Insulin-like growth factors in breast and prostatic cancer

by S I Helle and P E Lönnning

INTRODUCTION

While endocrine therapy has a well-defined role in the treatment of only three forms of cancer, its importance is illustrated by the fact that two of these diseases, carcinoma of the breast and carcinoma of the prostate, are among the most frequent cancers and are leading causes of cancer death in industrialised countries. Taking breast cancer into consideration, endocrine therapy with antiestrogens or oestrogen suppression (by gonadectomy in premenopausal women or use of aromatase inhibitors in postmenopausal women) has been found to cause tumour regression in advanced disease, and also to improve relapse-free and overall survival when used in the adjuvant setting. In prostatic carcinoma, androgen ablation or use of antiandrogens may cause tumour remission in the majority of patients treated for advanced disease.

There are several reasons to focus on different biochemical effects and alternative mechanisms of action in relation to endocrine therapy. Currently, we have a limited understanding of the mechanisms of resistance to endocrine therapy. While the response rate to first-line hormonal treatment may be about 50-70% in breast cancer patients whose tumours express the oestrogen receptor (ER), and as high as 70-80% in prostatic carcinoma (see Santen et al. 1990, Santen 1992 for references to the original work), for unknown reasons resistance inevitably develops, usually within 2 years. Nor do we know why at least 50% of breast cancer patients relapsing after an initial response to first-line treatment subsequently respond to second-line hormone therapy (Kvinnland et al. 1984). While no response to first-line hormone treatment in breast cancer may be due to the lack of ERs or the presence of a mutant form (Horwitz 1993), alternative mechanisms are more likely to be involved in the development of acquired resistance, as the lack of cross-resistance to different endocrine drugs like tamoxifen, aromatase inhibitors or progestins confirms drug-specific resistance not associated with general hormone resistance (Murray & Pitt 1982, Kaye et al. 1982, Iveson et al. 1993). These observations suggest that non-receptor-mediated mechanisms could be involved in the antitumour action of some of these drugs. Alternatively, a drug might promote growth factor stimulation of certain cell clones, over-ruling its antihormone action, and resulting in tumour growth after an initial treatment response.

Recent investigations have shown that treatment with tamoxifen modulates the expression of growth factors like transforming growth factor-β (TGF-β) (Butta et al. 1992), which is known to inhibit breast cancer cell growth, as well as that of insulin-like growth factor-I (IGF-I) (Colletti et al. 1989), a most potent mitogen to breast cancer cells.

There are several reasons to focus on IGF-I in relation to breast cancer in general, and in relation to endocrine treatment in particular. First, most human breast cancers contain receptors for IGF-I. Secondly, IGF-I has been confirmed to be a most potent mitogen to breast cancer cells in vitro. Thirdly, plasma levels of IGF-I have been found to be modulated by sex steroids (androgens and oestrogens) and by antihormone therapy used in breast cancer patients.

While the evidence linking IGF-I to tumour growth in prostatic cancer so far is limited, IGF-I has been found to stimulate the growth of certain prostatic carcinoma cells in vitro (see later).

This paper reviews data suggesting a role for IGF-I (and possibly IGF-II) in breast and prostatic
cancer growth, and the influence of hormone manipulation on these growth factors.

**STRUCTURE AND FUNCTION OF THE IGF SYSTEM**

**IGF-I and IGF-II**

The IGFs (IGF-I and IGF-II) are single-chain polypeptides structurally related to human proinsulin (Cohick & Clemmons 1993). Structural features of the mature peptides are shown in Table 1. They are encoded as prohormones, and the well conserved first 16 residues in the B domain seem to be critical for the capacity to bind to carrier proteins (see later).

The physiological role of the IGF system is incompletely understood. The main function of IGF-I is to act as an effector molecule for growth hormone (GH). IGF-I stimulates growth and differentiation in many tissues, and it reduces protein degradation and increases lipid oxidation in peripheral tissues (Hussain et al. 1993). Recent investigations have focused on the effect of IGF-I in glucose homeostasis, as administration of IGF-I causes a significant drop in plasma glucose levels (for review see Clemmons & Underwood 1994). IGF-II seems to be of importance in foetal growth. In vitro investigations have shown IGF-II to have mitogenic effects resembling those found for IGF-I (Mathieu et al. 1990), but little is known about the normal postnatal function of this growth factor.

**IGF receptors**

The biological effects of IGF-I and IGF-II are mediated through specific IGF receptors, namely the type I IGF receptor (IGF-IR) and the type II IGF receptor (IGF-IIR). Both IGF receptors have been detected in several different tissues. Structural characteristics, binding properties and functions of the receptors are given in Table 2.

