMT in mouse mammary epithelial cells.

MMP3 and lrECM alter integrin expression

Integrins are the primary cell receptors for the ECM (Schwartz, 2010). We previously found that $\alpha 1$ -integrin is necessary for the induction of EMT by MMP3, and that an auto-clustering mutant of $\alpha 1$ -integrin permits MMP3-induced EMT when cells are cultured in an otherwise inhibitory microenvironment (Lee et al., 2012). We therefore evaluated the levels of integrin expression in cells cultured on TCPS, which permits MMP3-induced EMT, and in those cultured on lrECM, which prevents EMT. We observed two significant changes resulting from exposure to MMP3 or culture on lrECM. First, treatment with MMP3 stimulated the upregulation of $\alpha 5$, αV , $\alpha 1$ and $\alpha 6$ integrins in cells cultured on TCPS, but not in cells cultured on lrECM (Figure 2a, b, d and Supplemental Figure 2a, b). Second, the expression of $\alpha 6$ -integrin was upregulated in cells cultured on lrECM, and the expression levels of this integrin did not change significantly in response to treatment with MMP3 (Figure 2c, d). These data led us to further investigate the role of $\alpha 6$ -integrin in lrECM-mediated inhibition of EMT and the role of $\alpha 5$ -integrin in MMP3-induced EMT.

Expression of $\alpha 6$ -integrin is required for the inhibition of MMP3-induced EMT by lrECM

To determine whether the expression of $\alpha 6$ -integrin is required for lrECM to block induction of EMT by MMP3, we used a small hairpin RNA (shRNA) approach to knock down its expression (Figure 3a, b). In contrast to untransfected cells or those transfected with a scrambled shRNA, in which lrECM prevented MMP3-induced EMT (Figure 3c, d), cells transfected with shRNA specifically targeting $\alpha 6$ -integrin (sh $\alpha 6$) upregulated Snail1 and SMA even in the presence of lrECM (Figure 3c, d). To explore whether constitutive

expression of $\alpha 6$ -integrin blocks EMT, we expressed $\alpha 6$ -integrin ectopically (Figure 4a) and monitored the progression of EMT in cells cultured on TCPS. We found that ectopic expression of $\alpha 6$ -integrin largely had no effect on the induction of EMT by MMP3 in this setting, although there was a modest attenuation in the expression of Snail1 (Figure 4b). These data suggest that $\alpha 6$ -integrin transmits signals from IrECM to block EMT, but is not sufficient to inhibit EMT in the absence of this ECM.

Expression of $\alpha 5$ -integrin is necessary for MMP3-induced EMT

Treatment with MMP3 induced EMT as well as increased the expression of $\alpha 5$ -integrin. To examine the role of $\alpha 5$ -integrin in MMP3-induced EMT, we used shRNA to decrease its endogenous expression (Figure 5a, b). In cells transfected with a scrambled shRNA, Snail1, SMA, and vimentin were all upregulated upon treatment with MMP3 (Figure 5c). In contrast, transfection with shRNA against $\alpha 5$ -integrin (sh $\alpha 5$) prevented the MMP3-induced upregulation of these same genes (Figure 5c). To investigate whether increased expression of $\alpha 5$ -integrin would be sufficient to promote EMT, we expressed $\alpha 5$ -integrin ectopically and monitored expression of EMT markers, and found no spontaneous EMT in cells cultured on IrECM (Supplemental Figure 3).

IrECM blocks Rac1b-induced EMT

MMP3 promotes EMT by inducing the expression and membrane localization of Rac1b, a highly activated splice variant of the small GTPase Rac1 (Lee et al., 2012; Radisky et al., 2005). Previous studies showed that specific microenvironmental cues could inhibit EMT by preventing association of Rac1b with the plasma membrane (Lee et al., 2012; Nelson et al., 2008). To investigate how IrECM affects this pathway, we transfected cells with a YFP-Rac1b expression construct and cultured them on IrECM. As expected, we found that IrECM prevented Rac1b localization to the cell membrane (Figure 6a–d) and inhibited EMT (Figure 6e–h), indicating that IrECM blocks EMT by altering signaling downstream of Rac1b.

