Deregulation of the IGF axis in cancer: epidemiological evidence and potential therapeutic interventions

L Jerome, L Shiry and B Leyland-Jones

Department of Oncology, McGill University, 546 Pine Avenue West, Montreal, Quebec, Canada H2W 1S6
1Insmed Incorporated, 4851 Lake Brook Drive, Richmond, Virginia, USA 23060

Abstract

The IGF system performs a fundamental role in the regulation of cellular proliferation, differentiation and apoptosis. These diverse biological actions are mediated primarily by IGF association with the type I IGF receptor (IGF-IR), which is in turn regulated by a group of high-affinity IGF-binding proteins (IGFBP-1 to -6). All of the IGFBPs can have growth-inhibitory effects by competitively binding IGFs and preventing their association with the IGF-IR. IGFBP-3 is the most abundant binding protein in the circulation and controls the actions of the IGFs by regulating their distribution and bioavailability to target tissues. Disruptions in the balance of IGF system components leading to excessive proliferation and survival signals have been implicated in the development of different tumor types. Epidemiological evidence indicates that increased levels of IGF-I, reduced levels of IGFBP-3 or an increased ratio of IGF-I to IGFBP-3 in the circulation are associated with an increased risk for the development of several common cancers, including those of the breast, prostate, lung and colon. The results of preclinical studies indicate that a diversity of interventions which antagonize IGF-IR signaling or augment IGFBP-3 function inhibit tumor cell growth in models of human cancers. A more comprehensive understanding of the interplay between cellular targets of the IGF system and antineoplastic agents will facilitate the development of novel strategies for the prevention and treatment of cancer.

Introduction

The insulin-like growth factor (IGF) system plays a pivotal role in normal growth throughout fetal and childhood development. In adult life, this system continues to function by regulating normal cellular metabolism, proliferation, differentiation and protecting against apoptotic signals. However, aberrant stimulation can contribute to the development and progression of malignant growth.

The IGFs stimulate cellular proliferation and promote survival largely through interaction with the IGF-I receptor (IGF-IR), which possesses ligand-activated tyrosine kinase activity (Sepp-Lorenzino 1998). IGF bioactivity is regulated by IGF-binding proteins (IGFBPs), primarily IGFBP-3, which exerts antiproliferative and proapoptotic activities dependent on, and independent of, IGFs (Firth & Baxter 2002). A number of recent epidemiological studies have shown that high levels of the primary circulating growth factor, IGF-I, low levels of its major regulatory binding protein, IGFBP-3, or an elevated ratio of IGF-I to IGFBP-3, are associated with increased risk of developing several common cancers, particularly those of the breast (Hankinson et al. 1998, Li et al. 2001), prostate (Chan et al. 2002), lung (London et al. 2002) and colon (Giovannucci et al. 2000). Hence, a renewed interest in this field as a potential target for the development of novel antineoplastic therapies has ensued.

Following an overview of the molecular structure and known physiological functions of the IGF family members, this review will present the epidemiological evidence linking the IGF system to cancer. Finally, we discuss the data from studies using cell culture and animal models of human cancers indicating that interference with the IGF axis, particularly via targeted upregulation of IGFBP-3 function and/or downregulation of IGF-IR activity, is inhibitory to cancer cell growth.

The IGF system

The IGF system involves the complex coordination of growth factors (IGF-I and IGF-II), cell surface receptors...
IGF-I and IGF-II

IGF-I is a 70 amino acid peptide that is mainly produced by the liver in response to growth hormone (GH) stimulation (Arany et al. 1994, Olivecrona et al. 1999), but like IGF-II, can be synthesized by almost any tissue in the body. Serum levels of IGF-I are age-dependent, increasing slowly from birth to puberty, at which point they peak and thereafter decline with age (Collett-Solberg & Cohen 2000). IGF-II is a paternally imprinted (i.e. maternally silent) 67 amino acid peptide whose serum concentration (400–600 ng/ml) is higher than IGF-I (100–200 ng/ml) at all ages, is not regulated by GH and remains stable after puberty (Moschos & Mantzoros 2002). Loss of imprinting in the IGF-II gene is often found in cancer (Jarrard et al. 1995, Oda et al. 1997, Cui et al. 1998, Kim et al. 1998, van Roozendaal et al. 1998), and most primary tumors and transformed cell lines overexpress IGF-II mRNA and protein (Werner & LeRoith 1996). The mitogenic effects of both IGFs are mediated primarily through the IGF-IR, with growth during the embryonic and fetal stages principally regulated by IGF-II and postnatally by IGF-I, which although present at lower levels, has higher affinity for the IGF-IR (Jones & Clemmons 1995). In addition to the well-established endocrine role for IGF-I, both IGFs play important paracrine and/or autocrine roles during normal development and malignant growth (see below). Liver-specific IGF-I knockout mice are viable and fertile, with a 75% reduction in circulating IGF-I levels but possessing normal tissue expression (Yakar et al. 1999). Heterozygous IGF-II gene knockout mice survive but are smaller than their wild-type littermates (DeChiara et al. 1990). Recently, IGF-II has been shown to bind with high affinity to the insulin receptor isoform A (IR-A), which predominates in fetal tissues and in a number of cancers (Frasca et al. 1999, Sciaccia et al. 1999, 2002). Unlike insulin, which elicits metabolic effects upon activation of IR-A tyrosine kinase activity, IGF-II stimulation of this receptor evokes a mitogenic response (Frasca et al. 1999). Consequently, the IR is the major receptor for locally produced IGF-II in certain cancers (Sciaccia et al. 1999).

IGFRs and signaling pathways

IGFRs are cell membrane-associated glycoproteins which differ significantly in structure and function. The IGF-IR, which is expressed in most cells and resembles the insulin receptor, is a tetramer consisting of two identical extracellular α-subunits and two identical membrane-spanning β-subunits (Fig. 1, Sepp-Lorenzino 1998). The IGF-IR also binds IGF-II, but with 10-fold lower affinity than IGF-I (Rubin & Baserga 1995). IGFs and insulin bind to each other’s receptor, IGF-IR and IR (which share 60% homology), with differential affinity (Steele-Perkins et al. 1988, Frattali & Pesson 1993). A hybrid IGF-IR/IR has been identified, which was initially thought to function as an IGF-IR, since it displayed higher affinity for IGF-I than insulin (Jones & Clemmons 1995). Pandini et al. (2002) have since shown that the IGF-I half-receptor β-subunit of IR/IGF-IR hybrids containing IR-A is activated by IGF-I, IGF-II and insulin, whereas hybrids containing IR-B are activated by IGF-I and to a lesser extent IGF-II. Thus, proliferation of tissues and malignancies which overexpress both receptors may be activated in response to insulin and IGFs via hybrid receptors.

