CMLS, Cell. Mol. Life Sci. 57 (2000) 932–942
1420-682X/00/060932-11 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

New concepts in regulation and function of the insulin-like growth factors: implications for understanding normal growth and neoplasia

H. Werner[a] and D. Le Roith[b,*]

[a]Department of Clinical Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, 69978 (Israel)
[b]Clinical Endocrinology Branch, Room 8D12, Bldg 10, NIH MSC 1758, Bethesda (Maryland 20892-1758, USA), Fax + 1 301 480 4386, e-mail: derek@helix.nih.gov

Received 21 November 1999; received after revision 11 January 2000; accepted 9 February 2000

Abstract. The insulin-like growth factors (IGFs) are a ubiquitous family of growth factors, binding proteins and receptors that are involved in normal growth and development. They are also implicated in numerous pathological states, including malignancy. IGF-II is a commonly expressed growth factor in many tumors and may enhance tumor growth, acting via the overexpressed IGF-I receptor, a cell-surface tyrosine kinase receptor. The IGF-I receptor may be overexpressed due to mutations in tumor suppression gene products such as p53 and WT-1 or growth factors such as bFGF and PDGF. Thus, this family of growth factors, especially the IGF-I receptor, may present an excellent target for new therapeutic agents in the treatment of cancer and other disorders of excessive cellular proliferation.

Key words. Insulin-like growth factors; IGF-I receptor; cancer; receptor signaling.

Introduction

Over the last several years, the insulin-like growth factor (IGF) field has witnessed an overwhelming influx of new information of both experimental and clinical nature (for a recent update see [1]). Some of these novel findings were quite unexpected, and they have compelled us to review and update a number of basic concepts that have prevailed for more than 30 years. The purpose of this essay is to review some of these new data and to evaluate their significance in the specific context of various physiological and pathological processes that involve the IGF system.

* Corresponding author.

The IGF family: old and new

The critical elements that regulate IGF function include ligands, receptors and IGF-binding proteins (IGFBPs). To date, this family comprises three ligands (insulin, IGF-I and IGF-II), three cell-surface receptors (the insulin, IGF-I and IGF-II/mannose-6-phosphate receptors) and at least six IGFBPs, which bind circulating IGFs and modulate their function. In addition to these 'classical' family members, which have been well characterized, more recent work has identified several other proteins as potential components of the IGF system. These 'nonclassical' members include two additional receptors (the insulin-receptor-related receptor (IRR) and the insulin-IGF-I hybrid receptor), and a steadily

growing number of IGFBP-related proteins. In addition, the biological activities of the IGFs have been shown to be modulated by a group of IGFBP-proteases that cleave the binding proteins, thereby regulating the overall availability of these ligands. The number and specificity of the various proteases involved has yet to be elucidated.

Both IGF-I and IGF-II are produced primarily by the liver, which is the major source of endocrine IGFs. IGF-I biosynthesis is tightly correlated with circulating levels of growth hormone (GH). Consequently, IGF-I gene expression levels increase 10- to 100-fold between birth and adulthood [2]. In contrast, IGF-II messenger RNA (mRNA) levels in rodents are high during the fetal and perinatal periods and decline thereafter [3]. In humans, however, significant levels of circulating IGF-II can be detected in adults. The original somatomedin hypothesis was based on the findings that GH can stimulate sulfate and thymidine incorporation into cartilage and that these effects are mediated by a plasma-borne factor ('sulfation factor', later termed IGF-I) [4]. Subsequent studies demonstrated that multiple tissues are capable of synthesizing IGFs, during both the fetal and adult stages of development. These findings provided evidence that IGFs can act locally (i.e. autocrine/paracrine) in addition to their endocrine modes of action [3, 5]. Finally, it was recently reported that ablation of IGF-I production specifically in the liver has essentially no effect on the growth and development of mice [6]. The potential implications of this unexpected finding will be discussed below.

The second component of the IGF family is the set of cell-surface receptors. There is ample consensus today that much of the biological action of the IGFs on growth and differentiation is mediated by the IGF-I receptor (IGF-I-R). A detailed description of the cellular events associated with activation of this receptor will be presented in the next section. The insulin receptor (IR) is very similar to the IGF-I-R both in overall gene and protein organization and with respect to the intracellular components that mediate insulin signal transduction. Despite these striking similarities, the IR is significantly less potent than the IGF-I-R in inducing mitogenesis. However, if IGF-II is present in abundance, then the IR can contribute significantly to mitogenesis [7]. The IGF-II/mannose-6-phosphate receptor (IGF-II/Man-6-P) receptor is a bifunctional binding protein that binds both IGF-II and ligands that contain Man-6-P at distinct binding sites. Whereas it has been clearly established that the IGF-II/Man-6-P receptor functions in lysosomal enzyme trafficking and IGF-II degradation, its role in IGF signal transduction remains controversial [8, 9].