$\alpha 6$ -integrin is required for IrECM to inhibit Rac1b membrane localization and induction of EMT

We next examined the effect of $\alpha 6$ -integrin expression on the membrane localization of Rac1b and EMT. In cells cotransfected with YFP-Rac1b and a scrambled shRNA, Rac1b localized to the membrane on TCPS but not on IrECM (Figure 7a, d). By contrast, in cells cotransfected with YFP-Rac1b and sh $\alpha 6$, Rac1b was localized to the membrane on both TCPS and IrECM (Figure 7b, c, e, f). Furthermore, the knockdown of $\alpha 6$ -integrin allowed Rac1b-induced EMT in cells cultured on IrECM (Figure 7g–j). These data suggest that IrECM blocks the membrane localization of Rac1b and subsequent EMT by signaling through $\alpha 6$ -integrin.

$\alpha 5$ -integrin is required for Rac1b membrane localization and induction of EMT

We then used the same approach to examine the effect of $\alpha 5$ -integrin on Rac1b membrane localization and EMT. We found that in cells cotransfected with YFP-Rac1b and a scrambled shRNA, Rac1b was localized to the membrane (Figure 8a, d). However, this localization was abrogated in cells cotransfected with YFP-Rac1b and sh $\alpha 5$ (Figure 8b, c, e, f). Furthermore, the knockdown of $\alpha 5$ -integrin prevented Rac1b-induced EMT (Figure 8g). Together, these results demonstrate that $\alpha 5$ -integrin is required for Rac1b to localize to the membrane and induce signaling that promotes EMT.

Discussion

Here we found that microenvironments rich in laminin prevent the induction of EMT by MMP3 and Rac1b, whereas those rich in stromal ECMs, including fibronectin and collagen I, act to promote MMP3-induced EMT. The mechanisms by which these ECM proteins regulate EMT appear to involve interactions with their corresponding integrin receptors. Laminin and IrECM suppress EMT while maintaining cells in a rounded shape, perhaps similar to that *in vivo*. The effects of laminin on EMT are mediated in part by $\alpha 6$ -integrin, the expression of which is increased in the presence of laminin. We also showed that $\alpha 6$ -integrin is required for sequestration of Rac1b from the cell membrane by laminin, as depleting $\alpha 6$ -integrin levels using shRNA enabled Rac1b membrane localization. It is likely that another receptor is involved, however, as the expression of $\alpha 6$ -integrin was not sufficient to block EMT in the absence of laminin. Two potential candidates are syndecan-1 and dystroglycan which bind to the same region of laminin as heparin, which inhibits laminin-mediated cell rounding (Muschler et al., 1999). Furthermore, dystroglycan anchors laminin-111 to the surface of mammary epithelial cells (Weir et al., 2006) and loss of its expression promotes aggressive tumor growth (Akhavan et al., 2012).

Why does $\alpha 6$ -integrin-mediated adhesion to laminin block EMT? It is instructive to consider the behavior of cells on mesenchymal ECM proteins (such as fibronectin), which permit EMT while simultaneously inducing cell spreading. These changes in cellular morphology are necessary for the induction of EMT by MMP3 (Nelson et al., 2008). Cell spreading and distention, which are promoted by increases in cytoskeletal tension and substratum stiffness, promote the membrane localization of Rac1b and production of ROS, which are necessary steps for MMP3-induced EMT (Lee et al., 2012). Here we found that laminin prevents cell spreading and EMT, whereas mesenchymal ECM proteins promote these phenotypic changes in mammary epithelial cells exposed to MMP3. It is therefore possible that IrECM suppresses EMT by maintaining cells in a rounded shape, preventing them from becoming mechanically stressed, and thus eliminating integrin clustering and Rac1b localization. Mesenchymal ECM proteins, by contrast, increase substratum stiffness and allow increased cellular tension, facilitating EMT progression. Consistent with this hypothesis, we previously found that the mechanical properties of the microenvironment act as important determinants for epithelial/mesenchymal phenotypes (Lee et al., 2012; Lui et al., 2012). In the experiments described above, exogenous IrECM gets deposited and consequently surrounds cultured mammary epithelial cells, creating a more compliant microenvironment than that on untreated or fibronectin-coated TCPS. It will be interesting to decouple the effects due to the biochemical properties from those due to mechanical properties of the substrata.

