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We have previously shown that IGF-I stimulates chemotaxis of MCF-7 and MDA-231 human breast cancer cells. We now report that PDGF is a potent stimulant of migration. FGF is less effective, and EGF has no effects. The integrins that are present on 4 additional cell lines were identified. Antibodies that bind to the Beta 3 integrin inhibited migration in 3 of the 4 cell lines. Anti-α5 antibodies were also potent inhibitors. The combination of estradiol and IGF-I potently stimulated chemotaxis in MCF-7 cells, and the response to both hormones was greater than that to either hormone alone. Two IGF binding proteins were shown to alter the chemokinetic response of MDA-231 cells to IGF-I. IGFBP-3 inhibited the response to IGF-I. In contrast, IGFBP-5 enhanced the response to IGF-I, suggesting that different binding proteins have different effects.

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David R. Clemens 7/13/00

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Introduction:

Insulin-like growth factor-I (IGF-I) is an important polypeptide growth factor that stimulates a diverse set of biological actions in many target tissues (1). IGF-I binds to its receptor, which is a 185 kiloDalton protein, which then undergoes tyrosine auto-phosphorylation and activates the IGF-I signaling cascade (2, 3). IGF-I has been shown to be a potent growth factor for breast carcinoma cells in culture, and most major breast carcinoma cell types that have been analyzed contain abundant IGF-I receptors. IGF-I has been shown to stimulate the growth of human breast cancer cells in long term culture, and physiologic levels of IGF-I are capable of stimulating DNA synthesis in the absence of other serum components (4, 5). This response has been shown to be mediated through the IGF-I receptor. IGF-I receptors have been shown to be more abundant in breast cancer biopsy specimens compared to surrounding tissues or normal breast biopsies, suggesting that increased levels of receptor may be related to cancer progression. The interaction between estrogen and IGF-I has been extensively studied in breast cancer cells. Stewart, *et al.* showed that estradiol sensitizes MCF-7 cells to the mitogenic effects of IGF-I by increasing IGF-I receptor synthesis and number (6). A blocking monoclonal antibody to the IGF-I receptor – one that blocks IGF-I binding to its receptor – has been shown to be a potent inhibitor of MCF-7 and M2-31 breast cancer cell line growth *in vitro* (7-9).

Several authors have reported that IGF-I can stimulate the migration of cancer cells, including melanoma cells and pancreatic carcinoma cells (10, 11). In previous studies, we have reported that IGF-I stimulates the migration of breast cancer cells and that this event is due to stimulation of chemotaxis (12-14). Likewise, the invasive capacity of these cells appears to be stimulated, since they can migrate through a collagen barrier, as well as through a membrane pore. IGF-I has been shown to increase protease expression on the surface of cancer cells, and this may account for its ability to increase cell invasiveness. In previous studies reported in this grant, we have shown that MCF-7 cells respond to IGF-I with increased migration, as do MDA-231 cells. MCF-7 cells will also migrate through type IV collagen or vitronectin-coated membranes. Although they respond well to IGF-I, they do not respond to 10% fetal calf serum, which is unusual for cancer cell lines. The increase in migration is directional and is due to chemotaxis, when an IGF-I gradient is established. Anti-IGF-I receptor antibodies will block this increase in chemotaxis for either cell line. Other growth factors, such as EGF, PDGF, and FGF, were shown to stimulate MDA-231 cells, but their effects were not as pronounced. In contrast, MCF-7 cells responded only to IGF-I.

Breast cancer cells have been shown to express integrin receptors. These are heterodimeric receptors composed of distinct alpha and beta subunits. Adhesion of cells to extracellular matrix (ECM) is primarily mediated by these receptors, and integrins have a central role in transducing the signal from ECM to growth factor stimulated phosphoprotein signaling pathways. Breast cancer cells have been shown to have altered integrin expression compared to normal breast epithelium (15). Coopman, *et al.* showed that basement membrane components influence the directional migration of MCF-7 cells (17). Laminen arrests migration, while type IV collagen and fibronectin have no effect. MCF-7 cells have been shown to express integrins capable of binding several ECM proteins, including laminen, vitronectin, and type IV collagen. Rossi, *et al.* showed that the presence of fibroblasts was necessary for MCF-7 cells to migrate (18). Interestingly, fibroblasts secrete IGF-I, suggesting that a paracrine effect is responsible for

their migration (19). Some breast cancer cell lines also express the αVβ3 integrin. The αVβ3 integrin has been shown to synergize with IGF-I receptor in stimulating cell migration of other cell types, suggesting that this type of synergism could also occur in breast cancer cells (20). Kelmke, *et al.* have shown that pancreatic carcinoma cells migrate in response to EGF, but only after attachment to vitronectin, suggesting that either αVβ3 or αVβ5 is involved (11, 21). Likewise, activation of the EGF receptor had to be present for these cells to migrate.