For a number of years it has been suggested that there are atypical insulin and IGF receptors that are capable of binding one or both ligands with relatively high affinity [10]. Receptor heterogeneity may result from primary structure variation, differential glycosylation, splicing events, hybrid formation, and additional gene products such as the IRR [11]. The IRR shares $\sim 55\%$ identity at the amino acid level with that of the IR and IGF-I-R [12]. The C-terminal domain downstream of the tyrosine kinase region of the IRR, however, is significantly shorter. The IRR does not bind significant levels of IGF-I, IGF-II, insulin, proinsulin or relaxin, and thus to date the IRR remains an orphan receptor with no identified ligand [13].

Among the various atypical receptors reported, attention has mostly been focused on the hybrid IR/IGF-I-R. It has now been clearly established that hybrid heterodimeric receptors comprising an IR α-β 'hemireceptor' and an IGF-I-R α-β hemireceptor form in cells. This has been demonstrated by several approaches, including sequential immunodepletion and immunoprecipitation experiments [14], immunoprecipitation of the IGF-I-R with a monoclonal antibody (αIR3) followed by microsequencing [15], and expression of full-length IGF-I-R and C-terminal truncations of the IR in HeLa cells [16]. IR/IGF-I-R hybrids seem to be widely expressed and, in certain tissues, even appear to be the most abundant form of receptor [17]. However, specific differences in signaling characteristics between the IGF-I-R and IR/IGF-I-R hybrids have not yet been established.

Finally, a virtual explosion of new information occurred in the field of the IGFBPs, the third component of the IGF system. This IGFBP superfamily includes, in addition to the classical members, IGFBP-1 through IGFBP-6, several other members which are now termed IGFBP-related proteins (IGFBP-rP)-1 through IGFBP-rP-5 [18, 19]. The IGFBP superfamily seems to have evolved from a common ancestor, with some of its current members showing high affinity for IGF, whereas others display low affinity. An important implication of this finding is that the IGFBP superfamily may influence cell growth in both IGF-dependent and IGF-independent fashion.

Signal transduction through the IGF-I receptor

The IGF-I-R is a member of the tyrosine kinase receptor family and is closely related to the insulin receptor. Both receptors form a subclass, because unlike other members of the family that are single transmembrane proteins, both the insulin and IGF-I-R exist as preformed dimers. Both receptors consist of two α and two β subunits joined by disulfide bonds [11, 20]. Ligand binding to the extracellular α subunits results in a conformational change that induces autophosphoryla-

tion of tyrosine residues within the β subunit, which is primarily intracellular. Autophosphorylation stimulates the receptor tyrosine kinase activity and leads to phosphorylation of other substrates. A number of various SH2 domain-containing proteins or 'docking proteins' bind to specific phosphotyrosine residues in the C-terminal portion of the β subunit. Some of these proteins also become phosphorylated by the receptor tyrosine kinase. The insulin receptor substrate (IRS) family of proteins (IRS-1 through IRS-4) and Shc are the best-characterized docking proteins (fig. 1). These proteins can then bind SH2-containing proteins in a manner dependent on the specific phosphotyrosine motif (Y-X-X-X) involved [21, 22]. These SH2-containing proteins include GRB-2, which together with mSOS can activate Ras, SH-PTP2 (a tyrosine phosphatase), the p85 regulatory subunit of phosphoinositide 3′-kinase (PI3′K) and other adapter proteins such as Crk and Nck [23]. Thus, enzymatic activation of the IGF-I-R tyrosine kinase results in stimulation of an array of various intracellular signaling cascades, including the Ras/Raf/MAP kinase and PI3′-kinase pathways. Other signaling pathways are also involved in IGF-I-R-mediated biological outcomes. Many of the protein kinase C (PKC) isoforms are regulated by IGF-I, and in vascular smooth muscle cells, for example, downregulation of these isoforms results in inhibition of IGF-I-stimulated DNA synthesis and cell migration. PKC may also activate the Ras/Raf/MAP kinase pathway. Adapter proteins, including the Crk family of protooncogenes, are involved in the IGF-

