Transactivation of CXCR4 by the Insulin-like Growth Factor-1 Receptor (IGF-1R) in Human MDA-MB-231 Breast Cancer Epithelial Cells*

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In the multimolecular environment in tissues and organs, cross-talk between growth factor and G protein-coupled receptors is likely to play an important role in both normal and pathological responses. In this report, we demonstrate transactivation of the chemokine receptor CXCR4 by the growth factor insulin-like growth factor (IGF)-1 is required for IGF-1-induced cell migration in metastatic MDA-MB-231 cells. The induction of chemotaxis in MDA-MB-231 cells by IGF-1 was inhibited by pretreatment of the cells with pertussis toxin (PTX) and by RNAi-mediated knockdown of CXCR4. Transactivation of the CXCR4 pathway by IGF-1 occurred independently of CXCL12, the chemokine ligand of CXCR4. Neither CXCR4 knockdown nor PTX had any effect on the ability of IGF-1 to activate IGF-1R, suggesting that CXCR4 and G proteins are activated subsequent to, or independently of, phosphorylation of IGF-1R by IGF-1. Coprecipitation studies revealed the presence of a constitutive complex containing IGF-1R, CXCR4, and the G protein subunits, G_α_i and G_β_γ, and stimulation of MDA-MB-231 cells with IGF-1 led to the release of G_α_i and G_β_γ from CXCR4. Based on our findings, we propose that CXCR4 constitutively forms a complex with IGF-1R in MDA-MB-231 cells, and that this interaction allows IGF-1 to activate migrational signaling pathways through CXCR4, G_α_i and G_β_γ.

The G protein-coupled receptor (GPCR) CXCR4 is the receptor for the chemokine CXCL12. Both molecules are essential for life, with genetic deletion in mice of either CXCR4 or CXCL12 resulting in a lethal phenotype (1–3). Activation of CXCR4 by CXCL12 has been implicated in the homeostasis and activation of the immune system, and influences a range of other biological systems under both normal and pathological conditions (4–6). These include angiogenesis (7–9), cell survival (10, 11), and more recently, tumor growth and metastasis (12–14). Indeed, it has recently been shown that CXCR4 is expressed in breast cancer tissues and cell lines, and that CXCL12 is expressed in several target organs of breast cancer metastasis (13). Additionally, treatment of mice with neutralizing Abs against CXCR4 inhibits metastasis in a mouse model of breast cancer, as does RNAi-mediated knockdown of CXCR4 on orthotopically transplanted breast carcinoma cells (12, 13). These data point to an important role for CXCR4 in cancer.

The cellular signal transduction pathways induced by CXCL12 have been well characterized in leukocytes. Interaction of CXCL12 with CXCR4 leads to the release of the G protein subunits G_α_i and G_β_γ from intracellular domains of CXCR4. These subunits then bind and activate downstream enzyme systems including phospholipase C, which leads to a transient increase in the level of intracellular Ca^{2+}, and phosphoinositide 3-kinase (PI3K), which results in activation of Akt and subsequently, cell migration (15–17). In contrast, the role of CXCR4, including characterization of signal transduction mechanisms in cell types other than leukocytes is less well established despite the fact that CXCR4 is expressed in most tissues and organs.

Cross-talk between GPCRs and growth factor receptor-tyrosine kinase (RTKs) induced signaling pathways has become increasingly well documented in different cellular systems. For example, EGFR is tyrosine-phosphorylated in response to CCL11, a ligand for the GPCR CCR3, leading to MAP kinase activation and IL-8 production in bronchial epithelial cells (18). In rat aortic vascular smooth muscle cells, both PDGFR and EGFR are phosphorylated by sphingosine 1-phosphate (S1P), a lipid mediator that is a ligand for the S1PR family of GPCRs, leading to activation of effectors downstream of PDGFR and EGFR including Shc, and the p85 regulatory subunit of the class IA PI3K (19). In contrast, examples of transactivation of GPCRs by RTKs are less abundant, although recently it has been shown that IGF-1 stimulated phosphorylation of CCR5 in MCF-7 cells. Chemotaxis induced by IGF-1 was inhibited by a neutralizing anti-CCL5 antibody, which indicates that transactivation of CCR5 by IGF-1 is indirect, requiring production of a CCR5 ligand (20).