As discussed below, this association between a growth factor and integrin signaling as a prerequisite for migration is almost certainly present with MCF-7 cells as well. Our laboratory performed immunoprecipitation studies to characterize the pattern of integrin expression in the MDA-231 and MCF-7 cells (Fig. 2, ref 14). Numerous migration assays done with anti-integrin antibodies have confirmed that integrins play a key role in IGF-I stimulated migration. Furthermore, the effects of anti-integrin antibodies are highly specific for the coating substance used and vary with the relative amounts of integrins expressed by a given cell line (Fig. 6, ref. 14).

Included in our original grant proposal was preliminary data related to the effect of calf serum exposure on MCF-7 cells. We found that MCF-7 cells maintained in 10% fetal calf serum (FCS), lost their capacity to migrate in response to IGF-I. Remarkably, the same MCF-7 cells, which had shown no migration, could be grown under identical conditions and split into two different plates--one containing 10% fetal calf serum and the other containing 10% calf serum. Ten days later, typically after two more passages, the cells in the calf serum would migrate well when stimulated with IGF-I while the group of cells continued in fetal calf serum would not migrate in any measurable numbers. No such loss of migratory ability was observed in the MDA-231 cells maintained for several months in 10% FCS. To determine the time period of exposure to calf serum that was necessary for cellular migration, an assay was done with seven separate populations of MCF-7 cells grown under different circumstances--six populations had been exposed to calf serum for varying periods of time and a control population which had always been in fetal calf serum. This assay revealed that exposure to calf serum for seven to ten days was associated with the most active migration. Interestingly, cells which had been in calf serum for over a month demonstrated nearly the same poor migratory response to IGF-I as cells which had always been maintained in fetal calf serum. As part of the work proposed in our grant, we performed cross-linking studies on MCF-7 cells grown in both 10% FCS and 10% calf serum.

The biologic activities of IGF-I are largely regulated by a group of high affinity IGF binding proteins (IGFBPs), six of which have been cloned and sequenced. These IGFBPs have negligible affinity for insulin and are present in the circulation and throughout the extracellular space. They are also found in the conditioned media and extracellular matrix of most cells in culture. These binding proteins have at least four major functions, which include acting as transport proteins and prolonging the half-life of IGF-I. In addition, they provide a means for IGF localization to specific tissues and cell types and directly modulate the interactions of IGF with its receptor (I). The role of these IGFBPs in HBC cell growth and metabolism has been an area of intense investigation in the past several years.

One published study reported a correlation between ER status and IGFBP secretion in breast cancer cell lines. It showed that ER negative cells secrete IGFBP-1 and IGFBP-3. In contrast, the ER positive lines were found to secrete IGFBP-2 and IGFBP-4 (22). Subsequent

studies sought to understand how IGFBPs affect the growth of HBC cells. One study demonstrated that IGF-I increased cell numbers of sparse MDA-231 cultures by 24% after 48 hours (23). Addition of IGFBP-1 led to a 45% increase while IGFBP-2 had no potentiating effect. However, IGFBP-1 in the absence of IGF-I had no effect on cell growth. A different group examined the actions and IGF-I and IGFBP-1 on MCF-7 cells. The MCF-7 cells normally require estrogen for growth; nevertheless, IGF-I can substitute for estrogen in short-term studies. In contrast to the findings for MDA-231 cells, IGFBP-1 was shown to block IGF-I induced mitogenesis in MCF-7 cells (24). After the identification of a sixth IGFBP, Sheikh et al. (25) examined four ER positive and three ER negative HBC cell lines for expression of IGFBP-5 and IGFBP-6. All cell lines except the MDA-231 expressed IGFBP-5 mRNA. In 1994 Chen et al. (26) reported that effects of multiple IGFBPs on IGF-stimulated DNA synthesis in MCF-7 cells. They found that IGFBP-2 and IGFBP-3 enhanced DNA synthesis in response to IGF-I while IGFBP-4 and IGFBP-5 had no effect.

