The Roles of Integrins and Extracellular Matrix Proteins in the Insulin-like Growth Factor I-stimulated Chemotaxis of Human Breast Cancer Cells*

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The effects of insulin-like growth factor I (IGF-I) on the migration of two human breast cancer cell lines, MCF-7 and MDA-231, were examined using a modified Boyden chamber. 10 ng/ml was the optimal IGF-I concentration for stimulation of migration. The majority of IGF-I-stimulated migration in both cell types was due to chemotaxis. MCF-7 cells failed to migrate on membranes coated with gelatin or fibronectin and migrated only in small numbers on laminin. In contrast, when vitronectin- or type IV collagen-coated membranes were used, the MCF-7 cells migrated in large numbers specifically in response to IGF-I but not to 10% fetal calf serum, epidermal growth factor, fibroblast growth factor, or platelet derived growth factor-BB. An IGF-I receptor-blocking antibody inhibited IGF-I-stimulated migration in both cell types. In addition, a blocking antibody to the αvβ5 integrin (a vitronectin receptor) inhibited migration of MCF-7 cells in response to IGF-I through vitronectin but not through type IV collagen. Similarly, blocking antibodies specific for α2 and β1 integrins significantly inhibited migration of both cell types through type IV collagen-coated membranes but not through vitronectin-coated membranes. We conclude that: 1) IGF-I stimulates migration of these two cell types through the IGF-I receptor; 2) interaction of vitronectin with the αvβ5 integrin or collagen with the α2β1 integrin is necessary for the complete IGF-I response in MCF-7 cells, and 3) because migration represents an in vitro model for metastatic spread, integrins, extracellular matrix proteins, and IGF-I may play coordinated roles in the metastasis of breast cancer in vivo.

Insulin-like growth factor I (IGF-I) has been shown to be a mitogen for human breast cancer cells including the MDA-231 and MCF-7 cultured cell lines (1–3). The increased growth of these cells in the presence of IGF-I requires the normal function of the IGF-I receptor and can be blocked by a monoclonal antibody to this receptor, αIR-3 (4). Terranova et al. (5) first reported on the correlation between malignant cells capable of traversing a porous membrane coated with extracellular matrix components and the ability of these cells to cause metastases. Using modified Boyden chambers or monolayer wounding migration assays, other authors have found that IGF-I can stimulate migration in human melanoma cells (6), FG pancreatic carcinoma cells (7), arterial smooth muscle cells (8, 9), and endothelial cells (10). Other chemoattractants, such as interleukin-1 and -6 for breast cancer cells (11) and epidermal growth factor (EGF) for FG cells (7), have also been demonstrated to stimulate cellular migration in these assays.

The integrins are a superfamily of heterodimer cell surface glycoprotein receptors composed of distinct α and β subunits (12). The integrins were originally described as cell adhesion receptors, but their functions in cell behavior including motility and invasion and their interactions with classical growth factor receptor signaling pathways have been increasingly recognized in the past few years (13). Although integrins themselves do not possess tyrosine kinase activity, activation of integrins by cell adhesion (14) or anti-integrin antibodies (15) causes tyrosine phosphorylation of p125<sup>FAK</sup> and other cytoplasmic proteins. The αvβ3 integrin, a vitronectin receptor, was recently shown to associate with insulin receptor substrate-1 in fibroblasts after the cells were stimulated with 100 nM insulin (16), a dose capable of activating IGF-I receptors (17). A connection between EGF stimulation and the activation of the αvβ3 integrin (another vitronectin receptor) with cell migration has also been described in pancreatic cancer cells (7). Our work began with the hypothesis that IGF-I would stimulate migration of MDA-231 and MCF-7 cells, which we have demonstrated utilizing microwell Boyden chambers. We have characterized the conditions required for optimal migration to IGF-I in these two cell lines. This has led to the conclusion that the integrins expressed by the cells play a key role in mediating the effect of IGF-I to stimulate migration.