Because investigating interactions between different receptor classes is essential for our understanding of the mechanisms by which cells process multiple signaling inputs, we have examined potential cross-talk in the signal transduction pathways induced following ligation of CXCR4 and IGF-1R. Our data demonstrate the existence of a physical association between IGF-1R, CXCR4, and the G protein subunits, G_α_i and G_β_γ in the breast cancer epithelial cell lines, MDA-MB-231. This interaction drives a unidirectional transactivation of CXCR4 and G proteins by IGF-1 leading to cell migration in MDA-MB-231 cells, which is independent of the CXCR4 chemokine ligand, CXCL12. These data indicate the existence of a novel form of transactivation between these two important receptors.

**MATERIALS AND METHODS**

**Cell Lines and Cell Culture Conditions**—Breast cancer cell lines, MCF-7 and MDA-MB-231, were obtained from the American Type Culture Collection. P6 cells (BALB/c3T3 cells overexpressing human IGF-1R) were kindly provided by Professor R. Baserga (Philadelphia, PA). MCF-7 and P6 cells were cultured in Dulbecco’s modified Eagle’s
medium supplemented with 10% fetal bovine serum whereas MDA-MB-231 cells were in RPMI 1640 with 10% fetal bovine serum, at 37 °C in a 5% CO₂ atmosphere.

**Reagents**—A hybridoma supernatant containing anti-IGF-1R (7C2 clone) was produced in the Monoclonal Antibody Facility in the School of Molecular & Biomedical Science, The University of Adelaide as described.³ A monoclonal anti-IGF-1R 24–31 (21) was a gift from Dr. Leah Cosgrove (CSIRO, Human Nutrition, Adelaide, South Australia). Monoclonal anti-human CXCR4 antibodies (clone 12G5) were purchased from R&D systems (Minneapolis, MN), and polyclonal CXCR4 antibodies were purchased from Chemicon International Inc. Monoclonal anti-IGF-1R antibodies (clone 2C8), antibodies to Gαs (T-19) and Gβ (M-14) and monoclonal control antibodies IgG (anti-hemagglutinin clone F-7) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Fluorescein isothiocyanate-conjugated anti-CXCR4 was from R&D Systems whereas PE-conjugated anti-mouse IgG and horseradish peroxidase-labeled donkey anti-rabbit IgG were purchased from Rockland (Gilbertsville, PA). DELFIA Eu-labeling kit reagents composed of europium-labeled anti-phosphotyrosine PY20 Abs and DELFIA enhancement solution were purchased from PerkinElmer Life Sciences. IGF-1 was obtained from GroPeP Pty Ltd (Adelaide, South Australia). CXCL12 was kindly provided by Professor Ian Clark-Lewis (UBC, Vancouver). Pertussis toxin (PTX) was purchased from Sapphire Bioscience, NSW, Australia.

**Retroviral-mediated RNAi Knockdown of CXCR4**—The shRNA retroviral expression vector was constructed by subcloning the human H1 gene promoter into the self-inactivating pMSCV plasmid. The resultant vector was digested with BglII and HindIII, and the annealed oligos 5′-gactgttggctctttttttgtcgcgcttcgaagacagacgcccaacagttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt
were washed with TBST, and the activated receptor complex formed was detected by incubating with europium-labeled anti-phosphotyrosine PY20 (10 ng/well) for 2 h at room temperature. After washing with distilled water, the plates were added with DELFIA enhancement solution (100 μl/well). Time-resolved fluorescence was then measured using 340-nm excitation and 610-nm emission filters on a BMG Lab Technologies Polarstar™ Fluorometer.