The expression of IGFBPs has also been examined in breast cancer tissues, where the correlation with ER status is not as clear-cut. Pekonen et al. (27) found that only IGFBP-3 expression could be correlated to ER status, being more common in ER negative tumors. They discovered that each of the tissue specimens, regardless of ER status, expressed all five of the IGFBPs that were known to exist at the time of publication. A more recent 1994 study by McGuire et al. (28) performed ligand blots for IGFBPs on eighty breast cancer tissue specimens. They were unable to detect IGFBP-1 in any of the specimens. A positive correlation of ER expression and IGFBP-4 expression was found, while IGFBP-3 was negatively correlated with ER status.

Two of the IGFBPs have been shown to have direct effects independent of IGF-I. In 1989, Blat et al. (29) reported that IGFBP-3 (then known as inhibitory diffusible factor 45) can inhibit serum stimulation of DNA synthesis in the chick embryo fibroblast cell line. Later, Jones et al. (30) found that IGFBP-1 stimulates migration of the Chinese hamster ovary (CHO) cells in woundings assays even in the absence of IGF-I. A single amino acid substitution in the IGFBP-1 sequence which prevented its binding to the $\alpha 5\beta 1$ integrin on the CHO cell surface reversed this stimulation. Oh and co workers (31) reported that IGFBP-3 has IGF-receptor independent activities in an ER negative cell line, Hs578T. These authors found that treatment of the Hs578T cells with exogenous IGFBP-3 significantly inhibited their monolayer growth. This inhibitory effect could be overcome by native IGF-I, but not by IGF analogs with normal affinity for the IGF-I receptor but decreased affinity for IGFBP-3. This finding suggested that the effect was independent of the IGF-I receptor but was blocked by the IGF-IGFBP-3 interaction (20).

BODY

METHODS

Migration Assays - The Boyden chamber migration assays were performed by filling the lower wells with a putative chemoattractant or control solution, over which the coated polycarbonate membrane (pore size-8 μm) is placed. Phenol-red free DMEM/F12 with 0.01% bovine serum albumin (BSA) is used as a negative control solution, and all growth factors are prepared in this solution. Monolayers of confluent or near confluent cells are trypsinized and re-plated twelve to sixteen hours prior to each assay. Each assay begins by rinsing the cells with PBS/EDTA (phosphate buffered saline containing a 5 mM concentration of EDTA) and separating them from the tissue culture dish with 2 ml of 0.1X trypsin in PBS/EDTA. Then 8 ml of a 10% serum containing media are added to inactivate the trypsin. The cell suspension is centrifuged once for 10 minutes; the serum containing media is then removed. Next the cells are resuspended and washed with the DMEM/BSA solution twice. After the second centrifug-ation, the supernantant is removed. The cell pellet is then re-suspended in a final volume of one to three ml. A hemacytometer is used to estimate the density of the cell suspension.

For those experiments in which an anti-integrin antibody is used, the cells are separated into microcentrifuge tubes and incubated with the antibody by gently tumbling at room temperature for thirty to sixty minutes. All anti-integrin antibodies were used at a concentration of 25 μg/ml. For those experiments in which IGFBP-1 or its [221 Tryp] mutant were used, the BP-1 (final concentration 10 nM or 50 nM) was added to the cell suspension. The suspension was then tumbled at room temperature for 45-60 minutes. A 50 μl volume of the suspension containing approximately 10,000-15,000 cells (MDA-231) or 50,000-60,000 cells (MCF-7) is loaded into each upper well, and the chamber is placed in a 37°C 5%CO2-enriched incubator. After a four hour period, the chamber is disassembled. The cells adherent to the upper surface of the membrane are scraped away so that only those cells which have migrated through the membrane remain. The membrane is then fixed in methanol, stained with Diff-Quik and allowed to air dry on a glass slide. A grid eyepiece in our microscope is then used to reliably demarcate several areas within each individual well in order to count migrated cells. Two full grids are counted for each of the MDA-231 cell wells and four grids are counted for each MCF-7 well.