Expression of the IGF-IR is stimulated by steroid and other hormones (estrogens, corticosteroids, GH, follicle-stimulating hormone (FSH), luteinizing hormone and thyroid hormones) and growth factors (basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF) and epidermal growth factor (EGF)). In contrast, IGF-IR expression is inhibited by many tumor suppressors, such as p53, Wilms’ tumor-1 and BRCA-1 and is subject to negative feedback regulation by high IGF-I levels (Yu & Rohan 2000, and references therein). The number of IGF-IRs on the cell surface is a major determinant of mitogenesis and cell survival (Rodriguez-Tarduchy et al. 1992). Malignant transformation is often associated with upregulated expression or constitutive activation of the IGF-IR (Kaleko et al. 1990, Macaulay 1992, Rubin & Baserga 1995).

Ligand binding to the extracellular domain of the IGF-IR (a type II receptor tyrosine kinase superfamilly member) signals a conformational change in the intracellular domain which induces receptor clustering, autophosphorylation and stimulation of the receptor’s tyrosine kinase activity (Sepp-Lorenzino 1998). Activation of a number of downstream signaling pathways is subsequently mediated by recruitment and tyrosine phosphorylation of IGF-IR adapter/ effector molecules. Notably, IGF-IR phosphorylation of IR substrate-1 (IRS-1) stimulates the phosphatidylinositol-3’ kinase (PI3K) pathway, associated with prevention of differentiation and apoptosis, via interaction with the p85 subunit of PI3K (Zhang & Yee 2000). The Ras–mitogen-activated protein kinase (MAPK) cascade, linked to cell growth and proliferation, is activated by IGF-IR-phosphorylated IRS-1 and Src- and collagen-homology (SHC) protein, via interaction.
Figure 1 Molecular structures of the IGFRs and signal transduction pathways activated by the IGF-IR. IGF bioavailability is determined by sequestration in ternary complexes with IGFBP-3 (or to a much lesser extent IGFBP-5) and ALS in the circulation, or in binary complexes with IGFBP-3 (or other IGFBPs) in the cellular environment. Modification of IGFBPs (e.g. proteolysis, phosphorylation, glycosylation, interaction with the cell surface or extracellular matrix) liberates IGFs, enabling their interaction with IGFRs. Ligand binding activates the tyrosine kinase activity of the IGF-IR, which phosphorylates itself and several downstream targets, including the adaptor proteins IRS-1 and SHC, which link the IGF-IR to the PI3K and Ras–MAPK signaling pathways leading to cell survival and proliferation respectively. IGFBP-3 also has IGF-independent antiproliferative and proapoptotic effects thought to be mediated by its association with incompletely characterized cell surface proteins or receptors. In contrast to the IGF-IR, ligand binding to the IGF-IIR, which lacks demonstrated tyrosine kinase activity, inhibits cellular growth by reducing the level of IGF-II available for interaction/signaling through the IGF-IR. Epidemiological evidence indicates that reduced circulating levels of IGFBP-3, increased levels of IGF-I or an increased ratio of IGF-I to IGFBP-3 are associated with increased cancer risk.
branee following proteolytic cleavage as a soluble fragment which acts as a scavenger receptor for IGF-II in the blood (Ellis et al. 1996). Targeted disruption of the IGF-IR gene results in elevated IGF-II plasma levels and has a positive effect on embryonic growth, with mutant embryos being larger than their wild-type counterparts, but leads to perinatal lethality (Lau et al. 1994). Homozygous deletion of either IGF-IR or IGF-II genes rescues the IGF-IR −/− body size effect (Li et al. 1993, Ludwig et al. 1996) supporting the role of this receptor in reducing IGF-II availability, thereby modulating its interaction and signaling through the IGF-IR. For this reason, the IGF-IR is hypothesized to behave as a tumor suppressor by favoring decreased activation of the IGF-IR pathway. As reviewed by Khandwala (2000), IGFBP-2 is locally overexpressed in a variety of cancers, and mutations in the IGF-IR have been demonstrated in breast and liver cancers (Devi et al. 1999).

**IGFBPs**

IGF bioactivity is not only dependent on interaction with IGF receptors, but is also influenced by the multifunctional family of IGFBPs (Table 1, reviewed by Firth & Baxter 2002). This superfamily includes six proteins (IGFBP-1 to -6) that bind IGFs with high affinity and a group of IGFBP-related proteins 1–9 that bind IGFs with low affinity. The IGFBPs have greater affinity than the IGFs for IGFBPs, and have endocrine, paracrine and autocrine effects dependent on, and independent of, IGF action.

When bound to IGFs, IGFBPs function by regulating their transport between intra- and extracellular spaces and interaction with their receptors (Zapf 1995), prolonging IGF half-life (Stewart & Rotwein 1996) and preventing excessive cell growth or promoting apoptosis (Rajah et al. 1999). Whereas IGFBP sequestration of IGFs is normally inhibitory, in certain target cells and conditions, IGFBPs (IGFBP-1, -3 and -5) can enhance IGF effects by presenting a slow-releasing IGF pool for interaction with the IGF-IR, while concomitantly repressing receptor downregulation by high IGF-I exposure (Conover & Powell 1991). Most circulating IGFs are bound by IGFBPs, with more than 75% forming a ternary complex with IGFBP-3 (the largest and most abundant IGFBP) and the acid-labile subunit (ALS), a liver-derived, GH-regulated glycoprotein (Baxter 1994). The half-life of the ternary complex is 12 h, as compared with free IGF-I (10 min) and free IGFBP-3 (30–90 min; Hasegawa et al. 1995). IGFBP-5 (present at levels 10% that of IGFBP-3) can also form a similar ternary complex with IGF-I or -II and ALS (Twigg & Baxter 1998), providing an additional mechanism for regulating IGF bioavailability. A small fraction of IGFs bind to IGFBPs as a binary complex, but less than 1% circulates in free form (Baxter 1994). Free or binary complexes exit the circulation rapidly, whereas ternary complexes are confined to the vascular compartment (Firth & Baxter 2002).