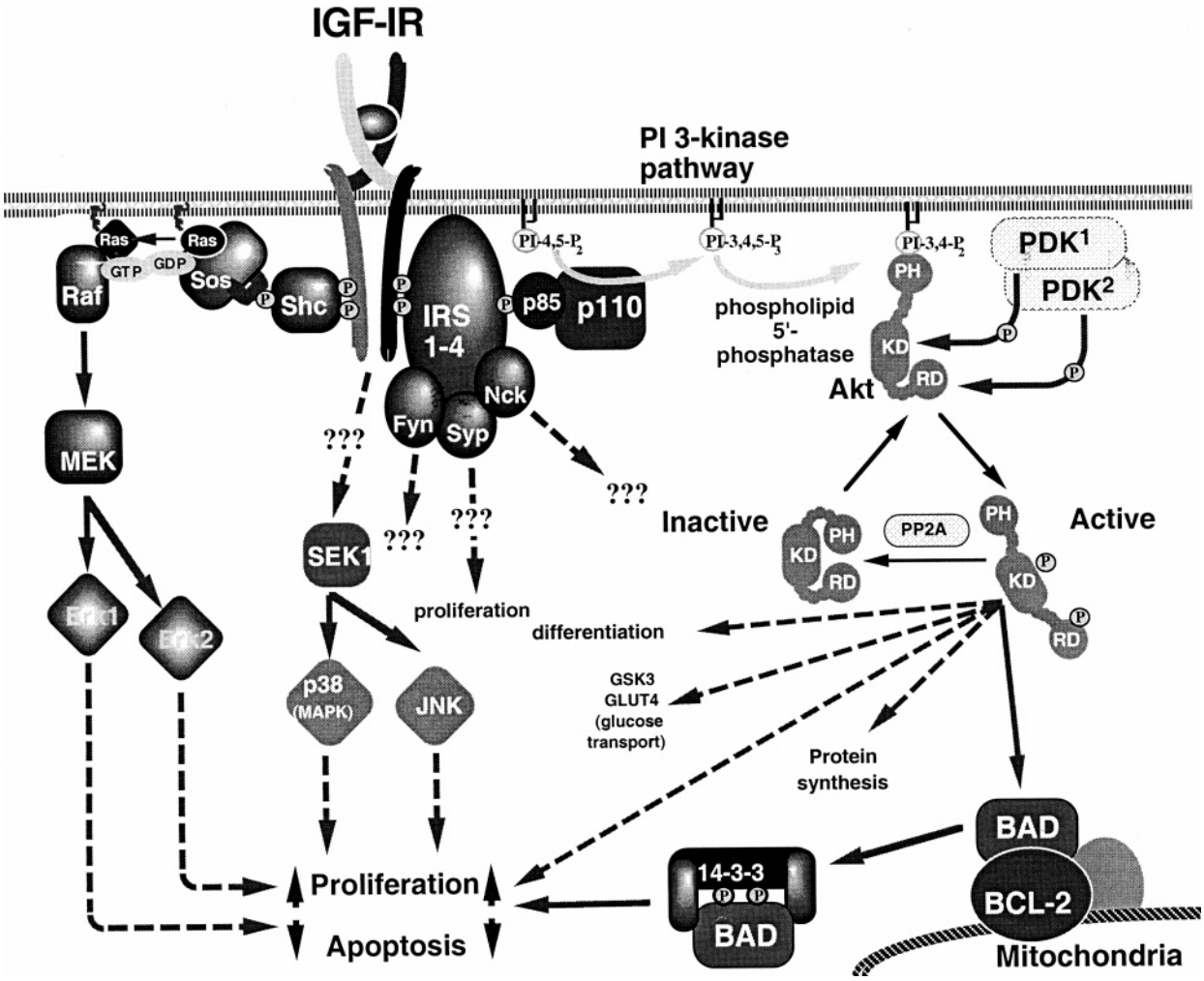

Figure 1. Schematic representation of intracellular signaling pathways emanating from the IGF-I receptor. The activated IGF-I receptor phosphorylates a number of adaptor proteins, including IRS-1, IRS-2 and SHC. These tyrosine phosphoproteins then interact with downstream molecules, e.g. p85, Syp, Grb2 and Nck, via their SH2 domains, thereby activating the Ras/Raf/MAP kinase, the PI3′-kinase and other kinase pathways.

Table 1. Characteristics of IGF-system knockout mice.