The IGF-IR is thought to mediate the metabolic and mitogenic effects of the IGFs. The binding affinities for the IGF-IR of IGF-II and insulin are about 2 and 100 times lower than that of IGF-I respectively (Steele-Perkins et al. 1988). The IGF-IR is structurally similar to the insulin receptor, and some tissues, like the placenta, express hybrid receptors containing subunits of both the IGF-IR and the insulin receptor (Siddle et al. 1994). An atypical IGF-IR, with high affinity for insulin, is also detected in the breast cancer cell line MCF-7 (Milazzo et al. 1992). The physiological roles of hybrid and atypical IGF receptors are not known.

Antibodies directed against the IGF-IR (like αIR3) inhibit IGF-mediated growth in breast cancer cells (see later). The importance of this receptor in the regulation of non-malignant cells may be demonstrated in 3T3 cells derived from mouse embryos (Baserga et al. 1994). These cells have a disrupted IGF-IR gene. For unexplained reasons, growth factors like platelet-derived growth factor (PDGF), epidermal growth factor (EGF), IGF-II, insulin, fibroblast growth factors and TGFs, which stimulate the wild type cells, do not support the growth of these cells in serum-free medium. This lack of effect is eliminated if the cells are transfected with a plasmid expressing the IGF-IR.

The biological role of the IGF-IIR has not yet been fully elucidated. The IGF-IIR may have a regulatory function in secretion and endocytosis of lysosomal enzymes (for review see Kornfeld 1992). This receptor has an affinity for IGF-II which is about 100 times higher than its affinity for IGF-I, and it does not bind insulin (Ballard et al. 1988).

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**Table 1 Structural characteristics of the IGFs.**

<table>
<thead>
<tr>
<th></th>
<th>Molecular mass (kDa)</th>
<th>Amino acids</th>
<th>Gene location</th>
<th>Domains</th>
<th>Disulphide bonds</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I</td>
<td>7649</td>
<td>70</td>
<td>12q</td>
<td>4 (ABCD)</td>
<td>3</td>
<td>48% homology with human proinsulin</td>
</tr>
<tr>
<td>IGF-II</td>
<td>7471</td>
<td>67</td>
<td>11p</td>
<td>4 (ABCD)</td>
<td>3</td>
<td>50% homology with human proinsulin</td>
</tr>
</tbody>
</table>
IGF-I and IGF-II in plasma and tissue

While circulating IGF-I is mainly synthesised in the liver (Schwander et al. 1983, Scott et al. 1985), IGF-I is also produced locally in many tissues. IGF-I may, in theory, act as an endocrine or paracrine growth factor, but the quantitative contribution from the different pathways to tissue IGF-I is not known. We are not aware of any study evaluating the uptake of circulating IGF-I by different tissues in vivo. However, studies on breast tissue samples in vitro suggest a rapid tissue uptake of IGF-I by passive diffusion from the medium (Hequet & Peyrat 1990).

It is noteworthy that the tissue uptake of circulating IGF-I may be different in breast cancer patients compared with healthy individuals, as many cancer patients seem to express an increased activity of a plasma protease modifying IGF-binding protein-3 (IGFBP-3), the major plasma binding protein for IGF-I (see later).

Factors influencing plasma IGF-I and IGF-II

Several endocrine factors seem to influence plasma IGF-I levels. As the production of IGF-I is stimulated by GH, plasma levels subsequently fall during life from peak levels in adolescents to lower levels in old age (Ho et al. 1987). The IGF-I level is low in patients with a poor nutritional status (for review see Clemmons & Underwood 1991). Oestrogens seem to have a dual influence on the IGF system. The IGF-I level decreases immediately after the menopause (Romagnoli et al. 1993), and treatment with luteinising hormone-releasing hormone (LHRH) analogues suppresses plasma levels of IGF-I in premenopausal women with breast cancer (Lien et al. 1992), and in girls with precocious puberty (Mansfield et al. 1988). This is consistent with the observation that oestrogens given by the transdermal route to postmenopausal women elevate serum IGF-I levels (Weissberger et al. 1991, Slowinska-Srzednicka et al. 1992). In contrast, oestrogens administered by the oral route suppress plasma IGF-I (Duursma et al. 1984, Dawson-Hughes et al. 1986). This is probably caused by a direct effect of large amounts of oestrogens on IGF-I synthesis in hepatocytes. Due to extensive first-pass metabolism, the amount of oestradiol reaching the liver after oral administration is about five times the amount reaching the systemic circulation (Longcope et al. 1985).