**Immunoprecipitations and Western Blot Analyses**—Cells were lysed at 4 °C for 20 min in Triton-lysis buffer (20 mM HEPES, 150 mM NaCl, 1.5 MgCl₂, 1 mM EGTA, 10% glycerol, and 1% Triton X-100) containing 2 mM Na₃VO₄, 5 mM NaF, 10 mM phenylmethylsulfonyl fluoride, and protease inhibitor (1:100, Sigma-Aldrich). The lysates were centrifuged at 1,400 × g at 4 °C for 10 min to remove insoluble materials, and the supernatants were collected. Total protein was determined using the BCA assay (Pierce). For immunoprecipitation, the lysates (1 mg of total proteins) were incubated with 1 μg of either anti-IGF-1R 2C8,
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anti-human CXCR4 1G5G, or IgG control antibodies, monoclonal anti-HA, at 4 °C overnight. Immunocomplexes were precipitated with protein G-coated microbeads at 4 °C for 1 h and purified on magnetic microcolumns (Miltenyi Biotec). The bound proteins were eluted from the column in preheated sample buffer (50 mM Tris-HCl pH 6.8, 50 mM dithiothreitol, 1% SDS, 0.005% bromphenol blue, and 10% glycerol). For whole lysate sample preparation, the lysates (50 μg of total proteins/well) were denatured by boiling for 5 min in sample buffer. The immunoprecipitates and whole lysates were then subjected to 15% SDS-PAGE, transferred to nitrocellulose membrane (Hybond™ P, Amersham Biosciences), and analyzed by Western blotting. The transferred membranes were blocked with 1% casein (Roche Applied Science) and incubated with primary Abs (1:1000 of polyclonal anti-CXCR4, 1:500 of anti-Gα and -Gβ) followed by horseradish peroxidase-conjugated donkey anti-rabbit IgG (1:1000). Membranes were visualized by enhanced chemiluminescence (Amersham Biosciences).

RESULTS

Expression of CXCR4 and IGF-1R on the MCF-7 and MDA-MB-231 Breast Cancer Cell Lines—Two breast cancer cell lines, the non-metastatic MCF-7 and metastatic MDA-MB-231, were characterized in terms of the expression and function of CXCR4 and IGF-1R. Flow cytometric analysis showed expression of both CXCR4 and IGF-1R on both cell types (Fig. 1 and TABLE ONE). MCF-7 cells expressed both receptors at high levels (94.74 ± 0.01% of positive cells with a geometric mean of 65.28 ± 17.08% for CXCR4 and 98.85 ± 0.69% of positive cells with a geometric mean of 26.75 ± 7.12% for IGF-1R) whereas MDA-MB-231 cells showed a high level of CXCR4 (95.71 ± 1.10% positive cells with a geometric mean of 77.32 ± 18.77%) and a lower level of IGF-1R expression (22.65 ± 9.34% of positive cells with a geometric mean of 13.10 ± 3.47%). Western blot analysis also confirmed the expression of CXCR4 and IGF-1R in both cell lines (data not shown).

Chemotactic Response of MCF-7 and MDA-MB-231 Cells to CXCL12 and IGF-1—The function of CXCR4 and IGF-1R on MCF-7 and MDA-MB-231 cells was examined by testing the migrational response of the cells to respective ligands, CXCL12 and IGF-1, using a modified Boyden chamber chemotaxis assay. Interestingly, even though CXCR4 expression was similar on MDA-MB-231 and MCF-7 cells, only the former responded to CXCL12 (Fig. 2A). In contrast, both cell lines migrated in response to IGF-1. However, in keeping with the lower level of IGF-1R expression, IGF-1-induced chemotaxis of MDA-MB-231 cells was lower than that observed in MCF-7 cells. Additional experimentation was conducted in MDA-MB-231 cells to determine the effect of combined stimulation with CXCL12 and IGF-1. The results of these experiments indicated an additive effect of those ligands on chemotaxis of MDA-MB-231 cells (Fig. 2B).

CXCL12 Does Not Transactivate IGF-1R on MDA-MB-231 Cells—To investigate potential cross-talk between CXCR4 and IGF-1R-induced signal transduction pathways, we initially determined whether there is cross-activation of IGF-1R by CXCL12 on MDA-MB-231 cells. Because activation of IGF-1R by IGF-1 leads to the rapid formation of a tyrosine-phosphorylated receptor complex, a KIRA assay was performed to compare the levels of IGF-1R activation induced by CXCL12 and IGF-1. Preliminary experiments indicated that in P6 (positive control), MCF-7 and MDA-MB-231 cells, maximal levels of activated IGF-1R complex formed after stimulation with 10 nM IGF-1 at 10 min (data not shown). Therefore, in subsequent experiments, the cells were stimulated with various concentrations of IGF-1 and CXCL12 for 10 min. The results of these experiments indicate that IGF-1 dose-dependently induced the activation of IGF-1R in all three cell lines (Fig. 3A) whereas CXCL12 failed to do so at any of the concentrations tested (Fig. 3B).