To assess the ability of these cells to respond to a chemokinetic stimulus as opposed to a chemotaxic stimulus, cultures were grown to confluency in 6 well plates. Both MCF-7 and MDA-231 cells were tested in this assay. The cultures were then wounded with a razor blade. Wounds had to be clean in that no cells could be attached across wound margin and no grooves could be cut in the plate, since the cells tend to migrate along the grooves. Following wounding, the wounds were scored by a blinded observer. Then the treatments, e.g. IGF-I or no IGF-I, were added at a concentration of 100 ng/ml in 0.2% calf serum. After 48 hours, the number of cells crossing the wound margin by at least 150 microns was determined. These cells were fixed to the plate by adding 70% methanol then stained with methylene blue and the number of cells migrating at least this far in each wound margin was determined.

Cross-linking Studies - Two populations of MCF-7 cells were studied, those which have been exposed to calf serum (CS) for 7-10 days and those which have always been in fetal calf

serum (FCS). Both groups were trypsinized and replated in the appropriate medium in a six well plate twelve to sixteen hours prior to cross-linking. This sequence of events reproduced the conditions under which MCF-7 cells migrate well to IGF-I. The cells were rinsed twice with binding buffer (HCO3-free EMEM with 0.1% BSA and 20 mM Hepes at pH 7.3), then incubated with 3 to 10 μCi of ^{125}I-IGF-I with increasing concentrations of unlabelled IGF-I. Following a 90 min incubation at 4°C, the wells were aspirated and rinsed twice with cross-linking buffer, which contained EMEM without amino acid with 20 mM Hepes at pH 7.3. Immediately prior to cross-linking, freshly prepared 10 mM DSS in DMSO was added to the cross-linking buffer to yield a final concentration of 0.5 mM. The cells were then incubated in the DSS in cross-linking buffer for 30 min at 4°C. The DSS was then aspirated and neutralized with 0.1 M Tris-HCl with 0.1M NaCl at pH 7.4. The wells were rinsed twice with this neutralizing buffer, and received a third rinse of 5 to 10 min duration. The buffer was aspirated and the cells were lysed in minimal volumes SDS sample buffer with 100mM DTT (dithiothreitol) to achieve reducing conditions. Standardized samples were resolved in a 6% SDS-PAGE.

Integrin Receptor Immunoprecipitation - To determine the presence of various integrins on MCF-7 or MDA-231 cells, the total cell surface proteins were labelled by adding 1 mCi of ^{125}I to confluent cultures in three 10 cm dishes. Chloramine T was then added and the reaction stopped by the addition of sodium metablsulfite. The iodinated proteins were harvested by adding 1 ml of RIPA buffer. Membranes were then centrifuged and the non-membranous material discarded. The radiolabelled proteins in the membranes were solubilized by adding 100 μl of laemmli buffer to each test sample then centrifuged in a microfuge for 10 minutes. After boiling, the supernatant was then analyzed by SDS-PAGE followed by direct autoradiography. To determine if particular integrins reacted with specific antibodies, prior to electrophoresis the RIPA lysate was incubated with a 1:500 dilution of various anti-integrin antibodies and then the complexes immunoprecipitated by the addition of protein A sepharose. The pellets were solubilized in 100 μl of laemmli sample buffer, and 60 μl of this mixture was loaded on SDS-PAGE gel 12.5% gel. Following electrophoresis, the gel was dried and a direct autoradiograph obtained to determine radiolabelled band intensities at various molecular weights. Correct standards were run in a parallel lane. SDS sample buffer contained dithiothreatol.

RESULTS

Task 1: Complete. The complete report of Task 1 was filed with the May, 1998 progress report.

Task 2: Months 5-28.

We have completed the anti-integrin antibody results that were incomplete on the last submitted report. The results are shown in Table I. Importantly, we discovered that the α3 integrin antibodies were potent inhibitors of IGF-I-stimulated migration in T47-D cells, and α5 was a potent inhibitor in HS578T cells. The initial integrin antibodies that had been tested were shown in Table IV of the last report. We include now, in Table II, a listing of the additional integrins that are present and the effects of anti-integrin antibodies in inhibiting the migration response to IGF-I stimulation.