IGFBPs are expressed in a diversity of tissues (Table 1; Moschos & Mantzoros 2002), including the liver (IGFBP-1 and -3), endometrium (IGFBP-1), central nervous system (CNS) (IGFBP-2 and -4), prostate (IGFBP-2 and -6), bones (IGFBP-4), kidneys (IGFBP-5) and ovary (IGFBP-6). In addition to the liver, IGFBP-3 is also expressed in a number of non-hepatic tissues. IGFBP gene transcription is complex and regulated by hormones (estrogen, glucocorticoids, parathyroid hormone, FSH, GH, thyroid hormone, insulin, vitamin D and cortisol), cytokines (interleukin-1 and tumor necrosis factor-α (TNF-α)) and growth factors (FGF, EGF, IGF, TGF-β, PDGF and IGFs; Yu & Rohan 2000).

Proteolysis of IGFBPs, influenced by such factors as insulin, IGF-I and estrogens (Yu & Rohan 2000), results in fragments with greatly reduced affinity for IGFs, making the latter more available to the cells (Rajah et al. 1995). Prostate-specific antigen (PSA) (synthesized by a number of tissues in addition to the prostate) is able to cleave IGFBP-3 and -5, cathepsin D can proteolyze all six IGFBPs, thrombin and plasmin cleave IGFBP-3, and matrix metalloproteinases (MMPs) cleave IGFBP-2, -3, -4 and -5 (Fielder et al. 1994, Fowlkes et al. 1994, Lalou et al. 1994, 1997, Booth et al. 1996, Okabe et al. 1999). The association of IGFBPs with the cell membrane (IGFBP-1 and -3; Delbe et al. 1991, Oh 1998) or extracellular matrix (IGFBP-2 and -5; Arai et al. 1996) may also affect the binding affinity of IGFBPs for IGFs. Furthermore, IGFBPs undergo substantial post-translational modification, with glycosylation and phosphorylation affecting cell interaction (IGFBP-3; Firth & Baxter 1999, Coverley et al. 2000) and phosphorylation affecting IGF- (IGFBP-1) and ALS-binding affinity (IGFBP-3) and susceptibility to proteases (IGFBP-3; Jones et al. 1991, Coverley et al. 2000).

Further to its central role in regulating IGF bioavailability via sequestration in circulating ternary complexes, IGFBP-3 also competitively inhibits IGF action at the cellular level. This has been demonstrated by a number of experiments in various cell types using an IGF-I analog, des-(1–3)-IGF-I, that binds the IGF-IR and stimulates DNA synthesis, but cannot bind IGFBP-3 (Barreca et al. 1996, Karas et al. 1997, Y M Li et al. 1997). In these studies IGFBP-3 inhibited cellular proliferation or estradiol production.

### Table 1 Expression and proteolysis of IGFBPs

<table>
<thead>
<tr>
<th>IGFBP</th>
<th>Tissue expression</th>
<th>Known protease(s)</th>
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<tbody>
<tr>
<td>IGFBP-1</td>
<td>Liver, endometrium</td>
<td>Cathepsin D</td>
</tr>
<tr>
<td>IGFBP-2</td>
<td>CNS, prostate</td>
<td>Cathepsin D, MMP</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>Liver, many others</td>
<td>PSA, cathepsin D, thrombin, plasmin, MMP</td>
</tr>
<tr>
<td>IGFBP-4</td>
<td>CNS, bones</td>
<td>Cathepsin D, MMP</td>
</tr>
<tr>
<td>IGFBP-5</td>
<td>Kidney</td>
<td>PSA, cathepsin D, MMP</td>
</tr>
<tr>
<td>IGFBP-6</td>
<td>Prostate, ovary</td>
<td>Cathepsin D</td>
</tr>
</tbody>
</table>
induced by IGF-I or IGF-I and FSH respectively, but not by des(1–3)-IGF-I.

IGFBP-3 is also active in the cellular environment as a potent antiproliferative agent where it functions by cell cycle blockade and induction of apoptosis, independently of IGF binding (Fig. 1). Valentinis et al. (1995) have shown dose-dependent growth inhibition of IGF-IR knockout mouse fibroblasts expressing different levels of IGFBP-3. Ricort & Binoux (2001) provided the first evidence that IGFBP-3 can specifically modulate IGF signaling independently of its binding to IGF-I. These authors demonstrated that IGFBP-3, but not IGFBP-5 or -7, dose-dependently inhibited IGF-I and des(1–3)-IGF-I stimulation of IGF-IR autophosphorylation and tyrosine kinase activity in MCF-7 breast cancer cells. An IGFBP-3 fragment with negligible binding affinity for IGF-I has been shown to block IGF-I-stimulated DNA synthesis in chick embryo fibroblasts (Lalou et al. 1996), induce apoptosis in MCF-7 breast carcinoma cells (Bernard et al. 2002) and inhibit mitogenesis in murine fibroblasts with a defective IGF-IR (Zadeh & Binoux 1997). A similar N-terminal IGFBP-3 proteolytic fragment with reduced IGF binding was characterized by Salahifar et al. (2000) and shown to inhibit DNA synthesis, in both the presence and absence of IGF stimulation in MCF-7 breast cancer cells.

In addition to its IGF-independent effects on DNA synthesis, IGFBP-3 has been reported to directly induce apoptosis in cells lacking the IGF-IR (Gill et al. 1997, Rajah et al. 1997). This effect is at least partially attributed to IGFBP-3-induced alterations in the ratio of proapoptotic (Bax) and antiapoptotic (Bcl-2) proteins (Butt et al. 2000).