Disrupted gene	Birth weight (% normal)	Perinatal viability	Characteristics
IGF-I*,†	60	5–70	genetic background affects perinatal viability, delayed ossification, underdeveloped muscles and lungs, infertility
IGF-II‡	60	100	normal postnatal (but not catchup) growth, develop into fertile dwarfs
IGF-I-R*	45	0	severe in utero growth retardation, hypoplasia, abnormal skin formation, delayed bone development, abnormal CNS
IGF-II/Man-6-P§	125–140	0	moderate fetal overgrowth, cardiac hypertrophy

* [29].
† [28].
‡ [27].
§ [34].

I-R signaling cascade, and CrkL expression, for example, results in a transformed phenotype [24, 25].

Activation of the IGF-I-R both increases mitogenesis and inhibits apoptosis. Until recently, IGF-induced mitogenesis was attributed primarily to the Ras/Raf/MAP kinase pathway, whereas the antiapoptotic effects of IGFs were thought to be mediated by the PI3′-kinase pathway [1, 11]. IGF-I-R activation has been shown to inhibit apoptosis and induce phosphorylation of downstream substrates such as AKT/PKB and Bad in a PI3′-kinase-dependent manner. Phosphorylation of Bad causes it to dissociate from Bcl2, thereby allowing Bcl2 to inhibit apoptosis. Whereas the PI3′-kinase pathway may be important in many cell lines for IGF-I-R inhibition of apoptosis, other pathways, including the MAP kinase pathway, have also been implicated in this effect of IGF-I. Still other pathways and substrates that have been implicated in IGF-I-R signaling include Grb10 and the Ca^{2+}/cyclic AMP response element-binding protein (CREB). Indeed, activation of CREB as well as the MAP kinase pathways leads to regulation of the expression of a large number of genes [1, 21, 22].

In the past, IGF-I-R signaling pathways (like growth factor receptor signaling cascades in general) have been studied and presented as linear tracts. However, it has become increasingly clear over the past few years that many if not all of these pathways interact with each other and that there is significant cross-talk between tyrosine kinase receptors and other cell surface receptors such as G-protein-related serpentine receptors, cytokine receptors and integrins. Thus, synthetic $\alpha V\beta 3$ integrin receptor antagonists inhibit IGF-I-stimulated smooth muscle cell migration and replication, β-arrestins can regulate IGF-I-R-induced mitogenesis, and antiestrogens affect the expression and phosphorylation state of the IGF-I-R, to mention just a few examples [11].

IGF knockout models

Homologous recombination has recently been used to selectively disrupt the expression of genes encoding ligands and receptors of the IGF family (table 1). This approach has proven invaluable to assess the specific roles of these proteins in embryonic and postnatal growth [26–29]. Thus, targeted disruption of the IGF-II gene resulted in mice that weigh just 60% that of their normal littermates at the time of birth. However, this reduction in growth rate was restricted to the embryonic period, and the animals developed into essentially normal and fertile dwarfs. The phenotype of null mutants for the IGF-I gene is apparently more complex, and seems to depend on the genetic background of the animals. Thus, some of these mice died shortly after birth, whereas others survived and reached adulthood. Surviving IGF-I-null mice showed, among other abnormalities, a delay in the ossification process, underdeveloped muscles and lungs, and infertility. Mice heterozygous for the disrupted IGF-I gene exhibited no major growth retardation. The phenotype of mice with a liver-specific disruption of the IGF-I gene and the implications of these observations on the somatomedin hypothesis will be described in the next section.

Homozygous IGF-I-R knockout mice exhibited the most severe developmental retardation. These animals, which invariably died at birth, weighed only 45% that of normal controls. They displayed hypoplasia, abnormal skin formation, delayed bone development and anomalous central nervous system morphology. Given the widespread distribution of the IGF-I-R during ontogenesis, it seems that this receptor can mediate the endocrine effects of IGF-II, which circulates in both the bloodstream and in cerebrospinal fluid, as well as the autocrine/paracrine actions of locally produced IGFs [30, 31]. The extensive damage resulting from disruption of the IGF-I-R gene is therefore consistent with its central role as a cell survival factor [32]. In contrast,

ablation of the IR gene resulted in mice that weighed ~90% of normal weight and showed no major developmental delay at time of birth. These animals, however, died during the first several days of postnatal life as a result of diabetic ketoacidosis [33]. Thus, these homologous receptors have clearly distinct functional roles in vivo.

