Review

The Potential Clinical Applications of Insulin-like Growth Factor-1 Ligand in Human Breast Cancer

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Abstract. *Insulin-like growth factor-1 (IGF-1) has become recognized as a growth factor with pro-mitogenic and anti-apoptotic effects on a variety of human cells. This article reviews the potential role of IGF-1 ligand in the clinical management of breast cancer patients. Many studies have shown that IGF-1 acts synergistically with oestrogen to stimulate breast cancer cells. Case-control studies have also demonstrated that premenopausal women with high levels of serum IGF-1 have an increased risk of developing breast cancer later in life. Serum IGF-1 levels can therefore be used as a potential biomarker for predicting breast cancer risk. Furthermore, there is evidence that serum IGF-1 levels can serve as a response biomarker in chemoprevention drug trials. The role of IGF-1 expression in breast cancer tissue as a prognostic marker is not clearly established. Identifying the IGF-1 gene polymorphism can potentially be used in predicting breast cancer risk.*

Despite its original role as a mediator of normal human growth and development, the insulin-like growth factor (IGF)-1 system has been heavily implicated in the development and progression of breast cancer. The IGF-1 system consists of the IGF-1 ligand, the receptor, binding proteins and proteases, which interact in dynamic equilibrium to regulate the effects of IGF-1 (52). This review aims to elaborate on the various laboratory and clinical findings regarding the IGF-1 ligand and discusses how measuring the IGF-1 ligand could be used in a clinical setting.

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The Insulin-like Growth Factor-1 (IGF-1) System

In normal physiology. The IGF-1 system plays an important role in normal growth and development. It is particularly important for the growth of specific organs such as the nervous system in which IGF-1 signaling regulates neuronal proliferation, apoptosis and cell survival (45). The IGF-1 system acts as a mediator for growth hormone (GH) which is fundamental to linear growth. Growth hormone, which is produced by the pituitary gland, stimulates production of the IGF-1 ligand in almost all tissue types, especially the liver which serves as the main source of circulating IGF-1 ligand (52). There is a negative feedback loop in which serum IGF-1 suppresses the secretion of GH (7) (Figure 1). In normal development, serum IGF-1 is expressed at low levels during embryonic growth, increases gradually from birth to puberty, surges in puberty and then declines with age thereafter. Its level can also be affected by nutritional status (72).

In human cancer. Initial evidence that the GH/IGF-1 axis contributed to breast cancer progression was provided thirty years ago when hypophysectomy was shown to favourably improve the outcome of metastatic breast cancer patients (58). Most of the IGF-1 research over the past two decades focused on its role in the development and progression in numerous types of cancer. IGF-1 acts as a potent mitogen that can stimulate normal breast and breast cancer cells. The IGF-1 system has been shown to promote malignant transformation of normal breast cells (4, 81), maintenance of malignant phenotype, increase metastatic potential (25, 47), resistance to apoptosis and cytotoxic drugs (1, 61, 62), multi-drug resistance (23, 26) and hormone independence (55-57). These are all features of more aggressive and resistant phenotypes and would eventually translate into poorer prognosis for patients with breast cancer showing increased IGF-1 activity. Several clinical studies have investigated the components of the IGF-1 system and looked at their ability to predict risk of developing breast cancer whilst other studies aimed to correlate IGF-1 levels with prognosis in breast cancer patients

