Reduction of Insulin-like Growth Factor I Receptors in MCF-7 Breast Cancer Cells Leads to a More Metastatic Phenotype

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ABSTRACT

Several lines of evidence suggest an important role for the insulin-like growth factor system in breast cancer. Alterations in insulin-like growth factor I receptor (IGF-IR) have been associated with breast cancer metastasis; however, the specific role played by the IGF-IR in this process remains unclear. To address this issue, we evaluated MCF-7 breast cancer cells stably transfected either with an antisense construct to the IGF-IR, which reduces the expression of the IGF-IRs by ~50% (SX13 cells), or with the empty vector as control (NEO cells). Using functional assays for motility, attachment, and aggregation, we found a 3-fold increase in migration using both the wounding assay and the Boyden chamber migration assay. In addition, the SX13 cells attached less, and there was a reduction in cellular aggregation. These functional changes were accompanied by ~50% decrease in expression of E-cadherin and ~80% increase in p120 protein levels. Moreover, there was a significant reduction in p120 present in the E-cadherin-catenin-p120 complex. There was a 2-fold increase in active Rac1 and Cdc42 and a 35% decrease in active Rho in the SX13 cells. Our findings strongly suggest that IGF-IR plays a role in the stabilization of the E-cadherin-catenin complex, thereby providing one possible explanation for the association between low levels of IGF-IR and a higher risk of mammary tumor metastasis.

INTRODUCTION

The IGF-IR is a member of the tyrosine kinase receptor superfamily. Its ligands (IGF-I and IGF-II) are potent mitogenic and antiapoptotic molecules involved in the regulation of cell proliferation and are important mitogenic factors for breast cancer cells. Whereas recent studies have suggested a role for circulating IGF-I in colon cancer metastasis, the role of the IGF-IR in the metastasis of breast cancer cells has not been fully elucidated.

Whereas inhibition of IGF-IR signaling restricts breast cancer cell growth both in vitro and in vivo, very low levels of IGF-IR in breast cancer cells are also associated with a higher risk and a less favorable clinical prognosis. Moreover, overexpression of IGF-IRs in human breast cancer cell lines inhibits cell scattering, even though it also increases the proliferative response to IGF-I in the presence of estradiol.

Adherens junctions, where E-cadherin molecules of adjacent epithelial cells contact each other, are important in the maintenance of normal differentiation in epithelial cells. Disrupted cell-cell adhesion is often observed in cancer cells, and loss of E-cadherin from epithelial tumor cells is correlated with an invasive phenotype. E-cadherin is a member of a superfamily of single transmembrane domain glycoproteins that mediate calcium-dependent cell-cell interaction and provide sites for attachment to the actin cytoskeleton. The cytoplasmic domain of E-cadherin associates with catenins. β-Catenin and γ-catenin (plakoglobin) interact directly with the COOH-terminal domain of the E-cadherin, and both proteins associate with α-catenin, which, in turn, associates directly or indirectly with actin filaments. β-Catenin also plays a key role in the wnt/wingless signaling pathway. The binding of wnt to its receptor triggers a cascade of events that leads to accumulation of β-catenin in the cytoplasm and the nucleus, where it interacts with several transcription factors. Thus, the amount of β-catenin available for nuclear signaling is influenced both by wnt signaling and by the amount of β-catenin bound to E-cadherin. Decreased turnover of the cytoplasmic pool of β-catenin, activation of the nuclear complex, and increased levels of free β-catenin have been associated with malignancy, supporting the potential role of β-catenin signaling in cancer.

An increase in the cytoplasmic pool of p120 would be predicted to decrease RhoA activity but increase Rac1 and Cdc42 activity, thereby promoting cell migration.

In this paper, we studied the role of the IGF-IR in cell-cell adhesion and motility. To this purpose, we used MCF-7 breast cancer cells that expressed a reduced number of IGF-IRs due to stable transfection of an antisense construct to the IGF-IR. Our results suggest that IGF-IR plays an important role in maintaining a noninvasive phenotype.