Pertussis Toxin Inhibits CXCL12- and IGF-1-induced Chemotaxis but Does Not Affect the Activation of IGF-1R Induced by IGF-1 in MDA-MB-231 Cells—To investigate the involvement of Giα in IGF-1-induced chemotaxis of MDA-MB-231 cells, the cells were treated with various concentrations of PTX, a specific inhibitor of Giα subunits. The cells were then tested for their chemotactic response to various concentrations of both CXCL12 and IGF-1. As shown in Fig. 4, A and B, PTX at a concentration of 10 ng/ml completely blocked the response to CXCL12 and partially inhibited that to IGF-1 in MDA-MB-231 cells. Similar levels of inhibition were observed when the cells were pretreated with 100 and 1,000 ng/ml PTX (data not shown). These data indicate a contribution of Giα to IGF-1-induced chemotaxis of MDA-MB-231 cells. Pretreatment of MCF-7 cells with PTX had no effect on IGF-1-induced chemotaxis at any of the three doses tested (Fig. 4C).

To test the possibility that blocking Giα with PTX inhibits the activation of IGF-1R by IGF-1, the lysates of cells untreated or treated with PTX were assayed for the level of tyrosine-phosphorylated IGF-1R complex formed in response to IGF-1 using the KIRA assay. Two different doses of PTX (10 and 100 ng/ml) failed to alter the level of IGF-1R activation in either MDA-MB-231 or MCF-7 cells (Fig. 5, A and B) indicating that Giα is not involved in IGF-1-induced formation of the activated IGF-1R complex.

RNAi of CXCR4 Inhibits Both CXCL12- and IGF-1-induced Chemotaxis but Has No Effect on the Activation of IGF-1R in MDA-MB-231 Cells—The involvement of CXCR4 in IGF-1-induced chemotaxis of MDA-MB-231 cells was examined using CXCR4-deficient cells. MDA-MB-231 cells were infected with a retrovirus expressing either RNAi to
knockdown CXCR4 or a retrovirus expressing specific target sequences for Renilla luciferase as a negative control. Individual clones were isolated and characterized for CXCR4 surface expression by flow cytometry and CXCR4 function was determined by assessing calcium mobilization and chemotaxis in response to CXCL12. Compared with wild-type MDA-MB-231 cells and the negative control clone, RNAi clones 11, 21, and 27 demonstrated a significant reduction of surface CXCR4 expression (Fig. 6A, shown only for clone 11) and of calcium mobilization in response to CXCL12 (data not shown). The surface expression of IGF-1R was not affected by RNAi CXCR4 knockdown in any of the clones (Fig. 6A shown only for clone 11). Compared with wild-type cells and the negative control clone, RNAi clones 11, 21, and 27 displayed a significant reduction in chemotaxis in response to CXCL12 (Fig. 6B) and IGF-1 (Fig. 6C). In contrast, knockdown of CXCR4 did not have any effect on IGF-1-induced IGF-1R activation as determined in the KIRA assay (Fig. 6D).

**FIGURE 4.** Pertussis toxin inhibits CXCL12- and IGF-1-mediated chemotactic responses of MDA-MB-231 cells but does not affect the IGF-1-induced response in MCF-7. The cells were pre-treated with various concentrations of PTX prior to testing their chemotactic ability using the Modified Boyden chamber assay. PTX completely blocked the response mediated by CXCL12 in MDA-MB-231 (A); partially inhibited that by IGF-1 in MDA-MB-231 cells (B); had no effect on the IGF-1-induced response in MCF-7 cells (C). The data are represented as the mean ± S.E. of migration index; n = 3 (A and B) and n = 5 (C) each performed in triplicate. Asterisks indicate statistically significantly different from control values (Student’s unpaired t test) at *, p < 0.05; **, p < 0.005; #, p < 0.0001.