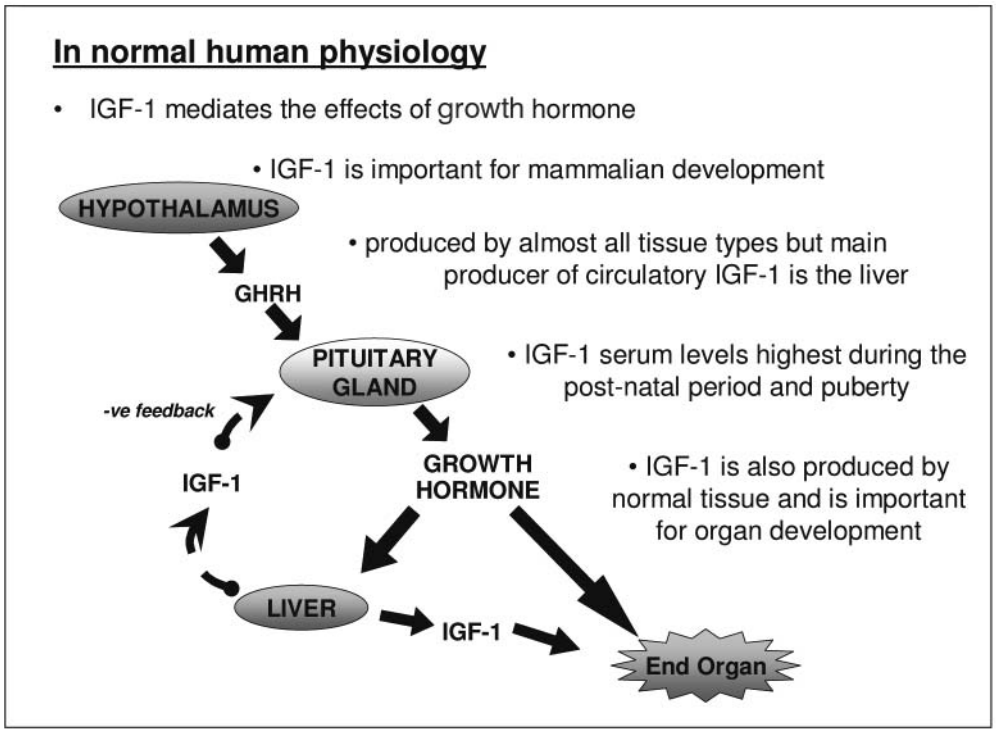

Figure 1. *The growth hormone axis.*

The Insulin-like Growth Factor-1 Ligand

The gene is located on chromosome 12q22. IGF-1 ligands contain A and B domains homologous to the A and B chains of the hormone insulin. It is a polypeptide hormone that is produced by almost any tissue. The liver produces the largest amount of IGF-1 as a result of GH stimulation and is the main contributor to serum IGF-1. Serum IGF-1 can stimulate normal breast cells, promote malignant transformation and contributes to breast cancer progression through an endocrine route (29). Studies have also shown that stromal cells adjacent to breast cancer cells can produce IGF-1 locally to stimulate tumour cells through a paracrine or autocrine route (9, 16, 22, 79, 82). In fact, several studies have shown that local tissue production could be an important source of IGF-1 which may play a role in the growth of normal tissue (69) as well breast cancer development and progression (16, 22, 79, 82).

The Relationship between the IGF-1 System and Oestrogen

Research has suggested that IGF-1 and oestrogen act synergistically to stimulate breast cancer and that IGF-1 may have little effect on proliferation in the absence of oestrogen (29). Oestrogen stimulation is thought to induce the expression of IGF-1R (76). Cell line studies from various normal and malignant human tissues have established that oestrogen sensitizes cells to the mitogenic effect of IGF-1 through several mechanisms. These include increasing the expression and binding of IGF-1R signaling components (63, 70), the promotion of cell cycle progression while decreasing cell cycle suppressors (14), and increasing the expression of intracellular signaling molecules including insulin receptor substrates (IRS)-1 and -2 (27, 42, 66) and increasing the activity of PI3K and Ras-Ref-MAPK intracellular pathways (71, 78). Anti-oestrogens such as tamoxifen can reduce plasma IGF-1 by 25-30% (10, 48, 60), while IGF-1 has also been shown to increase the expression of ER in breast tissue (84). A study in our department showed that there may be a reciprocal and cross-stimulatory relationship between IGF-1 ligand and oestrogen production (8). Some studies suggest that oestrogen can itself stimulate the proliferation of breast cancer cells directly or indirectly by stimulating the expression of a number of growth factors (6). In endometriomas, the growth promoting effects of oestrogen are mediated by the induction of IGF-1 expression (24, 44). Such findings suggest that that IGF-1 and oestrogen are important cofactors of the same pathway that may lead to development and progression of breast cancer.

IGF-1 and Breast Cancer Risk

Many studies support the role of IGF-1 in the malignant transformation of breast epithelia. Animal studies have shown that transgenic mice which overexpress GH and IGF-1 exhibit an increased rate of developing mammary tumours (28, 80). Likewise, liver-IGF-1-deficient mice showed a 75% reduction of circulating IGF-1 compared to control mice, which also correlated with a significant reduction in risk of mammary tumour development (81), while treatment of primates with growth hormone and IGF-1 led to mammary gland hyperplasia (54). Animal studies suggested that high levels of circulating IGF-1 could be responsible for an increased risk of breast cancer in humans and this hypothesis prompted studies into the relationship between serum IGF-1 and risk of breast cancer in human subjects