A direct role for IGFBP-3 in transcriptional regulation has also been proposed following its localization to the nucleus in several cell types (Jaques et al. 1997, W Li et al. 1997, Baxter 2001). It was subsequently shown that IGFBP-3 and IGFBP-5 have C-terminal nuclear localization signals (Schedlich et al. 1998) which allow their translocation to the nucleus by β-importin (Schedlich et al. 2000). Liu et al. (2000) recently demonstrated an essential role for the nuclear retinoid X receptor α (RXR-α) in mediating IGFBP-3-induced apoptosis, which is stimulated by RXR ligands. In contrast to IGFBP-3, IGFBP-5 appears to be antiapoptotic (Perks et al. 2000) and may compete with IGFBP-3 for nuclear trafficking and/or receptor binding. IGFBP-3 independent effects may be mediated by IGFBP-3 association with specific, incompletely characterized, cell surface proteins or receptors (Oh et al. 1993, Leal et al. 1997, Rajah et al. 1997, Yamanaka et al. 1999). However, characterization of a mutant form of IGFBP-3 revealed that cell surface binding and nuclear translocation are not required for IGFBP-3-mediated growth inhibition and apoptosis in T47D human breast cancer cells (Butt et al. 2002). These results suggest the existence of multiple pathways by which IGFBP-3 elicits its antiproliferative and proapoptotic effects. The complexity of IGFBP-3 function is further complicated by the finding that EGF activation of the Ras–MAPK signaling pathway ablates the growth-inhibitory effects of IGFBP-3 in breast epithelial cells (Martin et al. 2003).

**Epidemiological evidence implicating the IGF system in cancer**

Several years of research have demonstrated an association between excessive, aberrant signaling through the IGF-IR pathway and cancer. A number of recent epidemiological studies (summarized in Table 2) have suggested that reduced circulating levels of IGFBP-3, increased circulating levels of IGF-I or an increased ratio of IGF-I to IGFBP-3 are associated with an increased risk for the development of several common cancers, particularly those of the breast (Hankinson et al. 1998, Li et al. 2001), prostate (Chan et al. 2002), lung (London et al. 2002) and colon (Giovannucci et al. 2000).

**Breast cancer**

Results of a case-controlled prospective study of breast cancer showed no overall association between plasma IGF-I concentrations and the risk for developing the disease (Hankinson et al. 1998). However, the relative age-adjusted risk for breast cancer in premenopausal women in the top vs. the bottom tertile of plasma IGF-I concentrations was 2.33 (95% confidence interval (CI) 1.06–5.16; P = 0.08) and increased to 2.88 (95% CI 1.21–6.85; P < 0.05) after adjusting for plasma IGFBP-3 concentrations. Furthermore, in women less than 50 years old at the time of blood collection, the relative risks increased to 4.58 and 7.28 respectively. These results are similar to those reported by Li et al. (2001), who found a high ratio of IGF-I to IGFBP-3 was associated with an increased risk of breast cancer. This study highlighted the importance of unbound IGF-I as opposed to total IGF-I, as the odds ratios (ORs) for breast cancer patients with high levels of IGF-I after adjusting for menopausal status and IGFBP-3 were 2.00 for total IGF-I and 6.31 for free IGF-I.

**Prostate cancer**

A prospective case-controlled study found a strong positive association between plasma IGF-I levels and prostate cancer risk (Chan et al. 1998), with men in the highest quartile of IGF-I having a relative risk of 4.3 (95% CI 1.8–10.6; P = 0.001) compared with men in the lowest quartile, after adjustment for IGFBP-3 levels. Furthermore, in a prospective study in the Physicians’ Health Study, increased plasma levels of IGF-I and decreased levels of IGFBP-3 were predictors of advanced stage prostate cancer, but not of early stage disease, leading to the suggestion that alterations in the IGF axis are associated with more aggressive forms of prostate cancer (Chan et al. 2002). This idea is supported by the
results of Shariat et al. (2002), who found that in patients undergoing radical prostatectomy, preoperative IGFBP-3 levels were significantly lower in patients with aggressive forms of the disease and also decreased in patients with prostate cancer metastases. Similarly, Miyata et al. (2003) recently reported that serum IGF-I levels were significantly higher and serum IGFBP-3 levels were significantly lower in patients with advanced prostate cancer compared with those with benign prostatic hyperplasia. Other case-controlled studies have demonstrated a strong dose-dependent relation-

Lung cancer

Similar relationships between circulating IGF-I and IGFBP-3 have been reported in case-control studies in lung cancer. For example, Yu et al. (1999) reported that high plasma levels of IGF-I were associated with a 2-fold increase in the OR (OR = 2.06; 95% CI 1.19–3.56; P = 0.01) of developing lung cancer, whereas increased levels of IGFBP-3 were associated with a 50% reduction (OR = 0.48; 95% CI 0.25–0.92; P = 0.03), after adjustment for IGF-I levels. In a large case-control study nested within the Japan Collaborative Cohort Study, subjects in the highest quartile of circulating IGFBP-3 showed a reduced OR for developing lung cancer compared with those in the lowest quartile (OR = 0.55; 95% CI 0.37–0.81; P = 0.04; Wakai et al. 2002). In addition, after adjusting for IGFBP-3 levels, this risk was increased in the highest vs the lowest quartile of IGF-I (OR = 1.14; 95% CI 1.08–2.81).

In a prospective study of Chinese men, increased serum IGF-I levels were not associated with an increased risk for developing lung cancer (London et al. 2002). However, for subjects in the highest vs the lowest quartile of serum IGFBP-3 levels, the OR for lung cancer, adjusted for smoking and IGF-I levels, was 0.50 (95% CI 0.25–1.02). Finally, two recent studies published by Chang et al. (2002a,b) highlight the importance of IGFBP-3 expression in stage I non-small-cell lung cancer (NSCLC). Analysis of tumor samples from 74 patients with stage I NSCLC indicated that IGFBP-3 expression was reduced in approximately 57% of the samples. A statistically significant positive correlation between IGFBP-3 expression and disease-specific survival was observed, as well as a trend for patients with decreased IGFBP-3 expression to have a shorter overall, disease-free survival rate than patients with normal IGFBP-3 expression. It is known that hypermethylation of the promoter for IGFBP-3 reduces the expression of the gene (Hanafusa et al. 2002). These investigators used this information to determine whether the methylation status of the IGFBP-3 promoter in the tumor influenced prognosis of stage I NSCLC. Results from this study indicated that hypermethylation of the promoter was present in approximately 62% of the tumors. Furthermore, these patients had a significantly lower 5-year disease-specific, disease-free, and overall survival rate than patients without a methylated IGFBP-3 promoter.