**IGF-1R Is Physically Associated with CXCR4 and G Protein Subunits in Both MDA-MB-231 and MCF-7 Cells, but IGF-1 Activates the CXCR4 Signaling Pathway through G Proteins Only in MDA-MB-231 Cells**—The nature of the interaction between IGF-1R, CXCR4, and G proteins in MCF-7 and MDA-MB-231 cells was investigated. Immunoprecipitations were performed on cell lysates using an anti-IGF-1R mAb, anti-human CXCR4, or control IgG, followed by Western blots for either CXCR4, Gαi2, or Gβ. The immunoprecipitations with control IgG failed to coprecipitate CXCR4, Gαi2, and Gβ, despite the fact that these latter three proteins were readily detectable in whole cell lysates subjected directly to Western blot (Fig. 7A). In contrast, immunoprecipitation of IGF-1R and CXCR4 in both MCF-7 and MDA-MB-231 cells led to coprecipitation of all three proteins, indicating the existence of a constitutive complex between IGF-1R, CXCR4, Gαi2, and Gβ. Of note, the levels of Gαi2 and Gβ in immunoprecipitates of the two receptors consistently appeared to be higher in MDA-MB-231 cells than in MCF-7 cells.

**FIGURE 5.** Pretreatment with pertussis toxin has no effect on IGF-1-induced IGF-1R activation. MDA-MB-231 and MCF-7 cells were pretreated with various concentrations of PTX and the level of activated IGF-1R complex induced by incubation with the indicated concentrations of IGF-1 for 10 min was determined using a KIRA assay as described under “Materials and Methods.” MDA-MB-231 (A) and MCF-7 (B) cells were used. The levels of activated IGF-1R complex are expressed as fold-increase in relation to the level observed in unstimulated cells. The data are represented as the mean ± S.E. of n = 5 independent experiments each performed in triplicate.
The ability of IGF-1 to transactivate CXCR4 was investigated by examining the effect of stimulation with IGF-1 on the level of association of $G_{i2}$ and $G_{b2}$ with CXCR4 (Fig. 7). The results of these experiments showed that stimulation of MCF-7 cells with IGF-1 failed to release either $G_{i2}$ or $G_{b2}$ from the CXCR4/IGF-1R complex, whereas, in contrast, both $G_{i2}$ and $G_{b2}$ were released from the complex in MDA-MB-231 cells.

DISCUSSION

Here we present evidence of cross-talk between CXCR4 and IGF-1R in the human epithelial breast cancer cell line, MDA-MB-231. The basis of this cross-talk appears to depend on a physical association between CXCR4 and IGF-1R. It is unidirectional, involving activation of G protein subunits by IGF-1 that is dependent on the presence of a functional pool of CXCR4, but independent of the CXCR4 ligand CXCL12. These observations potentially have major implications for our understanding of CXCR4 and IGF-1R signaling in both normal and pathological situations.

In this study, two breast cancer cell lines, the non-metastatic MCF-7 and the highly metastatic MDA-MB-231 were characterized in terms of expression and function of CXCR4 and IGF-1R. MCF-7 cells exhibited a high level of IGF-1R expression and a strong chemotactic response to IGF-1 whereas MDA-MB-231 cells expressed a lower level of the receptor and a lower response to IGF-1. The lower level of IGF-1R expression in the metastatic MDA-MB-231 cells compared with the non-metastatic MCF-7 cells correlates well with the results of a recent study demonstrating that reduced expression of IGF-1R in MCF-7 cells leads to a more metastatic phenotype in those cells (26).

Although the two cell lines expressed high levels of CXCR4, only MDA-MB-231 cells responded functionally to CXCL12, indicating uncoupling of receptor expression and function in the MCF-7 cells. This phenomenon has been observed previously with respect to CXCR4 in the human hepatoma cell line HepG2 (27), and other chemokine receptors in a range of cell types (28, 29), although the molecular basis for this non-functional phenotype, at least with respect to cell migration, was not defined in those studies. However, the results of our studies suggest at least two mechanisms: differences in the level of expression of $G_{i2}$ and $G_{b2}$ in MB-MDA-231 cells and MCF-7 cells, which results in different levels of association of $G_{i2}$ with CXCR4 in those cells, and the failure of $G_{i2}$ and/or $G_{b2}$ to uncouple from CXCR4 upon activation of the receptor (data not shown).