MATERIALS AND METHODS

Cell Culture and Materials. MCF-7 cells, originally obtained from American Type Culture Collection (Manassas, VA), were stably transfected with an antisense IGF-IR cDNA (clones SX13 and SX8) and with the empty vector (NEO) and maintained as described previously.

Cell Migration Assay. NEO and SX13 cells were grown in 75-cm² tissue culture flasks (Falcon; Becton Dickinson Labware, Franklin Lakes, NJ) to 80% confluence. Cells were serum-starved overnight in IMEM without phenol red and harvested with dissociation solution (Sigma, St. Louis, MO). Six-well transwell chambers (BD Biosciences, San Diego, CA) with 8.0-µm pore size polycarbonate membrane were incubated with IMEM containing 0.01% bovine serum albumin and 0.01% FBS overnight. Cells (1 × 10³) were added to the upper well, which was placed into a lower well containing IMEM and 10% FBS as a chemoattractant. After 18–24 h of incubation at 37°C in 5% CO₂, cells remaining on the upper membrane surface were removed with a cotton swab. Cells on the lower surface of the filter were fixed and stained with Diff-Quik (Dade-Behring, Newark, DE). Five fields of adherent cells were counted.

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randomly counted in each well with a Nikon Diaphot-TMD inverted microscope at 10× magnification, and the results were numerically averaged.

**Wounding Assay.** Wounding assays were performed using a modification of the procedure described by Jones *et al.* (26). Briefly, two well chambers (LabTek; Nalge Nunc, Naperville, IL) were prepared by scratching registration marks onto the slide surface. NEO and SX13 cells were plated, grown normally for 48 h, and starved overnight. Cells were cut with a sterile razor, and five images were captured along the cut surface on a Zeiss Axiohot TV-100 (Carl Zeiss Inc., Thornwood, NY) equipped with a PentaMAX (Roper Scientific, Trenton, NJ) camera using a 20× objective. Additional images were captured 48 h later. For each experiment, the number of migrating NEO cells/μm cut membrane was set to 100%, and the number of migrating SX13 cells/μm cut membrane was compared with this number (percentage of SX13 cells/100 cells). Adhesion Assay. Adhesion to an uncoated surface was performed using a 48-well plate. Cells were starved overnight, treated with dissociation solution (Sigma), and resuspended in IMEM without phenol red plus 0.2% FBS. Cells (1.5 × 10^5) were plated in each well and allowed to attach for 5 h at 37°C. Time course (0.5, 1, 2, 3, and 5 h) and growth curve were performed to choose the appropriate time for the assay, and 5 h was chosen to avoid differences in growth.

After 5 h of incubation, floating cells were carefully aspirated, transferred to tubes, and spun, and both plates and tubes were washed with PBS and fixed in 5% formaldehyde. The fixed cells were then washed and allowed to dry. Dried fixed cells were stained using crystal violet (0.1%, w/v) in 20 mM 4-morpholinepropanesulfonic acid (Sigma) and then solubilized using 100 μl of 10% acetic acid. Absorbance was measured using an ELISA reader, and the percentage of attached cells was calculated.

**Growth Curve.** Cells (7.5 × 10^3) were plated in 6-well plates and allowed to attach and grow in regular medium, harvested after trypsinization, and counted at different time points (6, 20, 24, 30, and 48 h). Each condition was performed in quadruplicates.

**Aggregation Assay.** Poly-heme solution (12 mg/ml in ethanol; Sigma) was placed in a 48-well plate (95 μl/well) and allowed to dry. After overnight starvation, cells were treated with dissociation buffer and washed. Cells (1 × 10^6) were plated in each well in IMEM without phenol red plus 0.1% FBS and cultured for 48 h, and multiple fields were photographed in at least three experiments.

**Determination of Surface Integrins by Flow Cytometry.** Flow cytometry was used to measure cell surface integrins in NEO and SX13 cells. Cells were dispersed using dissociation solution (Sigma). Cells (1 × 10^6) were resuspended in fluorescence-activated cell-sorting buffer 1 and incubated with 15 μg/ml primary antibody (BD PharMingen, San Diego, CA). After washes, cells were incubated with 40 μg/ml FITC antimouse IgG (BD PharMingen). Fluorescence intensity was measured using a FACS calibur with CellQuest software (BD Biosciences, San Jose, CA).