The first study on this relationship was taken up by Hankinson *et al.* who carried out a case-control study by retrospectively measuring serum IGF-1 on blood samples collected from 397 women who later developed breast cancer against 620 age-matched controls (29). The results showed that when looking at the overall group, there was no relationship between serum IGF-1 and risk of developing breast cancer. However, on subanalysis based on menopausal status, there was a significant association between high serum IGF-1 and breast cancer risk only in women who were premenopausal at the time of blood collection (29). A subsequent larger case–control study by Schernhammer *et al.* involving 800 breast cancer patients and 1,129 age-matched controls also showed that serum IGF-1 levels were modestly associated breast cancer risk among premenopausal women only (67). Other studies have confirmed this observation that premenopausal women with relatively high levels of circulating IGF-1 were more prone to develop breast cancer later in life, but this risk was not present in postmenopausal women with high serum IGF-1 levels (3, 41, 43, 46, 64, 73). One study which examined postmenopausal women alone did show a significant association between high serum IGF-1 levels and a risk of breast cancer, but this was not significant once the hormone replacement therapy users were removed from the series (39). Another study confirmed that postmenopausal serum IGF-1 did not show any association with risk of developing breast cancer (41). A meta-analysis by Shi *et al.* involving 16 similar studies has concluded that premenopausal women with higher circulating IGF-1 have an increased breast cancer risk of nearly 40% (68). These findings reinforce the understanding that oestrogen may act as a cofactor in promoting the effects of IGF-1 on normal breast cells and may lead to malignant transformation.

IGF-1 Levels and Prognosis

Many previous studies have confirmed that premenopausal women generally have higher levels of serum IGF-1 compared to postmenopausal women (29, 53, 64). Some studies have shown that breast cancer patients in general have higher plasma IGF-1 levels at the time of diagnosis compared to normal (control) subjects (57, 59, 75).

Information looking at the relationship between serum IGF-1, clinical outcome and clinicopathological prognostic factors such as oestrogen receptor status, nodal status, tumour size and histological grading, are lacking. Vadgama *et al.* measured the serum IGF-1 in breast cancer patients after primary treatment and found that it correlated only to tumour size and progesterone receptor (PR) immunostaining (77). There was no association between serum IGF-1 with age, nodal stage or oestrogen receptor status. Their study also showed that patients who received adjuvant tamoxifen had lower serum IGF-1 levels which corresponded with a lower probability of recurrent breast cancer and longer overall survival (77). Coskun *et al.* showed that serum IGF-1 levels were higher in patients with metastasis compared to those without or 'normal' controls (12). There were no differences between ER+ and ER– metastatic or between the non-metastatic and control groups. However, this study involved only a small sample size and cancer cases were not matched to an equal number of controls (12). Holdaway *et al.* looked at serum IGF-1 levels at baseline and at 1 week post commencement of chemotherapy in patients with early and advanced breast cancer (33). In this study, there was no significant relationship between basal serum IGF-1 level and survival. Serum IGF-1 levels did not change with chemotherapy in the overall group. Contrary to serum IGFBP-3 levels, the fall in serum IGF-1 did not seem to have any association with overall survival (33).

Measuring local breast tissue IGF-1 expression seems logical considering that most studies conclude that the serum IGF-1 level falls after the onset of menopause and would not appear to contribute to late postmenopausal breast cancers. This leads to the hypothesis that local IGF-1 production may contribute to postmenopausal breast cancer. However, studies looking at the association between local breast tissue IGF-1 expression with clinicopathological features and prognosis are also limited. Yu *et al.* showed that tissue expression of IGF-1 in 135 tumour tissue cytosols using a radioimmunoassay technique did not show any significant correlation of IGF-1 expression with ER, PR or any other biochemical markers of poor prognosis such as p53, HER-1, HER-2 protein, S-phase fraction or DNA ploidy (83). An earlier study by Mizukami *et al.* who used immunohistochemistry also failed to show any correlation between IGF-1 expression, histological features and prognosis, but did show a positive correlation between tumour IGF-1 expression and ER content (51). Al-Sarakbi *et al.* showed that IGF-1 mRNA levels in breast tissue adjacent to breast tumours correlated with the number of metastatic lymph nodes only but not with any other pathological prognostic factor (2). Toropainen *et al.* measured the