Colorectal cancer

Recent epidemiological studies also point to an association between abnormalities in the IGF axis and an increased risk of developing colorectal tumors. Analysis of archived samples from the Physicians’ Health Study showed that after adjustments for age, smoking, body mass, alcohol intake and IGFBP-3 levels, men in the top quintile of plasma IGF-I had a relative risk of 2.51 (95% CI 1.15–5.46; P = 0.02) for colorectal cancer when compared with those in the lowest quintile (Ma et al. 1999). In terms of circulating IGFBP-3, the relative risk for men in the top vs the bottom quintile was 0.28 (95% CI 0.12–0.66; P = 0.005). Similarly, results of a prospective study in which plasma samples were obtained from women in the Nurses’ Health Study indicated that, after adjustment for IGFBP-3 levels, women in the top vs the bottom tertile of IGF-I levels were at an increased risk for developing intermediate to late stage colorectal neoplasia adenoma (relative risk = 2.78; 95% CI 0.94–5.08) and colorectal cancer (relative risk = 2.18; 95% CI 0.94–5.08; Giovannucci et al. 2000). Controlling for IGF-I levels, women in the top tertile of IGFBP-3 levels had a lower relative risk of intermediate to late stage adenoma (0.28; 95% CI 0.09–0.85) and cancer (0.28; 95% CI 0.10–0.83).

Acromegaly is a condition associated with increased circulating levels of GH and IGF-I. Although the exact mechanism(s) remain unclear, a number of studies have suggested that patients with this disease are at increased risk for developing benign and malignant tumors, particularly of the colon. A recent population-based cohort study of acromegalic patients in Sweden and Denmark reported an increased risk for the development of colon cancer (standardized incidence ratio = 2.6; 95% CI 1.6–3.8) and rectal cancer (standardized incidence ratio = 2.5; 95% CI 1.2–5.0) in addition to an increased risk of developing other neoplasias (Bari et al. 2002). In a relatively large retrospective study of acromegalic patients, no increase of overall cancer mortality was found in these patients above the general population, but a significant increase in mortality specifically due to colon cancer was observed (standardized mortality ratio = 2.47; 95% CI 1.31–4.22; Orme et al. 1998). In a smaller prospective study designed to establish the natural history of colorectal neoplasia in acromegaly and identify patients at an increased risk for developing neoplasia, it was determined that the development of new adenomas was associated with elevated serum IGF-I levels (P < 0.005; Jenkins et al. 2000). Another study of acromegalic patients reported that the prevalence of colorectal neoplasia detected during colonoscopy was significantly higher than would be expected in the general population, with an OR for developing colorectal cancer of 13.5 (95% CI 3.1–75) and of developing colorectal adenoma of 4.2 (95% CI 2.5–6.8; Jenkins et al. 1997). In a slightly larger extension study, significantly increased serum IGF-I levels were found in patients with a recurrent adenoma compared with those without (Jenkins 2000).

Other tumor types

Malignancies of the breast, prostate, lung and colon account for a significant percentage of human cancers and...
the associations observed in these epidemiological studies suggest that IGFBP-3 functions as a tumor suppressor in these diseases. Although most of the clinical information pertaining to dysregulation of the IGF axis and cancer has focused on these specific disease types, there are clinical reports to suggest this system is involved in many other types of cancer. For example, a strong relationship between circulating IGF-I levels and the risk of developing ovarian cancer before the age of 55 was reported in a prospective case-control study conducted in the United States, Sweden and Italy (Lukanova et al. 2002). Katsaros et al. (2002) recently reported that increased levels of IGFBP-3 in cancerous ovarian tissue were associated with favorable prognostic features of the disease and significantly reduced the risk for disease progression. Increased serum concentrations of IGF-I have been reported in women with atypical hyperplasia and endometrial carcinoma compared with controls (Ayabe et al. 1997, Waksanski et al. 2001). Increased expression of the IGF-IR has been reported in primary cervical cancer cells (Steller et al. 1996). Human hepatocellular carcinomas have been reported to contain low to undetectable levels of IGFBP-3 compared with non-neoplastic liver tissue (Huynh et al. 2002). Patients with increased expression of the IGF-IR in clear-cell renal cell carcinomas exhibit a significantly decreased disease-specific survival compared with patients with low IGF-IR tumor expression (Parker et al. 2002). Finally, increased expression of IGF-I and the IGF-IR have been detected in thyroid adenomas and carcinomas compared with normal thyroid tissue (Maiorano et al. 2000).

Thus, overall, the epidemiological evidence would suggest a link between increased circulating IGF-I levels and/or decreased circulating IGFBP-3 levels and the development of certain cancers. However, it is important to note that there are reports in the literature that do not support this hypothesis. For example, in a prospective study in women, Lukanova et al. (2001) found no association between serum IGF-I or IGFBP-3 levels and the risk of developing lung cancer. Similarly, Kaaks et al. (2002) found no association between IGF-I or IGFBP-3 levels and the risk of developing breast cancer in premenopausal women.

A significant amount of evidence also suggests that IGF-II may play an important role in cancer biology. Elevated levels of serum IGF-II have been reported in patients with colorectal cancer (el Atiq et al. 1994) with trends towards higher circulating levels in advanced disease (Renehan et al. 2000a,b). A prospective study with a cohort of Chinese men showed that individuals in the highest vs lowest quintile of IGF-II had an OR of 2.74 (CI 1.67–4.50; \( P = 0.0008 \)) for the development of colorectal cancer, after adjustment for body mass index, cigarette smoking and alcohol intake (Probst-Hensch et al. 2001). However, a prospective case-control study nested in the Physicians’ Health Study found no association between IGF-II levels and future colorectal cancer risk in the United States (Ma et al. 1999).

Increased circulating levels of IGF-II in patients with hepatocellular carcinoma have been associated with an increased occurrence of metastasis following treatment (Song et al. 2001). IGF-II mRNA has also been shown to be overexpressed (10-fold) in prostatic stromal cells from patients with benign prostate hyperplasia and may be important in the pathogenesis of this disease (Cohen et al. 1994). IGF-II mRNA levels become markedly increased during breast tumor growth in response to hormone and decreased during tumor regression following ovariectomy, in the N-nitrosomethylurea-induced rat mammary tumor model (Manni et al. 1994).

### Modulation of IGF-IR/IGFBP-3 function in models of human cancers

The plethora of evidence implicating the IGF system in the development and progression of human neoplasms suggests that it is a valuable target for the design of novel cancer treatments. The results of several studies support the notion that strategies aimed at interfering with the IGF axis are inhibitory to cancer cell growth and those directed at upregulating IGFBP-3 function and/or downregulating IGF-IR activity are reviewed here.