**Immunofluorescence.** Cells were stained for immunofluorescence as described previously (27). Rabbit anti-IGF-IR β antibodies (C-20) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and used at 7.5 μg/ml. Mouse anti-E-cadherin antibody (HECD-1) was obtained from Zymed Laboratories, Inc. (San Francisco, CA) and used at 15 μg/ml. Secondary antibodies conjugated to Alexa488 and to Alexa568 were from Molecular Probes, Inc. (Eugene, OR) and were used at 1:2000 dilutions. Cells were viewed in a Zeiss 410 confocal microscope (Carl Zeiss Inc.). Adobe Photoshop (Adobe Systems Inc., Mountain View, CA) was used to process the images.

**Subcellular Fractionation and Immunoblotting.** Cell lysates and subcellular fractions were prepared as described previously (25, 28). Protein concentration was determined with the BCA reagent (Pierce, Rockford, IL). Proteins were resolved by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked and probed overnight at 4°C with various antibodies as indicated in the figure legends. After washes, membranes were incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies. The detection of the immunocomplexes was performed using the enhanced chemiluminescence kit (ECL; New England Nuclear Life Science Products Inc., Boston, MA.). Densitometry was performed by scanning the radiographs, and the bands were analyzed with MacBas version 2.52 software (Fuji PhotoFilm; Master Scan System, Scanalitics, Billerica, MA).

**Activated RhoA, Rac1, and Cdc42 Assay.** The activities of Rac1, Cdc42, and RhoA were studied as described previously (29). Active Rac1 and Cdc42 were analyzed with GST fusion proteins of the Cdc42 and Rac1 interactive binding domain from PAK1, which binds only active Cdc42 and Rac1 kindly provided by Dr. R. A. Cerione (Department of Molecular Medicine, Cornell University, New York, NY). For active RhoA, a GST fusion protein was used containing residues 7–89 of murine rhotekin, which binds active RhoA (30), generously provided by Dr. K. Burridge (Department of Cell and Developmental Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC). Proteins were resolved by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were immunoblotted using antibodies for Cdc42, Rac1, or RhoA (Santa Cruz Biotechnology). Detection and analysis of the immunocomplexes were performed as described in “Subcellular Fractionation and Immunoblotting.”

**Immunoprecipitation.** Cell lysates were spun at 120,000 × g for 30 min, and supernatants containing 400 μg of total protein were mixed with 4 μg of either αrR3 (anti-IGF-IR antibody) or monoclonal anti-E-cadherin (BD Transduction Laboratories, San Diego, CA) antibodies and incubated at 4°C overnight. The immunocomplexes were precipitated with 30 μl of protein G-Sepharose (Amersham Pharmacia Biotech, Uppsala, Sweden) for 2.5 h at 4°C and spun at 12,000 × g for 5 min. After several washes, pellets were boiled for 5 min in reducing Laemmli buffer. Proteins were resolved by SDS-PAGE and transferred to nitrocellulose membranes. Blots were blocked and probed with several antibodies as indicated in the figure legends. After washings, immunoreactivity was detected and analyzed as described above. When appropriate, Western blotting was performed on whole cell lysates using 40 μg of protein.

**RESULTS**

**Aggregation and Adhesion Are Decreased, and Motility Is Enhanced in MCF-7 Cells Stably Transfected with an Antisense Construct for the IGF-IR.** Human MCF-7 breast cancer cells express a relatively high number of IGF-IRs and show a noninvasive

