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REGULATION OF BREAST CANCER GROWTH BY INSULIN-LIKE GROWTH FACTORS

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Summary—The IGFs may be important autocrine, paracrine or endocrine growth factors for human breast cancer. IGF-I and II stimulate growth of cultured human breast cancer cells. IGF-I is slightly more potent, paralleling its higher affinity for the IGF-I receptor. Antibody blockade of the IGF-I receptor inhibits growth stimulation induced by both IGFs, suggesting that this receptor mediates the growth effects of both peptides. However, IGF-I receptor blockade does not inhibit estrogen (E_2)-induced growth suggesting that secreted IGFs are not the major mediators of E_2 action. Several breast cancer cell lines express IGF-II mRNA by both Northern analysis and RNase protection assay. IGF-II activity is found in conditioned medium by radioimmuno and radioreceptor assay, after removal of somatomedin binding proteins (BP) which are secreted in abundance. IGF-I is undetectable. BPs of $\simeq$25 and 40 K predominate in ER-negative cell lines while BPs of 36 K predominate in ER-positive cells. Blockade of the IGF-I receptor inhibits anchorage-independent and monolayer growth in serum of a panel of breast cancer cell lines. Growth of one line (MDA-231) was also inhibited *in vivo* by receptor antibody treatment of nude mice. The antibody had no effect on growth of MCF-7 tumors. These data suggest the IGFs are important regulators of breast cancer cell proliferation and that antagonism of this pathway may offer a new treatment strategy.

INTRODUCTION

The insulin-like growth factors (IGFs) are a family of hormones with structural homologies to insulin. IGF-I is regulated by growth hormone, and it is a mediator of somatic growth [1]. The normal role for IGF-II is less clear, although it is mitogenic for cultured cells.

Experimental data suggest that the IGFs, as well as insulin, may be important regulators of breast cancer cell proliferation. Insulin and IGF-I have been reported to stimulate growth of several human breast cancer cell lines [2–5]. Multiplication stimulating activity (rat IGF-II) as well as purified or recombinant IGF-II are reported to stimulate proliferation of the MCF-7 and/or T47D cell lines [3, 6, 7]. Further, receptors for insulin, IGF-I and IGF-II have been found on human breast cancer cells, although which receptor(s) mediate the effects of these hormones has been the subject of debate [4, 6, 8].

In this paper we will review data primarily from our own laboratories that emphasize the potential importance of these growth factors for human breast cancer. We demonstrate that both IGF-I and IGF-II are potent mitogens for breast cancer cells, and that the growth effects of both are mediated predominantly via the IGF-I receptor. We also show that IGF-II (but not IGF-I) is expressed by certain breast cancer cell lines and can be detected in conditioned medium, consistent with the possibility of an autocrine growth pathway. Even though expression of IGF-II is stimulated by estrogen in MCF-7 and T47D cells, our data suggest that secretion of this factor does not mediate estrogen-induced growth. Interestingly, human breast cancer cell lines also express and secrete large amounts of IGF binding proteins (BP) which, theoretically, could alter the biological effects of these growth factors. We have found that the type of BP secreted is related to estrogen receptor (ER) status. Finally, the pathways of IGF-induced growth may offer new treatment strategies. Blockage of the IGF-I receptor inhibits growth of human breast cancer cells in both *in vitro* and *in vivo* experimental models.

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Growth effects of the IGFs

Both IGF-I and IGF-II are potent mitogens in serum-free medium for a variety of

ER-positive and ER-negative human breast cancer cell lines in DNA synthesis and cell proliferation assays [9, 10]. Dose–response studies show that as little as 0.05 nM of these peptides (recombinant or synthetic) is mitogenic, with maximal results observed with 1–10 nM concentrations [9]. IGF-II is consistently slightly less potent than IGF-I in these assays with 2-4 fold higher concentrations required for the same biologic effect.

Activity of both IGFs is mediated through the IGF-I receptor

The IGF-I receptor is a dimer possessing tyrosine kinase activity [11]. The IGF-II receptor has no tyrosine kinase activity and has been reported to be identical to the mannose-6′-phosphate (M-6P) receptor involved in lysosomal enzyme pathways [11]. It is not clear if the IGF-II/M-6P receptor mediates the cellular effects of IGF-II, and studies in other cells suggest that the proliferative effects of both IGFs are mediated through the IGF-I receptor.

We have shown that IGF-II, as well as IGF-I, can interact with the IGF-I receptor in human breast cancer cells [9]. In both competitive binding and affinity labeling studies, the affinity of IGF-II for the IGF-I receptor parallels its relative ability to stimulate cell proliferation. IGF-II is 2–3 fold less potent than IGF-I and insulin is 10-fold less potent in its ability to compete for IGF-I binding (Table 1).