Three forms of cross-talk between GPCR and RTK systems have been demonstrated in different cellular systems. First, RTKs can be transactivated by GPCRs. For example, EGF-R is phosphorylated in response to IGF-1 whereas MDA-MB-231 cells expressed a lower level of the receptor and a lower response to IGF-1.
CCL11, a ligand for the GPCR CCR3, leading to the MAP kinase activation and IL-8 production in bronchial epithelial cells (18). This appears to depend on activation of CCR3 by CCL11. Second, GPCRs can be transactivated by RTKs. For example, it has been shown that IGF-1 stimulates phosphorylation of CCR5 in MCF-7 cells. Chemotaxis induced by IGF-1 was inhibited by a neutralizing anti-CCR5 antibody. Transactivation of CCR5 by IGF-1 was therefore indirect, requiring the activity of the ligand (CCL5) for the second receptor (20). Finally, bidirectional transactivation between the same two receptor systems has also been observed. PDGFR is phosphorylated by S1P leading to activation of Class IB PI3Ks (33, 34). IGF-1 is known to induce activation of PI3K and this is dependent on tyrosine phosphorylation. In contrast, ligation of CXCR4 is independent of PI3K activation. A physical interaction between IGF-1R and Gi2 or Gαi2 has previously been demonstrated (35, 36). Moreover, in those studies, PTX was shown to inhibit IGF-1-induced activation of MAPK in neuronal cells (36), and IGF-1-induced mitogenesis of HIRcB cells and 3T3L1 adipocytes (35). In contrast to the conclusion from that study that IGF-1R functions as a G protein-coupled receptor (35), our findings suggest that the association of the G protein subunits with IGF-1R is indirect, and requires the presence of CXCR4. Certainly, our data indicate that the presence of functional CXCR4 is required for G protein-dependent cell migration in response to IGF-1.

Interestingly, CXCR4 and IGF-1R could be coprecipitated in both MCF-7 and MDA-MB-231 cells, indicating that the lack of involvement of CXCR4 in IGF-1-induced chemotaxis of MCF-7 cells was not because of a lack of association of CXCR4 and IGF-1R in those cells. Rather, our data indicate that the cross-talk in MDA-MB-231 cells is mediated at the level of G protein activity; both Gαi2 and Gβ are associated with the complex in both cell lines; however, activation of the complex, as determined by release of Gαi2 and Gβ from CXCR4, only occurs in MDA-MB-231 cells. This is consistent with our observation that MCF-7 cells do not respond to CXCL12, at least in terms of the migratory response.

In summary, our data provide evidence of a novel transactivation between RTK and GPCR signal transduction pathways. We have observed the coprecipitation of IGF-1R, CXCR4, and the G protein subunits, Gαi2 and Gβ, indicating a constitutive physical association between these molecules. Based on our data, we propose that this IGF-1R/CXCR4 complex allows CXCR4 and G proteins to act partially in IGF-1-induced chemotaxis of MDAMB-231 cells probably through activation of class IB PI3K activity. Our data also demonstrate that CXCR4 and G proteins operate independently of the activation of IGF-1R because neither PTX pretreatment nor CXCR4 knockdown affected the levels of tyrosine-phosphorylated IGF-1R complex formed.

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after IGF-1 stimulation. The fact that this pathway does not appear to be active in the non-metastatic MCF-7 cells suggests that CXCR4/IGF-1R receptor integration may play an important role in cancer metastasis. In addition, both IGF-1/IGF-1R and CXCL12/CXCR4 are essential for life (3, 37–39) raising the possibility that transactivation between IGF-1R and CXCR4 may be involved in development. Further experimentation comparing IGF-1R signaling complexes in both MCF-7 and MDA-MB-231 cells may provide further insights.

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