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**Fig. 1.** Increased migration of MCF-7 cells with reduced expression of IGF-IR. A, immunoblot analysis of nonstarved (time 0) and starved (24 h) NEO and SX13 cells. Blots were probed overnight at 4°C with a polyclonal antibody against the β subunit of the IGF-IR. No changes in the levels of IGF-IR were observed after 24 h of serum starvation. B, migration assay. Starved NEO and SX13 cells were seeded on top of Boyden chambers and allowed to migrate in the absence or presence of 10% FBS as a chemoattractant placed in the bottom chamber. There was a 3-fold increase in the number of migrating SX13 cells compared with NEO cells, with no random migration in the absence of chemoattractant. Results are expressed as the mean ± SE derived from three experiments performed in triplicates.
phenotype (31). Stable transfection of a vector containing the IGF-IR cDNA in an antisense direction (SX13 cells) leads to a decrease in the protein levels of the IGF-IR compared with the control (MCF-7 cells transfected with the empty vector NEO cells). As shown previously (6), NEO cells have approximately 50% more IGF-IR protein than SX13. Because many experiments required serum starvation, we also checked the level of IGF-IR in both cell lines after 24 h of serum starvation. Serum starvation did not affect the amount of IGF-IR (Fig. 1A).

We used Boyden chambers and monolayer wounding migration assays to study the effect of decreased numbers of IGF-IRs on motility in MCF-7 cells. Fig. 1B shows that neither NEO nor SX13 cells exhibited migration in the absence of chemoattractant in the bottom chamber. When 10% FBS was used as a chemoattractant, both NEO and SX13 cells migrated to the bottom of the membrane. However, the number of SX13 cells migrating toward 10% FBS was 3-fold greater than that in NEO cells, and this difference persisted and increased further over a period of 48 h (data not shown).

SX13 cells also exhibited greater migration than NEO cells in a wounding assay (Fig. 2). The number of cells/μm cut edge that had migrated 150 μm from the cut was counted. To avoid variability in the extent of migration, the number of migrating NEO cells in medium without IGF-I was set to 100%. The extent of migration under other conditions is given as the increase in migration relative to the NEO cells. Fig. 2A and B, shows an example of migrating NEO cells in the absence of IGF-I, whereas Fig. 2C and D, shows the migration of SX13 cells. There was an average increase in migration of 330% for SX13 cells (range, 130–660%) under basal conditions (Fig. 2E). In the presence of 100 ng/ml IGF-I, both NEO and SX13 cells increased their motility to about 500% more than unstimulated NEO cells (data not shown).

The integrin expression profile is often altered in transformed cells, and these changes are correlated with increased invasiveness (32, 33). Thus, we decided to analyze the profile of surface integrins of NEO and SX13 cell by fluorescence-activated cell sorting. Interestingly, there was no change in either the integrin subtypes or in the levels of integrins measured in SX13 cells compared with NEO cells (Table 1).

We next evaluated the ability of these cells to attach to a plastic tissue culture dish. To address the possibility that differences in the growth rates of SX13 and NEO cells could affect the interpretation of these results, we first performed a growth curve over 48 h to determine the optimal time to perform this assay. There was a statistical difference in number of cells after 24 h, suggesting that the growth rate of SX13 cells was decreased at this time (data not shown). A shorter time course was then performed to further optimize the best time to evaluate cell adhesion. Because a plateau was reached after 1 h of incubation in both SX13 and NEO cells (Fig. 3A) and because there was no difference in cell growth between SX13 and NEO cells.

Fig. 2. Increased migration of SX13 cells in the wounding assay. A, NEO cells at time 0. B, NEO cells at 48 h. The blue line shows the line cut at time 0, whereas the red line shows a distance of 150 μm from the original cut. The star indicates a representative registration mark. C, SX13 cells at time 0. D, SX13 cells at 48 h. The blue line shows the line cut at time 0, whereas the red line shows a distance of 150 μm from the original cut. The registration marks are beyond the portion of the image shown here. E, quantification of the wounding assays. The number of cells that had migrated 150 μm from the cut were counted and corrected for the μm of cut edge examined (number of cells/μm edge). The number of migrating NEO cells in medium without IGF-I was set to 100%. The number of migrating cells under other conditions is given as the percentage relative to the NEO cells. Results are expressed as the mean ± SE of three separate experiments.
between 30 min and 5 h, 5 h was chosen as the final time for this assay. Under basal conditions, a significantly lower percentage of SX13 cells attached to the plastic culture dish surface, as compared with NEO cells (Fig. 3B).

It has been reported previously that in MCF-7, overexpression of IGF-IR stimulates the ability to form globular aggregates characteristic of a noninvasive phenotype (8). As shown in Fig. 4, aggregation was decreased under basal conditions in SX13 cells, as compared with NEO cells.