expression of IGF-1 in tumour and breast stromal tissue using immunohistochemistry in a series of 211 breast cancer cases (74). They showed that IGF-1 immunostaining in tumour areas tended to be higher in axillary lymph node-negative cases compared to positive cases, and also higher in cases with low S-phase fraction compared to high S-phase fraction. In all cases, patients with positive tumour IGF-1 staining had significantly longer overall survival probability compared to those with negative tumour IGF-1 staining cases, but there was no effect on recurrence-free survival. IGF-1 immunostaining intensity in stromal tissue adjacent to breast tumours correlated with tumour size, nuclear pleomorphism, DNA diploidy and an increased likelihood of metastasis at the time of diagnosis but did not have any association with recurrence-free survival or overall survival (74). Eppler *et al.* measured IGF-1 using radioimmunoassay and found that IGF-1 expression was significantly lower in grade 3 tumours compared to grade 1 and 2 tumours (17). In all histopathological grades, IGF-1 immunoreactivity increased along with ER and PR level but was inversely related to S-phase fraction. In low grade tumours, the tumour IGF-1 level was associated with longer survival time (17). Overall, most studies suggest that IGF-1 expression is associated with favourable histopathological features and better prognosis. However, more studies are needed to validate these findings.

Serum IGF-1 as a Surrogate Biomarker of Primary and Secondary Breast Cancer Development

The strong association between breast cancer risk and serum IGF-1 has prompted clinical drug trials to use serum IGF-1 as a surrogate end-point biomarker for predicting the risk of developing primary breast carcinogenesis. In this way, circulating IGF-1 could be a cofactor in the development of breast cancer or may be a by-product of other processes that lead to carcinogenesis. As mentioned previously, several case-control studies have shown that serum IGF-1 in premenopausal women could potentially be used to predict the risk of developing breast cancer later in life. In the clinical setting, serum IGF-1 could be used as a risk biomarker, allowing the evaluation of breast cancer risk in the general population or at least in groups of patients at high risk of developing early breast cancer such as *BRCA1* and *BRCA2* gene mutation carriers and patients on exogenous oestrogen treatment.

Another use of serum IGF-1 is as a response biomarker in testing chemopreventive drugs. Chemoprevention is defined as the prevention of cancer by the use of pharmacological agents that inhibit or reverse the process of carcinogenesis. It aims to treat premalignant cells so as to interfere in the series of events involved in carcinogenesis that promote its progression to neoplastic disease. Traditionally, many drug chemoprevention trials recruited early-stage breast cancer patients who had completed breast cancer treatment and then prospectively looked at their risk of developing a contralateral or ipsilateral breast cancer in another quadrant as an end-point (75). Measuring a response biomarker can allow breast cancer risk to be evaluated before the incident occurs, which can play a very important role in chemoprevention trials. In addition, once the biomarker has been validated as a consistent predictor of risk it can also be utilized outside trial settings to help clinicians make decisions regarding initiation or continuation of chemoprevention. To date, serum IGF-1 and IGFBP-3 are two of the most widely used biomarkers of response in chemoprevention trials in addition to Ki-67, breast intra-epitheilial neoplasia morphology by FNA, nipple aspiration or biopy and mammographic density (18).

Many early studies showed that adjuvant tamoxifen treatment of breast cancer patients led to a reduction in serum IGF-1 (10, 19, 60) which suggests that oestrogen stimulation may be required in order to produce IGF-1 in circulation. The National Surgical Adjuvant Breast Project (NSABP) trial showed that women with a high Gail-risk of 1.7% or higher who were randomised to a 5-year treatment with tamoxifen enjoyed a 50% reduction in breast cancer incidence relative to those who received placebo (20, 21). Likewise, chemopreventive trials involving tamoxifen treatment of normal women showed a reduction of levels of biomarkers, including serum IGF-1 (13). However, whether tamoxifen lowers breast cancer risk directly or through modulation of serum IGF-1 levels still remains to be determined, and further drug trial studies are required before we can confidently use serum IGF-1 as a response biomarker.

Serum IGF-1 was used as a response biomarker in a phase III drug trial using fenretinide (a synthetic retinoid). The trial looked at the whether administration of the drug could reduce the risk of contralateral and recurrent ipsilateral breast cancer in treated breast cancer patients between the ages of 30-70. Fenretinide, which inhibits cell growth and induces apoptosis, was shown to reduce the risk of secondary breast malignancy in premenopausal women by 35%. Incidentally, this reduction in risk corresponded with a reduction in serum IGF-1 observed after one year of drug administration only in premenopausal women but not in postmenopausal women (75). The observed modulation of serum IGF-1 by fenretinide together with its clinical effects of secondary cancer risk suggests that a decline in IGF-1 levels may at least partially account for its chemopreventive activity. A 2x2 randomised trial of fenretinide and low dose tamoxifen and another randomized trial involving fenretinide and women on hormone replacement therapy are currently underway and aim to measure the change in serum IGF-1 levels to clarify the role of IGF-1 as a response biomarker of carcinogenesis (5).