### Downregulation of IGF-IR signaling

A number of endocrine maneuvers to inhibit IGF action have been investigated. For example, GH-releasing hormone (GHRH) antagonists reduce circulating levels of GH and have been shown to inhibit the growth of a variety of cancers, including osteosarcomas (Pinski et al. 1995), glioblastomas (Kiari et al. 2000), lung (Kiari et al. 1999), prostate (Lamharzi et al. 1998), colorectal (Schally & Varga 1999), and breast (Kahan et al. 2000) cancers. In vivo, this effect may be mediated by an inhibition in pituitary GH release, resulting in decreased hepatic IGF-I secretion with a concomitant reduction in serum IGF-I levels (Jungwirth et al. 1997b, Braczkowski et al. 2002). However, results from several studies suggest that GHRH antagonists may have a more direct effect by inhibiting IGF-I and/or IGF-II production by malignant cells (Jungwirth et al. 1997a,b, Lamharzi et al. 1998, Csernus et al. 1999, Chatzistamou et al. 2001, Braczkowski et al. 2002).

The recombinant protein pegvisomant is structurally similar to endogenous human GH and binds the GH receptor as a competitive antagonist. To date, pegvisomant has been primarily studied for its effectiveness in reducing circulating GH and IGF-I levels in acromegalic patients. Studies addressing the effectiveness of pegvisomant in tumor growth inhibition are limited. However, in athymic nude mice bearing human meningiomas, administration of pegvisomant was shown to significantly reduce tumor growth (McCutchion et al. 2001). In this study, IGF-II concentrations in the tumors...
did not vary with treatment and no autocrine IGF-I production by the tumors was detected. Therefore, the antitumor effects of pegvisomant were considered most likely a consequence of the observed decreased IGF-I levels in the circulation.

Somatostatin is a hormone that acts as a neurotransmitter in the CNS to inhibit GH and thyroid-stimulating hormone from the pituitary. In addition, somatostatin is synthesized and released throughout the gastrointestinal system where it inhibits the secretion of a variety of hormones implicated in tumor growth. The antiproliferative properties of long-acting somatostatin analogs have been demonstrated in a number of in vitro and in vivo studies (Siegel et al. 1988, Taylor et al. 1988, Liebow et al. 1989, Szende et al. 2003, Szereday et al. 2003). While these effects may be mediated by preferential binding to specific somatostatin receptors, results from both preclinical and clinical studies indicate that administration of these analogs can significantly reduce circulating IGF-I levels, which is likely to contribute to their growth-inhibitory effects (Pollak et al. 1989, Lamberts et al. 1996, Jungwirth et al. 1998, Lucas et al. 2003).

It has been demonstrated in a number of experimental systems that reducing the number of functional IGF-IRs through the use of antisense oligonucleotides is associated with decreased cell growth in vitro and delayed tumor formation with increased survival in vivo (Resnichoff et al. 1995, 1996, Scotlandi et al. 2002a). Through a frame-shift mutation, D’Ambrosio et al. (1996) engineered a human IGF-IR, designated 486/STOP, which exerted a strong dominant negative effect for several growth functions in vitro, including extensive apoptosis of cancer cells and inhibition of tumorigenesis in syngeneic rats. Later, Reiss et al. (1998) transfected human colon, lung, prostate, kidney and ovarian cancer cell lines with plasmids expressing the 486/STOP receptor. All transfected clones showed a significant inhibition in colony formation in soft agar in vitro, in addition to a significant decrease in tumorigenicity in vivo. Impairment of IGF-IR signaling in MDA-MB-231 486/STOP-transfected breast cancer cells also resulted in decreased cellular adhesion, invasion and metastasis in athymic nude mice (Dunn et al. 1998). Implantation of Ewing’s sarcoma cells that express dominant negative mutants of the IGF-IR into athymic nude mice results in a reduction in the tumor formation and metastatic capabilities typical of Ewing’s sarcoma cells (Scotlandi et al. 2002a).

It was recently reported that two recombinant adeno-viruses expressing dominant negative IGF-IRs inhibited the growth-stimulatory effects of IGF-I on human lung cancer cell lines and that intratumoral injections resulted in significant growth suppression in established lung cancer xenografts (Lee et al. 2003). Truncated IGF-IRs cloned into recombinant adeno-viruses have been shown to suppress tumorigenicity of colorectal cancer cells in vivo (Adachi et al. 2002). Similarly, retroviral expression of a kinase-defective IGF-IR into a CHO cell experimental system has been shown to impair cellular proliferation, transformation and tumor growth in athymic nude mice (Seely et al. 2002). Neutralization of IGF-I action has also been achieved by treatment with polyethylene glycol-conjugated recombinant human IGFBP-1 (Van den Berg et al. 1997). This conjugate with a prolonged serum half-life inhibited growth of MDA-MB-231 breast tumor xenografts and malignant ascites formation in the MDA-MB-435A model.

Still more evidence for the beneficial effects of interrupting IGF association with the IGF-IR arises from experimental data using αIR3, a monoclonal antibody which associates with the ligand-binding domain of the IGF-IR to inhibit IGF-I-mediated effects (Kull et al. 1983). It was demonstrated early on that αIR3 inhibited clonal growth and blocked the mitogenic effects of exogenous IGF-I in breast cancer cells in vitro (Arteaga et al. 1989). Inhibition of the IGF-IR signaling pathway by αIR3 has also been shown to decrease proliferation and increase apoptosis in neuroectodermal tumor cell lines (Scotlandi et al. 1996) and to enhance the cytotoxic effects of doxorubicin and paclitaxel in breast cancer cell lines and Ewing’s sarcoma cells (Bech et al. 2001, Benini et al. 2001). In vivo, administration of αIR3 has been shown to induce a complete regression of established tumors in nearly half of the mice inoculated with Ewing’s sarcoma cells (Scotlandi et al. 1998). A similar approach was taken by Li et al. (2000), who generated a chimeric humanized single-chain antibody (scFv-Fc) against the IGF-IR. These authors demonstrated that scFv-Fc inhibits IGF-I and IGF-II binding to the IGF-IR, exhibits dose-dependent growth inhibition of IGF-IR-overexpressing NIH-3T3 cells and significantly suppresses MCF-7 breast tumor growth in athymic mice. In a subsequent study, the chimeric antibody was shown to function via IGF-IR activation, causing receptor downregulation and refractoriness to further IGF-I stimulation (Sachdev et al. 2003).