Administration of dexamethasone, androgens and the synthetic progestin medroxyprogesterone acetate (MPA; 400 mg/week i.m.) has been reported to increase plasma levels of IGF-I (Meyer et al. 1982, Hobbs et al. 1993, Miell et al. 1993). However, when the activity of IGF-I in plasma samples

<table>
<thead>
<tr>
<th>Table 2 Structure and function of the IGF receptors.</th>
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<tbody>
<tr>
<td><strong>IGF-IR</strong></td>
</tr>
<tr>
<td><strong>Molecular mass (kDa)</strong></td>
</tr>
<tr>
<td><strong>Structure</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Signal transduction</strong></td>
</tr>
<tr>
<td><strong>Ligand affinity</strong></td>
</tr>
<tr>
<td><strong>Function</strong></td>
</tr>
<tr>
<td><strong>Comments</strong></td>
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**Table 3** Structural and functional features of IGFBPs.

<table>
<thead>
<tr>
<th></th>
<th>IGFBP-1</th>
<th>IGFBP-2</th>
<th>IGFBP-3</th>
<th>IGFBP-4</th>
<th>IGFBP-5</th>
<th>IGFBP-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular mass (kDa)</td>
<td>28</td>
<td>34</td>
<td>40, 43</td>
<td>24, 30</td>
<td>29, 31</td>
<td>34</td>
</tr>
<tr>
<td>Glycosylation</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Serum concentration (fasting levels)</td>
<td>20-40 µg/l</td>
<td>ca 150 µg/l</td>
<td>3-5 mg/l</td>
<td>ND</td>
<td>ND</td>
<td>ca 300 µg/l</td>
</tr>
<tr>
<td>Affinity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>IGF-II=IGF-I</td>
<td>IGF-II&lt;sub&gt;b&lt;/sub&gt; IGF-I</td>
<td>IGF-II=IGF-I</td>
<td>IGF-II=IGF-I</td>
<td>IGF-II=IGF-I</td>
<td>IGF-II&lt;sub&gt;b&lt;/sub&gt; IGF-I</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt; (nM)</td>
<td>(0.3)</td>
<td>(ND)</td>
<td>(0.26)</td>
<td>(0.06)</td>
<td>(0.067)</td>
<td>(0.067)</td>
</tr>
<tr>
<td>Cell association</td>
<td>Insulin (−)</td>
<td>Fasting (+)</td>
<td>GH (+)</td>
<td>IGF-I (+)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Regulated plasma levels&lt;sup&gt;c, d&lt;/sup&gt;</td>
<td>DEX (+/−)</td>
<td>Glucagon (+)</td>
<td>cAMP (+)</td>
<td>IGFBP-3</td>
<td>IGFBP-2</td>
<td>IGFBP-1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Clemmons et al. (1992).

<sup>b</sup>Concentration required to achieve 50% competition of native IGF at physiological concentration. Values for IGF-I are shown on the right, those for IGF-II on the left.

<sup>c</sup>Gargosky et al. (1993).

<sup>d</sup>Zapf et al. (1990).

ND, no data available; DEX, dexamethasone.

obtained from patients after treatment with dexamethasone was measured by use of a bioassay, it was found to be reduced compared with control samples (Gourmelen et al. 1982).

In contrast to IGF-I, IGF-II seems to be constitutionally expressed and serum levels are only slightly lowered by increasing age (Bennett et al. 1984). We are not aware of any reports on the influence of steroid hormones on plasma IGF-II.

**IGFBPs**

Only a small fraction of IGF-I and IGF-II circulates in plasma as unbound protein, the rest is bound to specific IGFBPs (Clemmons et al. 1993). Six IGFBPs (named IGFBP-1 to -6) have been identified in human plasma or other body compartments; some of their characteristics are given in Table 3.

Plasma protein binding of IGF-I and IGF-II occurs in two major forms. The growth factor may bind to a single binding protein to form a 40-50 kDa complex. Alternatively, it may associate with IGFBP-3 and an ‘acid-labile subunit’ to form a 150 kDa ternary complex (Baxter 1988). The different complexes may play a different role in the regulation of IGF-I and IGF-II bioavailability. While unbound IGF-I has been reported to have a plasma half-life of about 10 min, and the 40-50 kDa complex to have a half-life of 20-30 min, the plasma half-life of the 150 kDa ternary complex is as high as 12-15 h (Guler et al. 1989). As about 90% of the IGF-I in plasma is found in the 150 kDa complex, this complex may function as a depot of IGF-I in plasma, while the ‘smaller complexes’ may have a more instant buffer function.

While the 150 kDa complex is considered to be an inactive storage form of IGF-I in plasma, there is uncertainty as to whether IGF-I bound in the 40-50 kDa complexes may exert biological activity in its bound form or whether it has to be dissociated from its binding proteins. An important difference between the low and high molecular weight complexes is that the former may cross the endothelial barrier, while the latter may not (Arany et al. 1993). It is noteworthy that the transport of IGFBP-1 across intact capillaries is stimulated by insulin (Bar et al. 1990).