Finally, disruption of the IGF-II/Man-6-P receptor resulted in mice that were 25–40% larger than nomal at birth [34]. In addition, these animals displayed cardiac hypertrophy and died shortly after birth. The excessive growth in these animals could potentially be explained by the increased levels of circulating IGF-II (up to ~4.5-fold above normal values), which are likely to be a compensatory response to the absence of a functional IGF-II/Man-6-P receptor. Consistent with this interpretation, double knockouts of IGF-II and the IGF-II/Man-6-P receptor are rescued from perinatal lethality [35].

In humans, loss of one copy of the IGF-I-R gene has been reported in a number of infants with deletion of the distal long arm of chromosome 15 (q26.1 → qter) [36, 37]. An additional genetic rearrangement, ring chromosome 15, has been also documented to result in the loss of one allele of the IGF-I-R gene [38]. Most patients showing deletion of the distal 15q portion, as well as those with the ring chromosome, have severe intrauterine and postnatal growth retardation. Homozygous partial deletion of the IGF-I gene has been reported in a 15-year-old boy with severe prenatal and postnatal growth deficiency, sensorineural deafness and mental retardation [39]. This phenotype suggests that there is an important role for IGF-I not only in postnatal but also in prenatal growth. Moreover, the observed neurological deficiencies clearly point to a crucial role for IGF-I in normal development of the central nervous system [39].

Liver-specific IGF-I knockout: implications on the somatomedin theory

The original somatomedin hypothesis suggested that GH regulated the production of somatomedin (later called IGF-I) and that this factor was produced primarily by the liver, and reached peripheral target tissues via the circulation. Furthermore, the somatomedin hypothesis suggested that IGF-I was the major mediator of GH action. Subsequent studies demonstrated that IGF-I was produced by all tissues and probably has local autocrine/paracrine effects ('modified somatomedin hypothesis'), though distinguishing between the endocrine and the autocrine/paracrine roles of IGF-I has been elusive. Modern technology now makes it possible to address these questions by conditionally knocking out specific genes in specific tissues.

To create a liver-specific deletion of the IGF-I gene, we generated transgenic mice expressing Cre recombinase exclusively in the liver by expressing Cre under the control of the albumin promoter [6]. Cross-breeding of the loxP-flanked IGF-I mice and the albumin-Cre-expressing mice resulted in deletion of the IGF-I gene in the liver. IGF-I mRNA levels in liver were $< 1\%$ of the levels in wild-type animals. In contrast, IGF-I mRNA levels measured in nonhepatic tissues such as heart, muscle, fat, spleen and kidney were similar to those of control animals.

Circulating levels of IGF-I in these animals were markedly reduced (25% of those in wild-type animals) at 6 weeks of age. Postnatal growth and development, as assessed from age 3 to 6 weeks, was normal. Sexual maturation was normal, as demonstrated by normal fertility, normal-size litters and normal lactation and weaning. There were essentially no phenotypic distinctions between the liver-specific IGF-I-gene-deleted animals and their wild-type littermates. Thus, although liver production of IGF-I is the major contributor to circulating 'endocrine' IGF-I levels (~ 75%), liver production of IGF-I is not essential for normal postnatal and pubertal growth and development in the mouse. Rather, autocrine/paracrine IGF-I production is sufficient for normal growth and development. Thus, both the original and modified somatomedin hypotheses need to be reevaluated.

The IGFs as potential predictors of breast and prostate cancer

The role played by the IGF system in the biology of human cancer has generated a great deal of attention from both basic and clinical researchers [40, 41]. IGF-I functions as a progression factor during the cell cycle: once the cell is stimulated to enter G1 by a competence factor such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) or other stimuli, the cell will be able to traverse the cell cycle solely in the presence of subphysiologic concentrations of IGF-I [42]. In fact, most primary tumors and transformed cell lines express high levels of IGF-II mRNA and protein, with some tumors overexpressing the IGF-I gene [40]. IGF-I and IGF-II are assumed to act in an autocrine manner via the IGF-I-R.