The recent success of small molecule tyrosine kinase inhibitors has sparked interest in the development of compounds that effectively target the IGF-IR. Blum et al. (2000) reported on two competitive inhibitors of the IGF-IR kinase that inhibit IGF-IR autophosphorylation in intact cells. More recently, Stahl et al. (2002) identified a nakkijquinone analog that inhibits IGF-IR tyrosine activity with an IC50 of 500 nM. The ultimate success of small molecule tyrosine kinase inhibitors directed toward the IGF-IR remains to be determined. However, this approach is an attractive strategy in that these compounds are typically relatively inexpensive and orally active.

Upregulation of IGFBP-3 activity

Increased expression of natural IGFBP-3 or treatment with recombinant human IGFBP-3 (rIGFBP-3) has been shown not only to inhibit cancer cell growth, but also to induce...
cancer cell death in a variety of experimental systems. rhIGFBP-3 treatment leads to a significant reduction in HepG2 hepatocellular carcinoma cell proliferation while concomitantly attenuating IGF-I-induced IGF-IR autophosphorylation and trans-phosphorylation of its substrates (Huynh et al. 2002). Stable transfection and expression of IGFBP-3 cDNA in H1299 and NCI-H23 NSCLC and M12 prostate cancer cells was shown to inhibit cellular proliferation and induce apoptosis (Hochscheid et al. 2000, Devi et al. 2002, H Y Lee et al. 2002). H Y Lee et al. (2002) demonstrated that IGFBP-3 overexpression inhibited phosphorylation of IGF-IR substrates in NSCLC cells and that transfection with activated Akt (PI3K pathway) or MAPK kinase-1 (an upstream activator of MAPK), or treatment with IGF-I could partially block IGFBP-3-induced apoptosis. These studies suggest that the growth regulatory effects of IGFBP-3 were, at least in part, due to inhibition of IGF-dependent pathways.

IGFBP-3 may also play a direct role in inducing tumor cell death independently of IGF binding. IGFBP-3 is known to upregulate the cell cycle regulator p21/WAF1 (Boyle et al. 2001) and to increase the ratio of proapoptotic to anti-apoptotic Bcl family members (Butt et al. 2000). In addition, the results of several in vitro studies indicate that induction of IGFBP-3 expression in various cancer cells by some of the most potent antiproliferative factors known, including TNF-α, TGF-β, retinoic acid, vitamin D analogs and p53, is at least partially responsible for their growth inhibitory actions. Treatment of both prostate and breast cancer cells with TNF-α has been shown to induce apoptosis while up-regulating IGFBP-3 expression (Rozen et al. 1998, Rajah et al. 2002). In both cases, the TNF-α-induced apoptosis could be prevented by IGFBP-3-specific antisense oligonucleotides or IGFBP-3-neutralizing antibodies. McCaig et al. (2002) reported that the growth of Hs578T breast cancer cells was significantly inhibited by TGF-β and this was associated with a 14-fold increase in IGFBP-3 secretion. Treatment of prostate cancer cells with TGF-β has been shown to induce IGFBP-3 expression 6–12 h before apoptosis, and again, this effect could be prevented with IGFBP-3-specific antisense oligonucleotides or with IGFBP-3-neutralizing antibodies (Rajah et al. 1997). Gucev et al. (1996) demonstrated that treatment of MDA-MB-231 breast cancer cells with either TGF-β or retinoic acid increased the levels of IGFBP-3 mRNA (1.5- to 2-fold) and protein (2- to 3-fold) and this effect could be almost entirely blocked by IGFBP-3-specific antisense oligonucleotides. In this study, exogenous IGFBP-3 significantly inhibited MDA-MB-231 cell proliferation by 40%. The use of cDNA microarray hybridization and RT-PCR analysis, Dokmanovic et al. (2002) showed that the retinoid-induced growth arrest in MCF-7 breast cancer cells was associated with a strong induction of the IGFBP-3 gene. In a similar manner, the antiproliferative effect of a retinoic acid receptor-α agonist in hepatocellular carcinoma cells was also shown to be associated with significant upregulation of IGFBP-3 expression (Murakami et al. 2000).

In a study published by Colston et al. (1998), vitamin D analogs were observed to inhibit IGF-I-stimulated growth of MCF-7 cells, and this inhibition was associated with a concomitant increase in IGFBP-3 production. In this same study, growth of Hs578T cells, which are unresponsive to the mitogenic effects of IGF-I, was also inhibited by the vitamin D analogs and this inhibition was associated with an accumulation of IGFBP-3 in the conditioned medium. Addition of a vitamin D analog to LNCaP prostate cancer cells has been reported to upregulate IGFBP-3 at both the mRNA and protein level, an effect which could be abolished by IGFBP-3 antisense oligonucleotides or IGFBP-3-neutralizing antibodies (Boyle et al. 2001). The relationship between the tumor suppressor gene p53 and IGFBP-3 was first described by Buckbinder et al. (1995), who used a differential cloning approach to identify the IGFBP-3 gene as a p53-regulated gene, whose induction was associated with increased IGFBP-3 secretion and inhibition of IGF-IR signaling. Since that time, IGFBP-3 has been shown to both enhance and prolong p53-dependent apoptosis after irradiation in both human colonic adenoma-derived cells and in Hs578T breast carcinoma cells (Hollowood et al. 2000, Williams et al. 2000).

Inhibition of cultured androgen-independent PC-3 human prostate cancer cells by silybin, a naturally occurring flavonoid antioxidant found in milk thistle, is also associated with increased (9-fold) IGFBP-3 mRNA and protein accumulation, and reduced IGF-IR signaling (Zi et al. 2000). This finding was corroborated by a later study demonstrating dose-dependent growth inhibition of DU145 human prostate carcinoma xenografts by silybin, accompanied by increased (5.8-fold) plasma IGFBP-3 levels (Singh et al. 2002).