In vitro investigations have reported stimulatory as well as inhibitory effects of adding IGFBPs to cell cultures, depending on the experimental conditions. Preincubation with IGFBP-3 in cell suspensions has been reported to enhance the growth stimulatory
effect of IGF-I in fibroblasts, possibly because attachment of IGFBP-3 to the cell surface may facilitate binding of IGF-I to its receptor (Conover 1992). However, coinubcation of IGFBP-3 with IGF-I, as well as excessive cellular production of IGFBP-3 (demonstrated in transfection studies), may have an inhibitory effect on the bioactivity of IGF-I (Cohen et al. 1993a), probably because unbound IGFBP-3 may act as a scavenger, prohibiting the binding of IGF-I to its membrane receptor. Currently, we do not know the biological effects in different tissues.

Taking IGFBP-1 into consideration, this binding protein exists in a phosphorylated and a dephosphorylated form. The effects of IGFBP-1 depend on its phosphorylation state, as the dephosphorylated form has been found to enhance the effect of IGF-I on wound healing in rats, while the phosphorylated form did not (Jyung et al. 1994). It is noteworthy that this stimulatory function was only obtained with a molar ratio of IGF-I to IGFBP-1 of 11 to 1, while no effect was found when the proteins were added at equimolar concentrations.

Adding to the complexity of IGF-I bioregulation is the discovery of a serum protease that modifies the structure of IGFBP-3 by altering its binding affinity for IGF-I. Proteolytic cleavage of IGFBP-3 has been detected in the serum of pregnant women (Giudice et al. 1990, Hossenlopp et al. 1990), in serum from patients undergoing major surgery (Davies et al. 1991, Davenport et al. 1992), and in serum from patients suffering from different serious illnesses, including advanced cancer (Cwyfan Hughes et al. 1992, Frost et al. 1993a, Müller et al. 1993). Proteolytic enzyme activity has also been detected in conditioned media from fibroblasts and cell lines derived from different tumors, such as bladder carcinoma, squamous cell carcinoma, carcinoma of the cervix, choriocarcinoma and rhabdomyosarcoma (Frost et al. 1993b). The enzyme in the conditioned media was found to modify IGFBP-3 in a different manner from the plasma protease, but it was similar among the different cell lines. Proteolytically modified IGFBP-3 in serum has a lower binding affinity for IGF-I when compared with native IGFBP-3 (Hossenlopp et al. 1990).

The biological importance of alterations in the IGFBP-3 protease activity was illustrated in a recent investigation in which serum obtained from women late in pregnancy (with high serum protease activity) was found to stimulate DNA synthesis in cultured chick embryo fibroblasts, with a potency twice that of normal serum (Blat et al. 1994). This effect was blocked by adding monoclonal antibodies against IGF-I and IGF-II.

Recently, proteolytic activity for IGFBP-3 was identified in lymph obtained from normal individuals (Lalou & Binoux 1993), suggesting that such enzyme activity also exists in the tissues. The possibility of proteolytic enzymes acting on other IGFBPs should be considered; pregnancy serum was recently found to contain proteolytic activity for IGFBP-5 (Claussen et al. 1994). While none of these enzymes have been identified, they all seem to be calcium-dependent serine proteases. In contrast to characterised proteolytic enzymes like plasmin, kallikrein, cathepsin D and tissue-type plasminogen activator, the activity of IGFBP proteases found in these conditions does not seem to be influenced by protease inhibitors in serum (Frost et al. 1993b).

THE IGF SYSTEM IN BREAST CANCER

In vitro studies

Different cancer cell lines derived from carcinomas of the prostate, lung, ovary, colon and pancreas, gliomas and embryonal carcinomas have all been found to express the IGF-IR and also to secrete IGF-I or IGF-II (for review see Macaulay 1992).