Characterization of E-Cadherin Complexes, Localization, and Physical Interaction between E-Cadherin and IGF-IR. Loss or deactivation of adherens-type cell junctions has been associated with acquisition of metastatic phenotype (34). To investigate the mechanism by which aggregation is decreased in SX13 cells, total cellular levels of E-cadherin, β-catenin, and p120 catenin were measured by immunoblotting. As shown in Fig. 5A, E-cadherin levels were decreased (top panel), β-catenin levels were unchanged (middle panel), and p120 catenin levels were increased in SX13 cells (bottom panel), compared with NEO cells.

It has been reported that E-cadherin and the IGF-IR colocalize when IGF-IR is overexpressed (8). Immunofluorescence confocal microscopy revealed extensive colocalization of E-cadherin and IGF-IR in both NEO and SX13 cells at the cell surface, but only along the sites of cell-cell contact (Fig. 6). Interestingly, IGF-IR was only seen at these sites of cell-cell contact, although the predicted label would be the entire cell surface, including the free edges. Both proteins were also present in intracellular pools that do not colocalize. Thus, it appears that E-cadherin and IGF-IR form complexes where cells form contacts with each other.

To further investigate whether IGF-IR physically interacts with E-cadherin, we immunoprecipitated E-cadherin from cell lysates and immunoblotted these samples for IGF-IR, β-catenin, and p120 catenin (Fig. 7A). Immunoreactivity levels for IGF-IR and p120 catenin were significantly lower in SX13 cells, whereas the change in β-catenin levels was proportional to the change in E-cadherin levels (Fig. 7B). However, the amount of β-catenin found in the cytosolic fraction of SX13 cells was increased compared with that in the cytosolic fraction of NEO cells (19% versus 11%). The increase in the total amount of p120 catenin and the decrease in the amount of this protein that coimmunoprecipitated with E-cadherin suggest that there are absolute increased levels of free, cytoplasmic p120 catenin in SX13 cells. When the IGF-IR was immunoprecipitated from cell lysates, E-cadherin was coimmunoprecipitated, as determined by immunoblotting (data not shown). Similar results were obtained with a different clone stably transfected with antisense IGF-IR (SX8 cells; data not shown).

RhoA, Rac1, and Cdc42 Activity in MCF-7 Cells Stably Transfected with an Antisense for IGF-IR. We next measured the levels of active RhoA, Rac1, and Cdc42 using GST fusion proteins with binding domains that bind only activated forms of these GTPases. As shown in Fig. 8, there was an increase in the levels of active Cdc42 and active Rac1 in SX13 cells compared with NEO cells. In contrast, the levels of active RhoA were lower in SX13 cells. Serum starvation for 24 h had no effect on the activation state of any of these GTPases.

DISCUSSION

The initial process in the metastatic spread of breast carcinomas involves the invasion of malignant cells through the extracellular matrix of a basement membrane, followed by their migration into lymphatic or vascular channels (35, 36). The acquisition of migratory properties and weakening of cell-cell adhesion are imperative for tumor cell metastasis. Although several studies (3, 7) have suggested a role for the IGF system in the regulation of breast cancer growth, the impact of IGR-IR on the progression of the disease remains undefined.

Although MCF-7 breast cancer cells are poorly invasive (31), we show that a decrease in the expression of IGR-IR in these cells leads to increased motility and decreased ability to form aggregates in culture, resulting in characteristics consistent with a more metastatic phenotype. Because crossing a reconstituted basement membrane barrier is correlated with metastatic ability (37), measuring cell migration in Boyden chambers is a reliable method to test motility and invasiveness (35). Our experiments using modified Boyden chambers showed an increase in the migration of SX13 cells in response to FBS, but not in the absence of chemotractant. We obtained similar results