Treatment with MMP3 led to increased expression of $\alpha 5$ -integrin, which was necessary for promotion of EMT on mesenchymal ECM components. A role for $\alpha 5$ -integrin has been noted in mammary epithelial cells transformed by Ras and induced to undergo EMT by transforming growth factor- β (TGF β) (Maschler et al., 2005); in this system, the expression of $\alpha 5$ -integrin was required for cell survival, as treatment with function-blocking antibodies against this subunit induced apoptosis (Maschler et al., 2005). Here, we found that $\alpha 5$ -integrin was necessary for the membrane localization of Rac1b; shRNA against $\alpha 5$ -integrin blocked Rac1b localization and EMT. It is likely that $\alpha 5$ -integrin transmits biochemical signals to permit loss of epithelial phenotype.

Experiments in transgenic mice revealed that overexpression of MMP3 in mammary glands led to BM disruption, alteration of epithelial morphology, and decreased synthesis of the milk protein κ -casein (Simpson et al., 1994), though it was unclear whether these events occurred in parallel (all as a direct result of high levels of MMP3) or in series (some as a

consequence of the others). We found that MMP3-treated mammary epithelial cells remain rounded in morphology when in contact with laminin, which suggests that the changes in cellular morphology observed in transgenic mice could have resulted from loss of cellular interaction with the BM; interactions with non-laminin ECM proteins in the transgenic mice likely promoted the cellular distention *in vivo*. Consistent with this possibility, fibronectin has been shown to stimulate proliferation and to potentiate breast cancer progression, whereas BM promotes growth arrest and differentiation and maintains mammary epithelial architecture (Williams et al., 2008).

Sustained expression of MMP3 in transgenic mice was found to induce EMT, fibrosis, and eventual tumor development (Sternlicht et al., 1999). Several key steps in the signal transduction pathway can potentially be manipulated to disrupt EMT progression, including identifying the proteolytic targets of MMP3, blocking the membrane localization of Rac1b, and blocking the production of ROS and expression of Snail1 (Orlichenko and Radisky, 2008). The results presented here suggest a new target for intervention of MMP-mediated EMT and tumor progression: simply maintaining normal epithelial morphology and contact with the compliant BM could protect epithelial cells from undergoing pathological transdifferentiation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by the NIH (GM083997, CA128660, and HL110335), Susan G. Komen for the Cure, the David & Lucile Packard Foundation, the Alfred P. Sloan Foundation, and the Camille & Henry Dreyfus Foundation. C.M.N. holds a Career Award at the Scientific Interface from the Burroughs Wellcome Fund.

Abbreviations

SMA	alpha-smooth muscle actin
BM	basement membrane
ECM	extracellular matrix
EMT	epithelial-mesenchymal transition
EMyT	epithelial-myofibroblast transition
lrECM	laminin-rich ECM
MMP3	matrix metalloproteinase-3
ROS	reactive oxygen species
TGF	transforming growth factor-beta

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Figure 1.

IrECM inhibits MMP3-induced EMT. (a–d) Immunofluorescence images of control and MMP3-treated mammary epithelial cells cultured on uncoated TCPS or with IrECM, stained for cytokeratins (red), SMA (green), and nuclei (blue). (e) Quantification of relative levels of cytokeratins as determined by immunofluorescence staining. (f) Number of SMA-positive cells per 10^5 cells. (g–j) Quantification of mRNA levels of EMT markers as assessed by RT-PCR. Error bar = s.e.m. for 3 or more experiments. Fold change = MMP3-treated/control. Scale bar = 50 μm. * $p < 0.05$, ** $p < 0.01$.