IGF-I is one of the most potent mitogens to breast cancer cells in vitro (Furlanetto & DiCarlo 1984, Karey & Sirbasku 1988). This effect is most probably mediated through the IGF-IR, as monoclonal antibodies (like αIR3) directed against the IGF-IR inhibit IGF-I stimulated growth in both MCF-7 and MDA-231 cell lines (Artega et al. 1989). Whether breast tumour cells, on the other hand, are able to secrete IGF-I has been a subject of controversy. While previous studies suggested that IGF-I was secreted by breast cancer cells (Huff et al. 1986), more recent evidence indicates that these observations could be due to cross-reaction with other compounds, as mRNA for IGF-I has not been detected in any breast cancer cell line so far (Yee et al. 1989, van der Burg et al. 1990, Paik 1992). Thus, an autocrine action of IGF-I in breast cancer seems unlikely. However, the breast cancer cell line T 47-D
expresses mRNA for IGF-II (Yee et al. 1988), and IGF-II has been detected in conditioned media from different breast cancer cell lines, such as MDA-231, T 47-D, HBL 100 and MCF-7 McG (Lee et al. 1994). It is noteworthy that transfection of MCF-7 cells with cDNA for the IGF-II preprohormone causes oestrogen-independent growth. This effect is probably mediated through the IGF-IR, as it can be abolished with the antibody αIR3 directed against this receptor (Cullen et al. 1992). However, others found that IGF-II at low concentrations stimulated the growth of MCF-7 cells in the presence of αIR3, suggesting that a mitogenic effect could also be mediated by the IGF-IR in the same cell line (Mathieu et al. 1990). Five out of nine fibroblast cell lines derived from malignant breast tumours expressed mRNA for IGF-II, but only one out of nine expressed mRNA for IGF-I (Cullen et al. 1991). While IGF-II may, in theory, act as an autocrine and paracrine growth factor for breast cancer in vivo, further evidence supporting this theory is currently lacking.

All forms of IGFBP have been found to be secreted by different breast cancer cell lines, but the secretion pattern differs between different cell lines (De Leon et al. 1990, Sheikh et al. 1993). The secretion pattern seems to depend on the receptor status of the cancer cells. Studies of different breast cancer cell lines have revealed higher expression of IGFBP-3 in ER-negative cell lines like MDA-330, MDA-231 and HS578T, with higher expression of IGFBP-2 and -4 in ER-positive cell lines like ZR-75-I, BT-20, T 47-D and MCF-7 (Clemmons et al. 1990).

Conflicting evidence suggests that the IGFBPs modulate the effect of IGF-I on breast cancer cell lines. One study reported that IGFBP-2 and -3 (but not IGFBP-4 and -5) increased IGF-I-mediated DNA synthesis in MCF-7 cells (Chen et al. 1994), but others found human recombinant IGFBP-3 to attenuate oestrogen-stimulated proliferation of MCF-7 cells when added to the culture (Pratt & Pollak 1994).

Recombinant IGFBP-1 has been found to inhibit IGF-I-stimulated and also oestrogen-stimulated growth of MCF-7 breast cancer cells (McGuire et al. 1992). The inhibitory effect of IGFBP-1 on IGF-I, and also its effect on oestrogen-stimulated growth, was neutralised when IGF-I was added in excess (McGuire et al. 1992).

**In vivo studies**

Several studies have reported that IGF-IR and IGF-IIR are expressed in most human breast cancers (Peyrat et al. 1988, Foekens et al. 1989a, Cullen et al. 1990). The expression of the IGF-IR seems to be correlated with the expression of the ER and the progesterone receptor (PgR) (Pekonen et al. 1988, Papa et al. 1993). While one study reported that expression of the IGF-IR was associated with a better relapse-free survival compared with subjects lacking the receptor (Bonnetterre et al. 1990), others found expression of the IGF-IR to be associated with a poor prognosis in the subgroup of ER-negative cancers (Railo et al. 1994), or to be without prognostic importance (Foekens et al. 1989b).

Histopathological analysis of malignant as well as benign breast tumours revealed that the binding of 125I-labelled IGF-I was restricted to the epithelial cells, and this binding was found to be higher in malignant tumours compared with benign tumours and normal breast tissue (Jammes et al. 1992).

Although there seems to be an overexpression of IGF-IRs in breast cancer, amplification of the gene for this receptor is a rare event and reported to occur in only about 2% of the tumours (Berns et al. 1992, Papa et al. 1993). The importance of the IGF-IR for tumour growth in vivo has been demonstrated in ER-negative MDA-231 tumours in nude mice, where the monoclonal antibody αIR2, directed against the IGF-IR, was able to inhibit tumour growth (Arteaga et al. 1989). However, no effect of this antibody was found in ER-positive MCF-7 tumours. Like IGF-IR, expression of IGF-IIR in human breast cancer is not associated with gene amplification (Hebert et al. 1994). In situ hybridisation has shown most breast cancers to express mRNA for IGF-II at a higher concentration than that which is seen in normal breast epithelium (Zhao et al. 1993).