As in the previous report, IGF-I stimulated migration of all four cell types. The response to IGF-I was most potently inhibited consistently with anti-β1 integrin antibodies across all four cell lines. Anti-α2 antibodies had good effects against BT20 cells, but lesser effects with T47B cells. Both anti-β1 and -β2 antibodies were potent inhibitors of the migration of ZR-75 cells in response to IGF-I, whereas anti-α1 and -α6 antibodies were weak inhibitors. This completes the analysis of integrins that are present and the ability of these anti-integrin antibodies to inhibit IGF-I-stimulated migration in all six cell lines. This completes the revised statement of work proposal for Task 2.

Task 3:

Cross-linking studies for IGF-I receptors have been previously reported. Likewise, the migration studies proposed in 3B have been previously reported. In our last report, we reported the migration response of MCF-7 cells to estradiol, and the combination of estradiol + IGF-I. We have extended these in this report to determine the effect of Tamoxifen on IGF-I-stimulated migration. As shown in Table II, Tamoxifen is a potent inhibitor of MCF-7 cell migration in response to IGF-I. This was studied in an assay that measures chemokinesis. These results show that Tamoxifen blocks the effect of 30 ng/ml of IGF-I in stimulating this cell type, and the effect of Tamoxifen is dose-dependent – further reinforcing the importance of estrogen for IGF-I to mediate this effect, and that the effects of IGF-I and estrogen appear to be additive.

Task 4:

We have repeated the Boyden chamber assays for MCF-7 cells using IGFBP-3 and -5, as we had for MDA-231 cells previously. Likewise, we have utilized IGFBP-2 and -4, since these cells also produce these binding proteins, and we have studied the effects of IGFBP-2 and -4 on MDA-231 cells for comparison. We have utilized the des 1-3 analog to study its potency in the presence of all four binding proteins (i.e. IGFBP-2 through -5) that have been tested. The results show that, similar to MDA-231 cells, IGFBP-3 and -5 alter the migration response to IGF-I (Table III). Specifically, IGFBP-3 is a pure inhibitor, and a 4:1 molar excess inhibited the entire IGF-I-stimulated response. IGFBP-5, in contrast, enhanced the migration response to IGF-I. This is similar to the effect seen in MDA-231 cells. IGFBP-2 and -4 were tested on both MDA-231 and MCF-7 cells in a similar assay system (Table IV and V). Both proteins inhibited the migration response to IGF-I in each cell type. The proteins were approximately equally potent inhibitors in MDA-231 and MCF-7 cells. When chemotaxis was measured, IGFBP-2, -3, and -4 inhibited the response to IGF-I, although IGFBP-2 and -4 were significantly more potent than

IGFBP-3 (Table VI). When the effect of des 1-3 IGF-I, which binds poorly to these proteins, was assessed, IGFBP-3 and -5 were capable of inhibiting the effect of des IGF-I, but, in each case, the effect of des IGF-I was much greater than the effect of wild type IGF-I, suggesting that the inhibitory effects of these proteins were a function of their ability to inhibit IGF-I binding to the receptor (Table VII).

DISCUSSION

These results extend our previous experimental findings. They show that there is an additive response between estradiol and IGF-I in stimulating the migration of MCF-7 cells. This has now been demonstrated both with additive experiments, where the two factors are added together, and with use of Tamoxifen, a selective estrogen response modifier. Since IGF-I has been implicated epidemiologically in the propensity to develop breast cancer, this finding provides an interesting mechanism to be analyzed in future research into how this growth factor acts with estrogen to facilitate breast cancer cell invasiveness. The results also extend our previous findings with integrin receptors. They show that β1 integrins are important in mediating migration of several breast cancer cell types and that anti-β1 integrin antibodies are generally effective inhibitors. They also show some differential response between the display of β2 integrins on various breast cancer cell types, and the response to α2 and β2 integrins is reasonable in the cell types that possess these integrins. In contrast, the abundance of α1 and α6 integrins was considerably lower, and the inhibitory effects of their antibodies were also considerably less potent. Taken together with our previous data, they suggest that β3, α5, α3, and β1 integrins are generally the most abundant across these six breast cancer cell lines and that inhibiting these integrins often results in substantial inhibition of IGF-I-mediated migration. Since recent studies have shown that there is cooperative activity between growth factor signaling and several integrin receptors, including α2β1, αVβ3, αVβ5, and α3β1, multiple integrin receptors may function with this growth factor to mediate cell migration. Therefore, a therapeutic approach to inhibit IGF-I action by inhibiting integrin receptor activity may have to rely upon inhibition of multiple integrins, rather than one integrin. Likewise, these studies raise the possibility that inhibition of one integrin may lead to compensatory changes in another integrin that nullify the effects of that particular inhibitor. These possibilities should be considered in designing *in vivo* experiments in which anti-integrin receptor antibodies are tested.