The Relationship between IGF-1 Phenotype, Serum IGF-1 and Risk of Early Breast Cancer

There is evidence that serum IGF-1 levels vary considerably between healthy adults. Twin studies showed that 50% of the inter-individual variability of circulating IGF-1 is genetically determined (30, 40). Some studies have suggested that this variability may be due to inheritable polymorphism of the IGF-1 gene which may be due to the allelic variations upstream of the IGF-1 gene that lead to changes in the promoter region (11). The promoter region in the IGF-1 gene contains a CA-repeat sequence which ranges from 12 to 23 repeats (65). There is wide variation in the frequency of the 19-repeat allele between ethnic groups. Most white women have at least one copy of the 19-repeat allele while the frequency of 19-repeat allele differs between ethnic groups. The absence of a common 19-repeat allele in the IGF-1 gene is associated with high levels of serum IGF-1 during oral contraceptive (OC) use in nulliparous women (35). The risk of early-onset breast cancer after teenage OC use also varies considerably between ethnic groups and appears to correlate with the relative frequencies of the absence of this 19-repeat allele (34). Jernstrom *et al.* found that the absence of the IGF-1 19-repeat allele was more common in premenopausal women with breast cancer than those without breast cancer (38). Even though this IGF-1 polymorphism did not have any effect on serum IGF-1 on nulliparous non-OC users, women with absent 19-repeat alleles demonstrated higher levels of IGF-1 during OC use. This study suggested that there is an increased risk of breast cancer after hormonal exposure, especially in teenage OC use or pregnancy, in women who lack the 19-repeat allele (38). In addition, this study showed that the absence of the 19-repeat allele was more common in *BRCA1* mutation carriers than in other women. It also showed that women *BRCA1* carriers with absent 19-repeat alleles were more prone in developing early-onset breast cancer than *BRCA1* carriers with the presence of the 19-repeat allele. As in the case of circulating IGF-1, the IGF-1 genotype did not seem to affect risk of breast cancer in postmenopausal women (50).

Several studies have suggested that there is a relationship between breast density and risk of breast cancer. Measurement of breast densities using computer-assisted analysis of mammograms have shown a consistent association between high breast density and breast cancer risk (49). IGF-1 stimulates cell proliferation and reduces apoptosis and is associated with larger breast volumes (31, 36). Hartmann *et al.* showed that in women who underwent hormonal breast augmentation, only women who lacked the 19-repeat allele demonstrated a substantial increase in breast volume (32) whilst other studies have mentioned that larger breasts may be associated with higher risk of breast cancer (15). Jernstrom *et al.* showed that OC users with absent 19-repeat alleles had larger body-weight adjusted breast volumes than those with at least one copy of the 19-repeat allele (37). These findings do suggest that the IGF-1 genotype may play an important role in early breast cancer, and its effect on serum IGF-1 and breast cancer risk may rely on the availability of high levels of endogenous and exogenous oestrogen. A consequence of this interaction may be an effect on the proliferation of normal breast epithelia that leads to larger breasts and also the risk of breast cancer. In future, gene testing on the presence or absence of the 19-repeat allele may be useful in determining risk of breast cancer in high risk premenopausal women, such as those with a family history of breast cancer and those on oral contraceptives.

Future Prospects

Of all the components in the IGF-1 system, serum IGF-1 and IGFBP-3, not elaborated on in this review, have shown promising results that potentially allow them to be used in the clinical management of breast cancer (68). Measuring serum IGF-1 could be used to predict risk of premenopausal women, which is especially useful in high-risk women such as those with strong familial breast cancer histories, young oral contraceptive users and gene mutation carriers. Even though many studies have shown a strong association between serum IGF-1 and risk of recurrent new primary or contralateral breast cancer, further studies are needed to validate this. We look forward to results of chemopreventive drug trials which use serum IGF-1 as a response biomarker and further reinforce serum IGF-1 as a useful biomarker of measuring drug efficacy rather than using just clinical outcome as the end-point. So far, studies looking at IGF-1 expression in normal and malignant breast tissue and its prognostic value in breast cancer patients have been inconsistent but this may be due to the relatively few studies performed on this subject. Research has consistently shown that the IGF-1 system and the oestrogen hormone system interact substantially in stimulating breast cancer and that IGF-1 may be the key step between oestrogen stimulation and breast cancer carcinogenesis. If this is the case, then inhibiting the action of IGF-1 systemically or locally at breast tissue level by growth factor-targeted therapy would be the next step in IGF-1 research.

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