Immunohistochemical studies on human breast tissue have revealed that stromal cells express mRNA for IGF-I and IGF-II. Interestingly, expression of IGF-II seems to be most abundant in fibroblasts in the vicinity of the tumour cells (Paik 1992). Five out of the six IGFBPs (IGFBPs-1 to -5)
have been detected in human breast cancer biopsies, but whether tumour or stromal cells are the major source is not documented (Pekonen et al. 1992). mRNAs for IGFBPs-2, -3 and -4 have been detected in N-nitrosomethylurea (NMU)-induced mammary tumours in the rat, while only IGFBP-2 was found in the normal lactating breast in the same species (Manni et al. 1992). When NMU-induced tumours were investigated by in situ hybridization, mRNA for IGFBP-2 was located in the epithelial cells, while mRNA for IGFBP-5 and IGFBP-6 was found in the stromal cells (Manni et al. 1994). Receptor-positive and receptor-negative tumours differ in their expression of IGFBPs, paralleling the difference observed between ER-positive and ER-negative breast cancer cell lines in vitro (see above). Thus, studies on human breast cancer biopsies have reported a higher expression of IGFBP-3 mRNA in ER-negative compared with ER-positive tumours (Shao et al. 1992, Figueroa et al. 1993), while expression of IGFBP-4 was reported to be positively correlated with the expression of the ER as well as that of the PgR (McGuire et al. 1994). It is noteworthy that the content of all IGFBPs seems to be increased in malignant tumours compared with normal human breast tissue (Pekonen et al. 1992).

Interactions between IGF-I and sex steroids in breast cancer cell lines and tissue

Oestradiol and IGF-I have a synergistic effect on the proliferation of MCF-7 cells in vitro (Stewart et al. 1990). Oestradiol stimulation has been found to increase the cellular concentration of mRNA for the IGF-IR in this cell line. Interestingly, the stimulatory effect of oestradiol could be inhibited by adding an antibody (Sm 1,2) against IGF-I (Stewart et al. 1990). Oestradiol has been reported to increase the expression of IGF-II in the breast cancer cell lines T 47-D and MCF-7 McG (Lee et al. 1994).

Steroid hormones may influence the secretion of IGFBPs in breast cancer cell lines. Oestrogens enhanced the expression of IGFBPs-2, -4 and -5 in the ER-positive cell line T 47-D. 17β-Oestradiol increased the level of IGFBP-2 and -4 in conditioned media from the MCF-7 cell line, while the pure antioestrogen ICI 182,780, and, to a lesser degree, tamoxifen, increased the level of IGFBP-3, with a decline in the levels of IGFBPs-2 and -4 (Pratt & Pollak 1993). The relative concentration of IGFBP-3 compared with those of the other binding proteins in the conditioned media declined with oestrogen treatment and increased considerably with antioestrogen treatment.

Ovariectomy was found to decrease the expression of IGF-II but to increase the intratumour concentration of mRNA for IGFBPs-2 and -6 in NMU-induced mammary tumours (Manni et al. 1994). These effects could be reversed by hormone repletion.

LHRH antagonists have been found to inhibit oestrogen – as well as IGF-I-induced growth of MCF-7 cells in vitro, and also to suppress secretion of IGF-II by the same cell line (Hershkovitz et al. 1993).

Progestins may influence the expression of IGFBPs in breast cancer. The synthetic progestin R 5020 has been shown to downregulate IGF-IR mRNA in the PgR-positive cell line T 47-D by 50%, while the secretion of IGF-II into the medium was increased (Papa et al. 1991). In the same cell line, R 5020, in contrast to oestradiol, reduced the concentration of all IGFBPs in the conditioned medium, as measured by Western ligand blots (Owens et al. 1993), and MPA was recently found to decrease the mRNA for IGFBP-4 and IGFBP-5 (Cousts et al. 1994). The antiprogestin RU 486 also decreased the mRNA for IGFBP-5, but did not affect IGFBP-4 mRNA.

There is evidence that retinoids may influence the disposition of IGF-I. Retinoic acid has been found to inhibit IGF-I-stimulated growth of MCF-7 cells in vitro, and at the same time to increase the concentration of IGFBP-3 in the conditioned medium (Adamo et al. 1992). Retinoic acid also inhibits IGF-I-induced secretion of IGFBP-5 in T 47-D cells (Shemer et al. 1993).

EFFECTS OF BREAST CANCER DISEASE AND ANTIHORMONE TREATMENT ON THE DISPOSITION OF IGF-I AND IGF-II IN VIVO

Plasma levels of IGF-I may be influenced by the cancer itself as well as by endocrine manipulation. One study revealed IGF-I levels to be significantly higher
in plasma from breast cancer patients when compared with healthy controls (Peyrat et al. 1993). Whether this may be related to an elevated level of GH, which is reported to occur in about 40% of breast cancer patients (Emerman et al. 1985), is not known.

An important observation is that many patients suffering from advanced breast cancer seem to have an increased activity of the plasma IGFBP-3 protease (Frost et al. 1993a). Increased protease activity is not restricted to breast cancer patients only, as it has also been found in serum from patients suffering from lung cancers and cancers in the head and neck area (Frost et al. 1993b).