MATERIALS AND METHODS

Cell Culture and Materials—The MCF-7 (estrogen receptor positive) and MDA-231 (estrogen receptor negative) human breast carcinoma cells were obtained from the Lineberger Cancer Center, University of North Carolina. They were maintained in Dulbecco’s modified Eagle’s medium/Ham’s F-12 medium (1:1) (DMEM/F-12, Life Technologies, Inc.) containing 10% fetal bovine serum (Life Technologies, Inc.), penicillin, and streptomycin and passaged once or twice weekly using PBS-EDTA and trypsin. The MCF-7 cells were transferred into similar media containing 10% calf serum (Colorado Serum Co., Denver) 7–10 days prior to use in an assay. IGF-I was from Bachem, and the anti-IGF-I receptor monoclonal antibody αIR-3 was kindly provided by David Clemmens (University of North Carolina). The 48-well modified Boyden chamber and Costar porous polycyprylidone-free polycarbonate membranes (pore size, 8 μm) were purchased from Neuro Probe (Cabin John, MD). Membranes were coated by incubation at room temperature overnight just prior to use with gelatin (100 μg/ml) in 10

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1 The abbreviations used are: IGF-I, insulin-like growth factor I; EGF, epidermal growth factor; bFGF, basic fibroblast growth factor; PDGF-BB, platelet-derived growth factor-BB; DMEM, Dulbecco’s modified Eagle’s medium; BSA, bovine serum albumin; PBS, phosphate-buffered saline; HBS, Hepes-buffered saline.
IGF-I-stimulated Chemotaxis of Breast Cancer Cells

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tactic response in both cell types. We investigated the role of the IGF-I receptor in IGF-I-mediated chemotaxis by preincubating the cells with αIR-3, a monoclonal IGF-I receptor-blocking antibody. Compared with the insignificant inhibition associated with a murine IgG control, preincubation with αIR-3 led to dramatic reductions in migration to IGF-I (Fig. 5). The MDA-231 cells displayed a 65 ± 10% reduction in migration; the MCF-7 cells displayed a 91 ± 6% decrease.

FIG. 2. Immunoprecipitation of breast carcinoma cell integrins. To determine the presence and identity of integrin receptors on the surface of the MCF-7 (A) and MDA-231 (B) cells, proteins on the surfaces of intact cells were labeled with Na125I, and the labeled cells were lysed and immunoprecipitated with specific antibodies directed against integrin α or β subunits, as indicated beneath each lane. The immunoprecipitates were resolved under nonreducing conditions on 6% SDS-polyacrylamide gels. Autoradiographs of the dried gels are shown. The arrows indicate the locations in the gels of the integrin subunits. Because α and β integrin subunits are tightly associated until SDS-polyacrylamide gel electrophoresis, an antibody against a given α or β subunit will precipitate both subunits. In both cell lines the α2β1 and α3β1 collagen/laminin receptors are abundant. The abundance of the αVβ5 vitronectin-binding integrins is greatest in the MCF-7 cells. No αVβ3 integrins were detected in either cell line.

FIG. 3. MDA-231 and MCF-7 cells vary in their responses to chemoattractants. The effect of IGF-I on stimulation of migration through vitronectin-coated membranes was compared with that of other chemoattractants. Each is compared with the response of the cells to IGF-I (10 ng/ml) in the same assay, which is assigned an arbitrary value of 100%. EGF (10 ng/ml) elicited a response similar to that of IGF-I in the MDA-231 cells. The effects of bFGF (20 ng/ml) and PDGF-BB (10 ng/ml) were less pronounced. In contrast, the MCF-7 cells (hatched bars) had a significant response only to IGF-I.

FIG. 4. Directional migration of MCF-7 and MDA-231 cells. The graph depicts the effects on MDA-231 (open bars) and MCF-7 (hatched bars) cell migration of adding IGF-I (10 ng/ml) to either the upper chamber, lower chamber, neither chamber, or both chambers and was performed as described under “Materials and Methods.” The number of migrated cells when IGF-I was present in the lower well only is assigned a relative value of 100% and is a measure of chemotaxis. Migration in response to the addition of IGF-I to only the upper wells or to both wells is a measure of chemokinesis, stimulation of migration in the absence of a concentration gradient. For both cell lines, IGF-I is more potent in stimulating migration when there is a positive concentration gradient toward which the cells migrate.

FIG. 5. Inhibition of chemotaxis by an IGF-I receptor antibody. Chemotaxis in response to IGF-I was determined in the presence and the absence of the IGF-I receptor blocking antibody α-IR3 or control mouse IgG at 25 μg/ml. Taking the total number of migrated cells (in response to IGF-I, 10 ng/ml) with no added antibody as 100% migration, α-IR3 reduced migration of MDA-231 cells (open bars) to 36 ± 12%, whereas nonspecific mouse IgG had a minimal effect. This effect was more pronounced with the MCF-7 cells (hatched bars), where migration was reduced to 9 ± 5% by α-IR3.