| Table 1 Profile of integrins in NEO and SX13 cells |

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Fluorescence-activated cell-sorting analysis was used to measure cell surface integrins in NEO and SX13 cells. A mouse IgG1 isotype antibody was used to determine background staining. + or - indicates presence and quantitation of each integrin tested. There were no changes in the type or the amount of integrins expressed at the cell surface of SX13 compared with NEO cells.
using a monolayer wounding migration assay, further supporting the conclusion that changes in IGF-IR expression alter the motility properties of MCF-7 cells. Surprisingly, in our model there was no change in the integrin profile that could contribute to the increased motility and decreased attachment found in these cells, despite the importance of integrins in adhesion and migration. Whereas connections between components of insulin/IGF-I and integrin signaling pathways have been documented (38, 39), the contribution of specific extracellular matrix molecules and their integrin receptors to malignant transformation in vivo remains largely unknown. Other studies have shown that changes in integrins are not sufficient for cell migration and that the activation of cytokine or growth factor signal transduction pathways is also required (40). Thus, changes in the expression of integrins are not necessarily a requirement for changes in migration and adhesion to occur.

The continued expression and functional activity of E-cadherin are required for cells to remain tightly associated in the epithelium. In the absence of E-cadherin, many other cell adhesion and cell junction proteins expressed in epithelial cells are not capable of supporting intercellular adhesion (41). The majority of epithelial tumors show reduced E-cadherin expression, and a correlation between reduced E-cadherin expression, loss of tumor differentiation, and increased
invasiveness has been found (34). Several mechanisms have been proposed for the inactivation of cadherin-mediated cell-cell adhesion in human cancer. These include redistribution of E-cadherin protein (42, 43), down-regulation of cadherin expression or mutations within the gene (18, 44), biochemical modification by phosphorylation of components in the cadherin complex (45), or changes in the activation state of small GTPases Rac1 and RhoA (46, 47). A typical honeycomb distribution pattern of E-cadherin (31) was conserved in SX13 cells, suggesting that there was no redistribution of E-cadherin in our model. However, these cells did exhibit a 50% decrease in E-cadherin levels and a decreased ability to form aggregates in culture, presumably because of the loss of E-cadherin.

Previously, Guvakova and Surmacz (8) have shown an interaction between IGF-IR and E-cadherin in MCF-7 cells overexpressing IGF-IR, upon stimulation with IGF-I. These authors proposed that either catalytic function of the receptor or clustering of IGF-IR due to overexpression could be involved in the promotion of aggregation. We have extended these studies to show colocalization of IGF-IR and E-cadherin in cells with normal or reduced expression of IGF-IR even in the absence of IGF-I stimulation. Coimmunoprecipitation experiments provided further evidence for the formation of a complex containing IGF-IR, E-cadherin, and other members of the cadherin complex such as β-catenin and p120 catenin. To our knowledge, this is the first report showing ligand-independent interaction between the IGF-IR and a cell-cell adhesion molecule such as E-cadherin.

Whereas the expression of E-cadherin was reduced in SX13 cells, the total amount of p120 catenin was increased, and β-catenin was shifted from the membrane to the cytoplasmic compartment. Moreover, decreased amounts of β-catenin and even smaller amounts of p120 catenin were coimmunoprecipitated with E-cadherin in SX13 cells, as compared with NEO cells. These results suggest that in SX13, decreased levels of IGF-IR lead to a decreased E-cadherin complex formation. Thus, because both cytoplasmic β-catenin levels and p120 catenin levels were increased, there was a net increase in free β-catenin available for signaling in SX13 cells as well as an increase in the cytoplasmic pool of p120 catenin.

The effect of an increase in the free pool of β-catenin on gene transcription has been well documented (36, 48). Recently, it has been demonstrated that overexpression of the integrin-linked protein kinase p59(ILK) stimulates the action of the β-catenin and lymphocyte enhancer factor/T-cell factor complex, causing a down-regulation of E-cadherin gene expression (18). In our model, this could be one possible feedback loop that would accelerate the loss of E-cadherin.