Several antihormone drugs commonly used in breast cancer may influence the plasma concentrations of IGF-I and its binding proteins. Tamoxifen reduces plasma IGF-I levels by 25-30% on average (Colletti et al. 1989, Kiang et al. 1992, Lønning et al. 1992, Friedl et al. 1993). Several mechanisms may be involved. Tamoxifen has been found to inhibit the expression of the IGF-I gene in liver and lung in rats (Huynh et al. 1993), but the drug also inhibits the pulsatile secretion of GH in vivo in the rat (Tannenbaum et al. 1992).

It is noteworthy that the effect of tamoxifen on plasma IGF-I parallels findings obtained when oestrogens are administered by the oral route in pharmacological doses (see above). Tamoxifen stimulates IGF-I gene expression in the uterus, while the pure antioestrogen ICI 182,780 is inhibitory (Huynh & Pollak 1993). Previous studies have shown that tamoxifen exerts oestrogen-agonistic effects on the synthesis by the liver of plasma proteins like sex hormone-binding globulin, thyroxine-binding globulin and the lipoproteins (Fex et al. 1981, Love et al. 1991), and the effect of tamoxifen on plasma IGF-I may be an oestrogen-agonistic effect of the drug.

While the effect of tamoxifen on the total plasma concentration of IGF-I is modest, tamoxifen may also influence the disposition of IGF-I by modifying the concentration of IGFBPs. We found tamoxifen to elevate the plasma level of IGFBP-1 by a mean value of 80% (Lønning et al. 1992), an observation recently confirmed by others (Lahti et al. 1994).

Treatment with LHRH agonists caused a decrease in plasma levels of IGF-I in breast cancer patients (Lien et al. 1992) and in girls with precocious puberty (Mansfield et al. 1988). This is in accordance with a fall in plasma IGF-I in the menopause and the increase seen in women treated with parenteral oestrogen substitution (see above).

The effect of aromatase inhibitors on plasma IGF-I is conflicting. The steroidal aromatase inhibitor formestane (4-hydroxyandrostenedione; 4-OHA) has little effect on IGF-I or IGF-II, IGFBP-3 and IGFBP-1 in patients treated with a drug schedule of 250 mg i.m. fortnightly (Frost et al. 1993a, Ferrari et al. 1994). While one study reported that administration of 4-OHA at 500 mg i.m. fortnightly increased plasma IGF-I levels (Ferrari et al. 1994), others found that short-term treatment with the same regimen did not cause any significant alteration in plasma IGF-I (Reed et al. 1992).

The aromatase inhibitor aminoglutethimide (AG) causes a moderate (25%), albeit significant, increase in plasma IGF-I levels (Lien et al. 1992, Frost et al. 1993a). The discrepancy between the effects of 4-OHA at 250 mg every fortnight on the one hand, and AG at 1000 mg daily (and possibly 4-OHA at 500 mg i.m. every fortnight) on the other, on plasma IGF-I is difficult to explain with regard to the effects of these drug regimens on plasma oestrogen levels (Dowsett et al. 1989, MacNeill et al. 1994), and may be related to other biochemical effects. Whether an elevation of plasma IGF-I may be detrimental to the antitumour effects of AG is not known. Neither AG nor 4-OHA caused any consistent change in the pattern of IGFBPs or in the plasma protease activity (Frost et al. 1993a).

Treatment of postmenopausal breast cancer patients with the synthetic progestin MPA at 500 mg daily was found to cause a significant increase in plasma IGF-I (Reed et al. 1992). Additionally, treatment with another progestin, megestrol acetate (MA), at 160 mg once daily, was found to cause a significant increase (81% on average) in plasma IGF-I (Frost et al. 1993a). Treatment with MA caused a significant reduction in the IGFBP-3 plasma protease activity (Frost et al. 1993a). Accordingly, the increase in plasma IGF-I caused by MA treatment may be secondary to a reduced release from its major binding protein, as a redistribution of IGF-I to the high molecular weight IGFBP-3/acid labile subunit complex will prolong its half-life in plasma and limit its release to the tissues.

While most studies have evaluated the IGFBP-3 protease activity in plasma alone, such enzyme activ-
ity has also been detected in lymph (see above). It is possible that this enzyme may be found in different tissues, playing a role in the local regulation of IGF-I bioavailability. If this is the case, any alteration in the activity of this enzyme may be of importance not only to the release of IGF-I from IGFBP-3 but also to the local regulation of IGF-I action in the tissues.

Several drugs currently evaluated as antitumour agents in breast cancer may influence breast cancer growth by affecting the disposition of IGF-I.