Anti-integrin Monoclonal Antibodies Inhibit IGF-I-stimulated Chemotaxis of MCF-7 Cells—Because a membrane coating of either vitronectin or type IV collagen was necessary for optimal IGF-I-stimulated migration, we sought to elucidate the role of integrin receptors in this process. Immunoprecipitation studies clearly showed that the MCF-7 cells have an abundance of the α2β1 collagen/laminin receptor and the αvβ5 vitronectin receptor (Fig. 2). We investigated the contributions of these integrins by using monoclonal anti-integrin antibodies with
well defined functional blocking properties (19–22). As shown in Fig. 6A, preincubation with the anti-αVβ5 antibody caused a 70% decrease in IGF-I-stimulated migration through vitronectin, whereas the effect of the antibodies against αVβ3, α2, and β1 were minimal. In Fig. 6B, the results of migration assays utilizing membranes coated with type IV collagen are summarized. The antibody against α2, which specifically blocks the α2β1 integrin, and the antibody against β1, which blocks signaling by multiple β1 integrins including both the α2β1 and the α3β1 collagen receptors, had the most profound inhibitory effects. This was not a nonspecific toxic effect of these antibodies on the MCF-7 cells, because neither had an effect on cells migrating through vitronectin in response to IGF-I (Fig. 6A).

**DISCUSSION**

This is the first report to our knowledge on the effects of anti-integrin antibodies. IGF-I-stimulated (10 ng/ml) chemotaxis was determined in the presence and the absence of monoclonal anti-integrin blocking antibodies (25 µg/ml) against αVβ3 (clone LM609, Chemicon), αVβ5 (clone P1F6, Becton Dickinson), α2 (clone P1E6, Becton Dickinson), and β1 (clone mAb13, Becton Dickinson) and control nonimmune mouse IgG. Experiments were performed utilizing membranes coated with either vitronectin (A) or type IV collagen (B). Migration in the absence of antibodies was taken as 100%. Through vitronectin-coated membranes, (A), anti-αVβ5 inhibited migration by 70 ± 18%, whereas the other antibodies and IgG had small effects. In contrast, through collagen-coated membranes, both anti-α2 and anti-β1 had major effects, reducing migration by 63 ± 5% and 96 ± 6%, respectively, whereas anti-αVβ5 and IgG had minimal effects.

ability to induce chemotaxis in these cells has not previously been studied. 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. IGF-I, synthesized by surrounding stromal cells, has been implicated in the development of tumors from breast cancer cells seeded into nude mice (4). The modified Boyden chamber assays allow us to examine the cellular processes of invasion and migration and provide a valid in vitro model of the in vivo process of metastatic spread (5), which is responsible for nearly all of the mortality from breast cancer (23). In addition, the Boyden chamber assays avoid any confusion of migration with proliferation even in these rapidly proliferating transformed cells by virtue of its 4-h duration. It also allows for precise definition of the extracellular matrix proteins over which the cells migrate.

We selected the MCF-7 and MDA-231 cell lines because their increased growth in response to IGF-I has been well characterized (1–3) and because they were both isolated from metastatic sites. Other authors have used the MDA-231 cells for study based on their high degree of invasiveness (24). Our results are in agreement with this, because we found that the MDA-231 traversed every membrane coating used, including gelatin, fibronectin, laminin, vitronectin, and type IV collagen. Furthermore, the MDA-231 cells migrated in the absence of growth factors and demonstrated chemotaxis toward bFGF, EGF, PDGF-BB, and 10% fetal bovine serum as well as toward IGF-I.