Figure 2.

MMP3 and IrECM alter integrin expression. (a–c) Quantification of mRNA levels of integrin subunits by RT-PCR. Error bar = s.e.m. for 3 or more experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (d) Relative levels of integrin subunits in cells treated with or without MMP3 and IrECM, as determined by immunoblot analysis.

Figure 3. Expression of $\alpha 6$ -integrin is required for the inhibition of MMP3-induced EMT by IrECM. (a) Quantification of shRNA-mediated knockdown of $\alpha 6$ -integrin as determined by RT-PCR. (b) Relative levels of $\alpha 6$ -integrin in control and sh $\alpha 6$ -expressing cells, as determined by immunoblot analysis. (c–d) Quantification of mRNA levels of EMT markers in cells transfected with nothing, scrambled shRNA, or sh $\alpha 6$, as determined by RT-PCR. Error bar = s.e.m. for 3 or more experiments. * $p < 0.05$.

Figure 4.

Ectopic expression of $\alpha 6$ -integrin blocks induction of Snail1, but has no effect on other markers of EMT. (a) Relative levels of $\alpha 6$ -integrin in control and transfected cells, as determined by immunoblot analysis. (b) Quantification of mRNA levels of EMT markers in cells transfected with an empty vector or ectopically expressing $\alpha 6$ -integrin, as determined by RT-PCR. Error bar = s.e.m. for 3 or more experiments. * $p < 0.05$.

Figure 5.

Expression of α5-integrin is necessary for MMP3-induced EMT in cells cultured on TCPS. (a) Quantification of shRNA-mediated knockdown of α5-integrin as determined by RT-PCR. (b) Relative levels of α5-integrin in control and sh α5-expressing cells, as determined by immunoblot analysis. (c) Quantification of mRNA levels of EMT markers in cells transfected with a scrambled shRNA or sh α5 as determined by RT-PCR. Error bar = s.e.m. for 3 or more experiments. * $p < 0.05$, *** $p < 0.001$.

Figure 6.

IrECM inhibits Rac1b-induced EMT. (a–d) Immunofluorescence images of GFP-(control vector) and YFP-Rac1b-transfected mammary epithelial cells cultured on uncoated or IrECM-coated substrata. Arrows indicate Rac1b membrane localization. (e–h) Quantification of mRNA levels of EMT markers in cells transfected with GFP or YFP-Rac1b, as determined by RT-PCR. Error bar = s.e.m. for 3 or more experiments. Scale bar = 25 μ m. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 7.

6-integrin is required for inhibition of Rac1b membrane localization and induction of EMT. (a–f) Immunofluorescence images of cells cotransfected with YFP-Rac1b and a scrambled shRNA or sh 6 cultured on TCPS and on IrECM. Arrows indicate Rac1b membrane localization. (g–j) Quantification of mRNA levels of EMT markers as determined by RT-PCR. Error bar = s.e.m. for 3 or more experiments. Fold change = Rac1b/GFP. Scale bar = 25 μ m. * $p < 0.05$, ** $p < 0.01$.

Figure 8.

5-integrin is required for Rac1b membrane localization and induction of EMT. (a-f) Immunofluorescence images of cells cotransfected with GFP (control vector) or YFP-Rac1b and a scrambled shRNA or sh 5, cultured on TCPS or on IrECM. Arrows indicate Rac1b membrane localization. (g) Quantification of mRNA levels of EMT markers by RT-PCR. Error bar = s.e.m. for 3 or more experiments. Fold change = Rac1b/GFP. Scale bar = 25 μ m. * $p < 0.05$, ** $p < 0.01$.