To further investigate the role of the IGF-I receptor in mediating the proliferative effects of the IGFs, we used a monoclonal antireceptor antibody (alpha-IR-3) provided by Drs F. Kull and S. Jacobs (Wellcome Research Laboratories). This antibody blocks the hormone binding domain of the IGF-I receptor. At a concentration of 0.1 μM, alpha-IR-3 completely abolished IGF-I-induced stimulation of DNA synthesis in a panel of breast cancer cell lines [10]. The inhibitory effect of the antibody could be completely reversed by equimolar concentrations of IGF-I, indicating that the inhibitory effect was due to an interaction with the IGF-I receptor, and excluding the possibility of nonspecific antibody-induced cytotoxicity.

Table 1. Relative binding affinities* for IGF-I, IGF-II and insulin in human breast cancer cell lines

	MCF-7 (nM)	MDA-231 (nM)
IGF-I	3	5
IGF-II	7	8
Insulin	30	80

*Potency estimated from half-maximal displacement in competitive binding studies [9].

Table 2. Effect of alpha-IR-3 on IGF-II-induced growth

	[^{3}H]-thymidine incorporation (% above control)	
	MCF-7	MDA-231
IGF-II (13 nM)	63	48
IGF-II + alpha-IR-3 (10 nM)	22	19

Since we had shown that IGF-II could bind with high affinity to the IGF-I receptor in human breast cancer cells, we hypothesized that, if this receptor mediated IGF-II effects, blockade of the receptor with alpha-IR-3 ought to inhibit IGF-II induced growth. IGF-II dose–response studies in MCF-7 and MDA-231 cells demonstrate that alpha-IR-3 also significantly inhibits growth stimulation by this peptide [9]. Table 2 demonstrates that at a concentration of 10 nM, alpha-IR-3 partially inhibited growth stimulation by 13 nM IGF-II. Similar data have recently been reported by others [12]. Thus, in human breast cancer cells the growth effects of both IGF-I and IGF-II are mediated predominantly through the IGF-I receptor. We cannot exclude from these studies that some growth effects are also transmitted via the IGF-II/M-6P receptor. However, these data suggest that blockade of the IGF-I receptor might offer a new therapeutic strategy by inhibiting the growth-promoting activity of two related polypeptide growth factors.

Expression of IGFs by human breast cancer cells

Earlier reports suggested that cultured human breast cancer cells expressed IGF-I mRNA and secreted an IGF-I-like activity into conditioned medium [5, 13]. Later studies, however, suggested that breast cancer cells do not make genuine IGF-I, although stromal tissues in breast cancer specimens may be an abundant source [14]. The initial studies may have been confounded by cross-hybridizing mRNA species on Northern blots and by the presence of large amounts of IGF-BPs present in conditioned medium that could give false-positive results in an IGF-I RIA. We have not been able to detect immunoassayable IGF-I activity in conditioned medium when these BPs are removed. Thus, IGF-I seems unlikely to have an autocrine role in human breast cancer growth regulation, although paracrine/endocrine functions may be important.

However, recent data suggest that autocrine as well as paracrine pathways may involve

IGF-II. We have demonstrated by a sensitive and specific RNase protection assay, as well as by Northern blot analysis, that at least two human breast cancer cell lines (MCF-7 and T47D) expressed genuine IGF-II mRNA [9]. Furthermore, estrogen treatment of these cells results in a 4–5 fold increase in IGF-II mRNA. These data are similar to those reported by Yee [7], who also showed that breast cancer epithelial cells as well as stromal cells in breast cancer tissue specimens expressed IGF-II mRNA.

We also found IGF-II immunoactivity and receptor binding activity in conditioned media from a panel of breast cancer cell lines after removal of IGF-BPs using Sep-Pak C-18 cartridges [9]. Table 3 shows the IGF-II activity in six different cell lines. The highest levels were detected in the two cell lines in which IGF-II mRNA was also found. The small amount of IGF-II activity in the other cell lines may represent residual BPs.

Secreted IGFs do not mediate estrogen-induced growth

The observation that the expression and secretion of IGF-II by the ER-positive MCF-7 and T47D cell lines is estrogen-regulated raised the possibility that IGF-II, through an autocrine mechanism, could mediate the growth effects of estrogen. However, blockade of the IGF-I receptor by alpha-IR-3, which blocks significantly the growth effects of exogenous IGF-I and IGF-II, has no effect on estrogen-induced growth in either MCF-7 or T47D cells [9]. Since estrogen stimulation also increases the expression and secretion of transforming growth factor alpha (TGF-alpha), we also examined the effect of blocking its receptor (the EGF receptor) alone or in combination with the IGF-I receptor with a cocktail of monoclonal anti-receptor antibodies (Table 4). Despite being able to block the effects of exogenous TGF-alpha and/or IGF-I, these antibodies were unable to inhibit estrogen-induced growth. These data suggest that secreted TGF-alpha and IGFs are not the major mediators of estrogen-induced growth in these cell lines. Either other secreted factors are important, or estrogen induces cell proliferation by alternative mechanisms.