In contrast, migration of the MCF-7 cells was found to have more specific requirements. The MCF-7 cells migrated only in response to IGF-I not toward a negative control, 10% fetal bovine serum, or three other growth factors. These other growth factors, EGF, bFGF, and PDGF-BB, have each been shown to be capable of inducing increased migration in other cell types (7, 8, 25). Furthermore, the MCF-7 cells showed a brisk response to IGF-I only when migrating through membranes coated with vitronectin or type IV collagen. While not causing the complete arrest of MCF-7 cell migration reported by other investigators using laminin-coated glass coverslips (18), the use of membranes coated with laminin in our assays led to dramatic reductions in cell migration compared with vitronectin or type IV collagen. Furthermore, there was no detectable migration of MCF-7 cells on membranes coated with gelatin or fibronectin. The marked differences in conditions required for migration between these two cell lines may be related to their differences in estrogen receptor status. Studies using estradiol and anti-estrogens to modify the migration response of MCF-7 cells will be required to determine the dependence of this response on estrogen receptor activation. Alternatively, the observed differences in growth factor responses between the two cell lines may be due to the highly undifferentiated state of the MDA-231 cells compared with the MCF-7 line, which retains some epithelial characteristics. The two cell lines also have differences in IGF-binding protein expression (26). Because IGF-binding proteins can have direct, integrin-mediated effects on cell migration (27) as well as effects on IGF interactions with the IGF-I receptor (17), further studies into the effects of IGF-binding proteins on IGF-stimulated cell migration are warranted.

The results of the checkerboard analyses (Fig. 4) established that the majority of increased migration we observed in response to IGF-I was due to chemotaxis, directional migration toward a higher concentration of a solubilized chemotaxant. The finding that a positive concentration gradient of IGF-I, rather than its mere presence, is necessary for optimal migration may have important physiologic implications. IGF synthe-
sist in tumor specimens has been shown to originate from surrounding stromal cells, suggesting the presence of IGF concentration gradients in breast carcinomas in vivo. The blocking antibody to the IGF-I receptor, αIR-3, has been previously been shown to be capable of inhibiting the increased growth of the MDA-231 and MCF-7 cells associated with their exposure to IGF-I (28). Therefore, it was not surprising that preincubation of the cells with this antibody would also markedly limit their migratory response to IGF-I. We performed these experiments to be certain that the chemotaxis induced by IGF-I was an IGF-I receptor-dependent phenomenon.

It has recently become clear that the integrins function as true receptors, transmitting signals to the cell interior upon ligation by extracellular matrix components (29). Our results demonstrate that both integrin receptors and IGF-I receptors are involved in IGF-I-stimulated migration of MCF-7 cells. The immunoprecipitation studies (Fig. 2) demonstrated that the MCF-7 cell line has a proportionately higher amount of the αvβ5 integrin, a vitronectin receptor, compared with the MDA-231 cells. The predominant vitronectin receptor expressed by the MCF-7 cells is the αvβ5 integrin, and preincubation of these cells with a blocking antibody to αvβ5 markedly reduced their migratory response to IGF-I through vitronectin-coated membranes (Fig. 6A). As predicted, cells preincubated with the same anti-αvβ5 antibody were not significantly affected when migrating toward IGF-I through type IV collagen, confirming the specificity of the requirement for αvβ5 for migration on vitronectin (Fig. 6B).

The α2β1 integrin can serve to mediate IGF-I-stimulated migration when the cells migrate through collagen. The α2β1 and α3β1 integrins are both capable of binding to type IV collagen but not to vitronectin. The antibody to the β1 subunit, common to both receptors, produced the most significant inhibition of migration when assays were conducted with membranes coated with type IV collagen (Fig. 6B), almost eliminating the response to IGF-I. In addition, the anti-α2 antibody inhibited migration on type IV collagen by 63%. Neither antibody had a significant effect on the MCF-7 cells migrating through vitronectin (Fig. 6A), confirming that the requirement for α2β1 (and possibly α3β1) functionality is specific for migration on type IV collagen in these cells.

We therefore conclude that integrins are activated during the process of IGF-I-stimulated chemotaxis in breast cancer cells. The identity of the integrin that is essential for cell migration is specific to the particular extracellular matrix substance used. This is the first description of this association between IGF-I receptor stimulation and integrin function in breast cancer cells. Breast cancer tissues have been shown to have altered patterns of integrin expression (90) and higher numbers of IGF-I receptors (31) when compared with normal breast tissue. It is likely that integrins are involved in the response of these malignant cells to IGF-I, mediating their ability to traverse basement membranes and metastasize.

The role of IGF-I in the stimulation of migration is an important area of study that heretofore has been relatively neglected compared with the role of IGF-I in cellular growth and proliferation. Although the precise mechanisms by which the IGF-I receptor and integrin receptors interact are not yet known, this area of study warrants continued investigation. If the nature of the cross-talk between these two signaling systems can be elucidated, our understanding of malignant behavior will be greatly enhanced.

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