In contrast to the well-known role of β-catenin, the function of p120 catenin remains controversial. Although it has been suggested that p120 catenin plays a critical role in the regulation and stabilization of adhesion, conflicting data have been reported regarding the nature of this effect (49, 50). Recently, Noren et al. (24) proposed a model in which p120 catenin can shuttle between a cadherin-bound state and a cytoplasmic pool in which it can interact with regulators of the Rho family of GTPases. Our findings are compatible with this model. In SX13 cells, the decreased levels of p120 catenin found

Fig. 6. IGF-IR and E-cadherin colocalize in both NEO and SX13 cells. NEO and SX13 cells were fixed, permeabilized, and stained with a mixture of rabbit anti-IGF-IR and mouse anti-E-cadherin antibodies, followed by Alexa488-conjugated goat antirabbit and Alexa568-conjugated goat antimouse antibodies as described in "Materials and Methods." A, IGF-IR immunostaining in NEO cells. B, E-cadherin immunostaining in NEO cells. C, merged image with IGF-IR shown in green and E-cadherin shown in red. D, IGF-IR immunostaining in SX13 cells. E, E-cadherin immunostaining in SX13 cells. F, merged image with IGF-IR shown in green and E-cadherin shown in red. In both cell lines, there is extensive colocalization of the two proteins along the areas of cell-cell contact.
within the E-cadherin complex and the resulting increase of its "unbound" pool were accompanied by an increase in Rac1 and Cdc42 activities as well as a decrease in RhoA activity, as compared with NEO cells. Rac1, Cdc42, and RhoA activities determine cellular motility characteristics. The changes in the activities described here have been associated with enhanced cell motility (51, 52) and a decrease in assembly of stress fibers with increased migration (53).

In sum, our findings provide at least a partial explanation for the

Fig. 7. IGF-IR interacts with the E-cadherin-catenin complex. A, whole cell lysates from nonstarved (time 0) or starved (24 h) NEO and SX13 cells were immuno-precipitated with an antibody against E-cadherin, followed by immunoblot analysis for E-cadherin, IGF-IR, β-catenin, and p120 catenin. IGF-IR was present in precipitates from both SX13 and NEO cells. There was a decreased amount of IGF-IR and p120 catenin present in precipitates from SX13 cells compared with NEO cells, with a proportional change in the amount of β-catenin. B shows the ratio between the amount of IGF-IR (left panel), β-catenin (middle panel), and p120 catenin (right panel) detected in the precipitates and the amount of E-cadherin coprecipitated in the same experiment. Each bar represents the mean ± SE derived from three separate experiments.

Fig. 8. Differential activation of small GTPases of the Rho family in SX13 cells. A, 500 µg of cell lysates from nonstarved (0) and starved (24 h) SX13 and NEO cells were incubated with glutathione-Sepharose beads containing the Cdc42, Rac1, and RhoA interactive binding domains that bind only active Cdc42, Rac1, and RhoA, followed by immunoblot analysis. Alternatively 40 µg of protein obtained from whole lysates of non-starved (time 0) and starved (24 h) NEO and SX13 cells were analyzed. In both cases, the membrane antibodies for Cdc42, Rac1, or RhoA were used for immunoblotting. There was an increase in the active forms of Cdc42 (top panel) and Rac1 (middle panel) and a decrease in the activity of RhoA (bottom panel) in SX13 cells compared with NEO, with no changes in the total amount of these small GTPases. B shows the ratio between the active and the total protein tested in the same sample. Each bar represents the mean ± SE derived from three separate experiments.
enhanced motility seen in SX13 cells. E-cadherin-mediated cell-cell contact formation is accompanied by sequestration of β-catenin and p120 catenin to the junction regions. We propose (Fig. 9A) that in MCF-7 cells under basal conditions, IGF-IR is also required for the formation of fully functional and stable E-cadherin-catenin complexes. E-cadherin-mediated cell-cell contact formation is accompanied by sequestration of β-catenin and p120 catenin to the junction regions, which results in strong cell-cell contacts. A decrease in IGF-IR expression (B) leads to a decreased amount of the E-cadherin-catenin complexes and to changes in the stoichiometry of these complexes. As a consequence, β-catenin and p120 catenin are shifted into the cytosol, where increased levels of p120 catenin result in differential activation of Rho family members and enhanced motility. Increased free β-catenin could down-regulate E-cadherin gene expression → indicates inhibition, ↔ indicates balance, ↑ indicates possible mechanism or pathway, ↑ indicates increase, ↓ indicates decrease, ↔ indicates shifted equilibrium.

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