Table 3. IGF-II activity in conditioned media

Cell line	RIA (ng/ml × 10^7 cells)	RRA (ng/ml × 10^7 cells)
T47D	135.4	111.5
MCF-7	4.7	14.4
BT20	0.91	4.1
ZR75-1	0.64	0.56
MDA-330	0.19	0.06
MDA-231	0.36	2.2

Table 4. Effect of IGF-I and EGF receptor blockade on estrogen-induced growth in MCF-7 cells

Group	[^{3}H]-thymidine incorporation (cpm × 10^{-3})
Control	3.4 ± 0.4
IGF-I (13 nM)	17.6 ± 0.5
IGF-I + alpha-IR-3	7.7 ± 0.2
TGF-alpha (10 nM)	8.0 ± 0.3
TGF-alpha + 528 Ab[a]	5.5 ± 0.2
IGF-I + TGF-alpha	25.0 ± 0.9
IGF-I/TGF-alpha + alpha-IR-3/528 Ab	11.8 ± 0.4
E_2 (1 nM)	36.3 ± 1.9
E_2 + alpha-IR-3	36.7 ± 1.9
E_2 + 528 Ab	36.7 ± 1.4
E_2 + alpha-IR-3 + 528 Ab	41.8 ± 0.5

[a]528 Ab is an EGF receptor antibody provided by Dr J. Mendelsohn. Both antibodies were present at a concentration of 100 nM. Each antibody by itself had no effect on basal [^{3}H]thymidine incorporation.

IGF-BP profiles in human breast cancer cells

In our studies of IGF activity in conditioned media, we discovered that all cell lines tested secreted abundant IGF-BPs (manuscript submitted). To characterize the type of BPs secreted, a series of studies using ligand binding blots, immunoblotting, RIA and Northern blotting to detect mRNA were performed. The data are summarized in Table 5. The type of IGF-BP detected correlates with the ER status of the cell line. The ER-negative cell lines secrete IGF-BP-1, IGF-BP-3 and a 24,000 M_r form. In contrast, the ER-positive lines secreted predominantly IGF-BP-2 as well as the 24,000 M_r form. (The BT20 cell line is ER-negative by biochemical assay, but is positive for ER mRNA by PCR.) Estrogen stimulation of MCF-7 cells resulted in a 2-fold increase in IGF-BP levels in conditioned medium. Other groups have also detected IGF-BPs in conditioned medium from human breast cancer cell [15, 16], but the physiologic relevance of IGF-BP secretion remains to be defined.

Table 5. Types of IGF-BP secreted by human breast cancer cells

	Type of IGF-BP			
	24K	BP-1	BP-2	BP-3
HS578T[a]	+	+	−	+
MDA-330[a]	+	+	−	+
MDA-231[a]	+	+	−	+
MCF-7[b]	+	−	+	−
ZR75-1[b]	+	−	+	−
T47D[b]	+	−	+	−
BT20[b]	−	−	+	−

[a]ER-negative.
[b]ER-positive.

Blockade of IGF pathways as a potential new treatment strategy

The cumulative data suggest that IGF pathways may be important for growth regulation of breast cancer by autocrine, paracrine, and/or endocrine mechanisms. Just as antiestrogen blockade of ER is now an effective treatment for human breast cancer, blockade of IGF pathways also has the potential for inhibiting tumor growth.

As an experimental model, we have used the alpha-IR-3 antibody to block the IGF-I receptor in cultured human breast cancer cells and in breast cancer cells growing as xenografts in athymic mice [10, 17]. This antibody inhibits DNA synthesis, monolayer growth and anchorage-independent growth of several ER-positive and ER-negative breast cancer cell lines cultured *in vitro* in the presence of serum [10].

Two cell lines, MCF-7 and MDA-231, were studied in more detail. Despite the fact that alpha-IR-3 was a potent inhibitor of the anchorage-independent growth of MCF-7 cells, the antibody had no effect on growth of this cell line in estrogen-supplemented athymic mice when injected i.p. twice weekly at a dose as high as 500 μg/mouse [17]. In contrast, the MDA-231 cells were inhibited by alpha-IR-3 *in vitro* and *in vivo*. Tumor growth was inhibited by as little as 20 μg/mouse twice weekly, and nearly complete inhibition was seen with 500 μg/mouse. No growth inhibition was observed with a control antibody (alpha-IR-1) that recognizes a non-binding domain of the insulin receptor. Histologic examination of treated tumors demonstrated tumor cell loss, fibrosis and marked collagen deposition. Other reports have shown that *in vivo* blockade of the EGF receptor or neutralization of bombesin, a possible autocrine growth factor, can inhibit growth of other tumors [18, 19]. Obviously, antagonism of these polypeptide growth factor pathways may have potential toxic effects that need to be carefully explored, but the data suggest that additional study of these possible new forms of "endocrine therapy" is warranted.

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