Our findings extensively document the chemokinetic and chemotactic effects of IGF binding protein modification of IGF-I response in both MCF-7 and MDA-231 cells. In general, IGFBP-2 and -4 are consistently inhibitory for both types of responses in both cell lines. In contrast, IGFBP-3 and -5 have more variable effects. IGFBP-5 has the potential to potentiate migratory responses, at least across a lateral surface, which is consistent with its ability to be incorporated into extracellular matrix and potentiate growth responses to IGF-I. In contrast, IGFBP-3, while generally inhibitory, is substantially less inhibitory than IGFBP-2 and -4, suggesting that either it may be proteolytically cleaved in the medium or that it is incorporated into extracellular matrix and that this incorporation leads to simultaneous stimulation, as well as inhibition of IGF-I action. These findings point to the complexity of understanding IGF binding protein regulation of IGF-I action. Since parenchymal cells around breast tumors and breast cancers themselves can produce IGF binding proteins, this complex set of biologic responses may be important modifiers of IGF-I actions and may modify IGF-I actions in bifunctional ways that lead to complex and subtle changes in the responsiveness of these cells. Finally, we conclude that chemokinetic assays with a long time course are much more reliable indices of assessing binding protein function in this system as compared to chemotactic assays and should probably be used to predict the responsiveness of *in vivo* systems to IGF-I in various combinations of binding proteins.

CONCLUSIONS

1. Breast cancer cells tested herein express multiple integrin receptors, and inhibiting ligand occupancy of these integrin receptors inhibits the migration response to IGF-I. Some of the differences in cell responsiveness to IGF-I may be partially mediated by changing integrin receptor display or changing extracellular matrix composition, along with integrin display.

2. Inhibiting one integrin may attenuate IGF-I action, but inhibiting multiple integrins will probably be required to achieve a marked reduction in IGF-I effectiveness.

3. Serum estrogen response modifiers, such as Tamoxifen, may be important in altering cell migration responsiveness to IGF-I.

4. IGFBP-2 through -5 modulate IGF-I migration. Since these proteins are produced by breast cancer cells in culture and *in vivo*, these are important factors to consider when predicting the migratory response of breast cancer cells to IGF-I in any test system.

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APPENDICES

TABLE I

Migration Responses of Breast Cancer Cell Lines Exposed to Anti-Integrin Antibodies

Cell line Integrin	T47D	BT-20	HS578T	ZR-75-1
B1	-61%	-39%	-80%	-70%
B2	ND	ND	ND	-66%
α2	-22%	-44%	ND	ND
α1	ND	-15%	ND	-11%
α6	ND	ND	-19%	-20%

ND = not detected.

The results are expressed as the percentage inhibition of the mean of 3 separate experiments. The percentage was calculated by dividing the number of cells migrating in the Boyden chamber assay by the number of cells migrating in response to 100 ng/ml of IGF-I in control cultures.

TABLE II

Chemotactic Response of MCF-7 Cells to Tamoxifen and IGF-I

	Number of migrating cells	% increase or decrease compared to control
Control	311	—
IGF-I, 10 ng/ml	584	87%
IGF-I, 30 ng/ml	737	136%
Tamoxifen (10^{-7} M)	334	0
IGF-I (30 ng) + Tamoxifen (10^{-7} M)	590	-20%*
IGF-I (30 ng) + Tamoxifen (10^{-6} M)	399	-46%*

* Percent decrease compared to the effect of IGF-I, 30 ng/ml, alone.

The results represent the mean of three separate experiments measuring cell migration across a lateral surface in response to IGF-I or estradiol. 9-12 wounds were used per experiment.