Somatostatin is known to suppress GH secretion in vivo (Manni et al. 1989), and somatostatin analogues have been reported to suppress plasma IGF-I levels by about 30%. While results so far suggest that somatostatin analogues have a moderate antitumour activity when given as a monotherapy to breast cancer patients (Vennin et al. 1989, Stolfi et al. 1990), the use of these drugs in concert with other antitumour agents merits further evaluation.

Suramin, a polyanionic antiparasitic drug, inhibits growth factor-induced mitogenesis in several tumours including human breast cancer cell lines. The drug binds several growth factors including PDGF, TGF-β, EGF and IGF-I, and interferes with the binding of the growth factors to their receptors (Ravera et al. 1993).

The synthetic retinoid fenretinide was found to reduce the plasma IGF-I level in breast cancer patients by about 15% compared with matched controls (Torrisi et al. 1993).

THE IGF SYSTEM IN THE PROSTATE

While increasing evidence suggests a role for IGF-I and IGF-II in the growth regulation of prostatic carcinomas, data are still sparse compared with the results in breast cancer.

Studies on cells and biopsies from benign prostatic tissue

The IGF-IR has been demonstrated in membrane preparations from prostatic epithelial cells (Cohen et al. 1991), and tissue from patients with benign prostatic hyperplasia has been found to express two classes of binding site for IGF-I (Fiorelli et al. 1991). Treatment with LHRH agonists was found to increase the capacity of both binding sites. Interest-

ingly, immunohistochemical localisation of IGF receptors demonstrated binding to the basal epithelial cells in untreated patients, while patients treated with LHRH agonists also showed binding to the glandular epithelium.

Prostatic epithelial cells have been found to produce IGFBP-2 and IGFBP-4 (Cohen et al. 1991), and human prostate epithelial cells and stromal fibroblasts have been found to express IGFBP-3 (Birnbaum et al. 1994). In situ hybridisation studies of tissue from patients with prostatic hyperplasia demonstrated mRNA for IGF-I only in the stromal compartment (Barni et al. 1994), suggesting a paracrine effect of IGF-I in prostatic tissue.

Studies in prostatic cancer tissue and cell lines

Specific receptors for IGF-I have been detected in the rat prostatic cancer cell line PA-III (Polychronakos et al. 1991) and in the human prostatic cancer cell lines DU-145 and PC-3 (Kaicer et al. 1991, Connolly & Rose 1994). IGF-I expression has been demonstrated in vitro in several prostatic cancer cell lines, such as PC-3, DU-145, and LNca.FGC (Pietrzkowski et al. 1993), and the growth of these cell lines may be inhibited by adding an antisense oligodeoxynucleotide to IGF-IR or inactive IGF-I analogues (occupying the IGF-IR). IGF-I has been found to stimulate both androgen-independent cell lines such as PC-3 and DU-145 and androgen-dependent cell lines such as LnCaP (Iwamura et al. 1993). The androgen-independent cell lines expressed the IGF-IR at higher concentrations than the androgen-dependent cell line LnCaP. Dihydrotestosterone had no effect on the expression of binding sites in any of the cell lines (Iwamura et al. 1993).

One prostate cancer cell line, PC-3, was found to secrete IGFBP-3 (Kaicer et al. 1993). It is noteworthy that, when added to the medium at a concentration of 50 ng/ml, IGFBP-3 was found to stimulate the growth of PC-3 cells. At a concentration of 150 ng/ml, IGFBP-3 had no influence on the growth of PC-3 cells, but was shown to inhibit the growth of normal prostate epithelial cells (Kaicer et al. 1993). These findings show how the net effect of a binding protein on IGF-I bioavailability may depend on its local concentration.

Patients suffering from advanced prostatic carcinoma seem to show abnormalities in their IGF
SUMMARY AND CONCLUSION

IGF-I and IGF-II are both potent mitogens to breast and prostatic carcinoma cells. Evidence suggests that the disposition of IGF-I is different in patients suffering from advanced breast and prostatic carcinoma when compared with healthy controls. Steroid hormones and endocrine manipulation used in the treatment of breast and prostatic carcinomas may influence the plasma concentration of IGF-I and its binding proteins. However, due to the complex regulation of IGF bioavailability, it is difficult to evaluate the impact of alterations in IGFBPs. Further studies are warranted to evaluate the influence of hormone treatment on IGF-I, IGF-II and their binding proteins in plasma and, in particular, in tumour tissue from patients suffering from breast and prostatic carcinomas. While most studies so far have evaluated the influence of short-term hormone therapy on the disposition of IGF-I, it would be of particular interest to look at the effect of long-term treatment, and to evaluate possible alterations in the IGF system in relation to the emergence of resistance to endocrine therapy.

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