IGFBP-3 and anticancer therapy

Recent independent studies have demonstrated that IGFBP-3 can induce cell cycle arrest and enhance the efficacy of radiative, proapoptotic and chemotherapeutic agents (Table 3). For example, IGFBP-3 reduces cell survival and enhances apoptosis in response to radiation in MCF-7 and T47D breast cancer cells (Butt et al. 2000, Shiry et al. 2002). Studies have also demonstrated IGFBP-3 sensitization of human retinoblastoma and gastric cancer cells to apoptosis by the topoisomerase inhibitors, etoposide, camptothecan and amscarine (Giuliano et al. 1998, D Y Lee et al. 2002) and accentuation of apoptosis induced by ceramide in Hs578T human breast cancer cells (Perks et al. 2002). IGFBP-3 was shown to potentiate paclitaxel-induced cell cycle arrest and apoptosis in Hs578T human breast and gastric carcinoma cells (Fowler et al. 2000, D Y Lee et al. 2002). In addition, through sequestration of IGF-I, rhIGFBP-3 has been shown to restore sensitivity to Herceptin in resistant breast cancer cells.
Table 3  Evidence for the utility of IGFBP-3 as an anticancer agent or modulator of antitumor therapies

<table>
<thead>
<tr>
<th>Experimental system</th>
<th>IGFBP-3 effect</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>In vitro</td>
<td></td>
<td></td>
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<tr>
<td>MCF-7 human breast cancer</td>
<td>Dose-dependent sensitization to radiotherapy</td>
<td>Shiry et al. (2002)</td>
</tr>
<tr>
<td>T47D human breast cancer</td>
<td>Potentiation of radiation-induced apoptosis</td>
<td>Butt et al. (2000)</td>
</tr>
<tr>
<td>Y79 human retinoblastoma</td>
<td>Sensitization to apoptosis by camptothecin, etoposide, amsacrine</td>
<td>Giuliano et al. (1998)</td>
</tr>
<tr>
<td>SNU-484 and SNU-668 human gastric carcinoma</td>
<td>Potentiation of cell cycle arrest, apoptosis and growth inhibition by paclitaxel or etoposide</td>
<td>DY Lee et al. (2002)</td>
</tr>
<tr>
<td>Hs578T human breast cancer</td>
<td>Dose-dependent enhancement of ceramide-induced apoptosis</td>
<td>Perks et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>Restoration of Herceptin sensitivity in resistant cells</td>
<td>Lu et al. (2001)</td>
</tr>
<tr>
<td>In vivo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1299 human NSCLC xenograft</td>
<td>Single-agent antitumor activity</td>
<td>HY Lee et al. (2002)</td>
</tr>
<tr>
<td>3LL Lewis lung carcinoma allograft</td>
<td>Single-agent tumor growth inhibition</td>
<td>Our unpublished observation</td>
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<tr>
<td>LoVo human colorectal xenograft</td>
<td>Single-agent and combinatorial antitumor activity with CPT-11</td>
<td>Our unpublished observation</td>
</tr>
<tr>
<td>MCF-7 human breast carcinoma xenograft</td>
<td>Potentiation of paclitaxel antitumor activity</td>
<td>Our unpublished observation</td>
</tr>
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</table>

expressing both the IGF-IR and HER2 (a member of the EGF family of receptors; Lu et al. 2001).

IGFBP-3 has also been shown to have potent antitumor activity in vivo, either alone or in combination with standard chemotherapeutic agents. Direct injection of an IGFBP-3-expressing adenovirus into NSCLC xenografts induced massive destruction of tumors (H Y Lee et al. 2002) and endogenous overexpression of IGFBP-3 significantly reduced tumor formation and/or growth of NSCLC and prostate carcinoma xenografts (Hochscheid et al. 2000, Devi et al. 2002). More recently, rhIGFBP-3 treatment was shown to induce significant tumor growth inhibition in mice bearing 3LL Lewis lung and LoVo colorectal carcinomas and to enhance the chemotherapeutic effects of paclitaxel and CPT-11 in MCF-7 breast and LoVo colorectal xenografts respectively (L Jerome, L Shiry and B Leyland-Jones, unpublished observations). Collectively, these studies emphasize the value of developing IGFBP-3 as a potential therapeutic modality for the treatment of a variety of cancers.

Conclusions

Clearly there are multiple signaling pathways involved in neoplastic growth, of which there is a significant amount of knowledge yet to be gained. Mounting evidence suggests that the loss of IGF-I/IGFBP-3 autocrine/paracrine loops may lead to tumor growth and progression. Thus, therapies aimed at modulating the balance between the actions of IGF-I and IGFBP-3 (the most prevalent endocrine growth factor and binding protein, respectively) at the cellular level represent novel areas of cancer treatment and prevention in high-risk groups (i.e. high IGF-I/IGFBP-3 ratios). Suggested potential therapeutic agents include GHRH antagonists, somatostatin analogs, GH receptor antagonists (e.g. pegvisomant), IGF-IR antibodies and analogs of IGF-I (Khandwala et al. 2000). Clinical studies have demonstrated the efficacy of the antiestrogen tamoxifen, which decreases the IGF-I/IGFBP-3 ratio via increased IGFBP-3 (Campbell et al. 2001) and reduced IGF-I levels (Osborne 1998), in the treatment and prevention of breast cancer (Fisher et al. 1998). Another estrogen receptor modulator, raloxifene (used for the treatment and prevention of osteoporosis in postmenopausal women) was shown to decrease the IGF-1/IGFBP-3 ratio and has been proposed for future studies in breast cancer prevention (Torrisi et al. 2001). Finally, preclinical studies indicate that other factors which upregulate IGFBP-3 expression (e.g. vitamin D and retinoic acid analogs), or more recently IGFBP-3 itself, are potential candidates that deserve further attention as anticancer agents, alone or in combination with other antineoplastic drugs. Clearly, further studies exploring the complexity of cellular consequences as a result of such interventions are essential for the development of more specific therapeutic strategies which will allow targeting on the basis of tumor type.
References


Colston KW, Perks CM, Xie SP & Holly JM 1998 Growth inhibition of both MCF-7 and HeLaS7 human breast cancer cell


expression of insulin-like growth factor II in tumors. PNAS 95 8864–8868.


Leaf SM, Liu Q, Huang SS & Huang JS 1997 The type V transforming growth factor beta receptor is the putative insulin-like growth factor-binding protein 3 receptor. Journal of Biological Chemistry 272 20572–20576.


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Jerome et al.: Deregulation of the IGF axis in cancer