TABLE III

Chemokinetic Response of MCF-7 Cells to IGFBP-3 and IGFBP-5

	Number of migrating cells	% Increase or decrease in migration
Control	298	0
IGFBP-3 (100 ng/ml)	299	0
IGFBP-3 (500 ng/ml)	202	-32%
IGF-I (30 ng/ml)	555	86%
IGF-I (30 ng/ml) + IGFBP-3 (100 ng/ml)	385	29%
IGF-I (30 ng/ml) + IGFBP-3 (500 ng/ml)	290	-2%
IGFBP-5 (100 ng/ml)	336	13%
IGFBP-5 (500 ng/ml)	301	0
IGFBP-5 (500 ng/ml) + IGFBP-5 (100 ng/ml)	599	101%
IGFBP-5 (500 ng/ml) + IGFBP-5 (500 ng/ml)	638	114%

The results represent the mean of three separate experiments. 9-12 wounds were used per data point.

TABLE IV

Chemotactic Response of MDA-231 Cells to IGFBP-3 and IGFBP-4

	Number of migrating cells	% Increase or decrease in migration
Control	246	
IGFBP-2 (100 ng/ml)	230	-6.5%
IGFBP-2 (500 ng/ml)	211	-14%
IGF-I (30 ng/ml)	403	64%
IGF-I (30 ng/ml) + IGFBP-2 (100 ng/ml)	336	37%
IGF-I (30 ng/ml) + IGFBP-2 (500 ng/ml)	234	-1%
IGFBP-4 (100 ng/ml)	250	0
IGFBP-4 (500 ng/ml)	217	-12%
IGF-I (30 ng/ml) + IGFBP-4 (100 ng/ml)	289	17.5%
IGF-I (30 ng/ml) + IGFBP-4 (500 ng/ml)	227	-7%

The results represent the mean of three separate experiments. 9-12 wounds were used per data point.

TABLE V

Chemotactic Response of MCF-7 Cells to IGFBP-2 and IGFBP-4

	Number of migrating cells	% Increase or decrease in migration
Control	341	
IGFBP-2 (100 ng/ml)	338	0
IGFBP-2 (500 ng/ml)	290	-16%
IGF-I (30 ng/ml)	566	66%
IGF-I (30 ng/ml) + IGFBP-2 (100 ng/ml)	509	49%
IGF-I (30 ng/ml) + IGFBP-2 (500 ng/ml)	392	15%
IGFBP-4 (100 ng/ml)	317	-7%
IGFBP-4 (500 ng/ml)	387	-16%
IGF-I (30 ng/ml) + IGFBP-4 (100 ng/ml)	511	50%
IGF-I (30 ng/ml) + IGFBP-4 (500 ng/ml)	419	23%

The results represent the mean of three separate experiments. 9-12 wounds were used per data point.

TABLE VI

Chemotactic Response of MDA-231 Cells to IGFBP-2, IGFBP-3 and IGFBP-4

	*Number of migrating cells	% Increase or decrease in migration
Control	17	0
IGF-I (30 ng/ml)	184	0
IGFBP-2	19	-89%
IGFBP-3	15	-90%
IGFBP-4	20	-89%
IGF-I (30 ng/ml) + IGFBP-2	111	-40%
IGF-I (30 ng/ml) + IGFBP-3	152	-16%
IGF-I (30 ng/ml) + IGFBP-4	93	-49%

* The number of cells counted on the opposite side of the Boyden chamber at the end of the chemotaxis assay. The results represent the mean of 3 separate experiments with 3 replicates per experiment.

TABLE VII

Chemotactic Response of MDA-231 Cells to des 1-3 IGF-I in the Presence of IGFBP-3, IGFBP-4, and IGFBP-5

	*Number of migrating cells	% Increase or decrease compared to IGF-I
Control	16	—
IGF-I	104	0
des (1-3) IGF-I	111	5%
IGF-I + IGFBP-3	52	-50%
des (1-3) IGF-I + IGFBP-3	89	-15%
IGF-I + IGFBP-4	47	-55%
des (1-3) IGF-I + IGFBP-4	109	4%
IGF-I + IGFBP-5	63	-39%
des (1-3) IGF-I + IGFBP-5	115	10%

* The number of cells counted on the opposite side of the Boyden chamber at the end of the chemotaxis assay. The results represent the mean of 3 separate experiments with 3 replicates per experiment.