The recent finding that higher circulating IGF-I concentrations (and lower IGFBP-3 levels) are associated with an increased risk of breast and prostate cancer sparked an ardent debate on the issue of whether endocrine levels of IGF-I can be used as predictors of specific types of cancer [43, 44] (table 2). The first of these prospective studies included 121,700 women (aged 30–55 years), with 397 cases of breast cancer confirmed

among individuals in which the levels of IGF-I and IGFBP-3 were measured on average 28 months before diagnosis. The relative risk (RR) of breast cancer in premenopausal women less than 50 years old was 4.6 in the upper tertile of IGF-I values (compared with individuals in the lower tertile). When the concentrations of IGFBP-3 were incorporated into the multivariate analysis, the RR increased to 7.3. The second prospective study included 15,000 men (aged 40–82 years), with 152 cases of prostate cancer confirmed (on average after 7 years) in individuals in which the concentrations of IGF-I and IGFBP-3 were determined. The RR of prostate cancer in the upper quartile of IGF-I values was 2.4 compared with the lower quartile, although it increased to 4.3 when the concentrations of IGFBP-3 were included in the analysis. In men over 60 years of age the RR was 7.9.

The results of these investigations, if confirmed by additional large prospective studies, may shed light on the role of circulating IGF ligands and binding proteins on the etiology of a variety of human cancers. Furthermore, such studies may potentially facilitate the design of more rational hormonal therapies intended to lower IGF concentrations and/or to decrease the sensitivity of the target organs to the mitogenic action of the IGFs.

The IGF-I receptor and cancer

A fundamental role of the IGF-I-R in malignant transformation has now been well established. Several clinical and experimental observations support this notion: (i) the IGF-I-R is highly overexpressed by most tumors and cancer cell lines [40, 45]; (ii) fibroblast cell lines (R^-) established from mouse embryos in which the IGF-I-R was disrupted by homologous recombination cannot be transformed by any of a number of oncogenes, including the SV40 large T antigen, activated *ras* and the bovine papillomavirus E5 protein [46–48]. Reintroduction of a functional receptor renders R^- cells susceptible to the transforming activities of these oncogenes; and (iii) overexpression of the IGF-I-R results in a ligand-dependent transformed phenotype which includes the formation of tumors in nude mice [49].

The transforming activity of the IGF-I-R depends, to a large extent, on its potent antiapoptotic activity, in addition to its mitogenic effects. The ability of the IGF-I-R to protect cells from apoptosis has been shown in several different systems, including fibroblasts, neurally derived cells, hemopoietic cells and others [50, 51]. Especially impressive was the antiapoptotic activity displayed by the IGF-I-R in vivo [52, 53]. These experiments showed that R^- cells undergo apoptosis when placed in a biodiffusion chamber in the subcutaneous tissue of a rat. In contrast, fibroblasts overexpressing the receptor, or tumor-derived cells with elevated numbers of receptors, had the capacity to double over a 1-day period. Furthermore, the major single factor determining cell survival in these studies proved to be the number of IGF-I-Rs [52]. IGF-I-R levels have been shown to be the critical determinant that causes cells to switch from a 'nonmitogenic' to a 'mitogenic' mode. Thus, one study demonstrated that cells with less than 15,000 IGF-I-Rs will not grow in serum-free media supplemented with IGF-I, whereas cells with 22,000 binding sites will grow in the sole presence of IGF-I. Furthermore, cells expressing more than 30,000 receptors are able to grow in soft agar, suggesting an increase in their transforming capacity.

In light of the central role played by this receptor in many transforming events, targeting the IGF-I-R as a potential anticancer therapy appears to be a promising approach. Potential strategies include the use of anti-receptor antibodies, ligand analogs and antisense methodologies. Induction of apoptosis appears to be the common theme of these different modalities. In view of the potential relevance of the endocrine IGF-I and IGFBP levels on cancer predisposition, it may be that logical and comprehensive therapeutic approaches designed to simultaneously target ligands, receptors and binding proteins may have the greatest probability of success [54].

Table 2. Correlation between circulating levels of IGF-I and risk of breast* and prostate† cancer.

Plasma IGF-I	RR	RR‡
Breast cancer (premenopausal, <50 years old)		
<158 ng/ml	1.0	1.0
158–206 ng/ml	2.64	3.12
>207 ng/ml	4.58	7.28
RR = relative risk		
Prostate cancer		
99–184 ng/ml	1.0	1.0
185–236 ng/ml	1.32	1.94
237–293 ng/ml	1.81	2.83
294–500 ng/ml	2.41	4.32

* [44].
† [43].
‡ Adjusted for IGFBP-3.

Interplay between oncogenes and tumor suppressors in control of IGF-I receptor gene expression

Some of the transcription factors that regulate expression of the IGF-I-R gene have now been identified. Characterization of the mechanisms of action of these factors has provided important information that is facil-

itating our understanding of the molecular events responsible for IGF-I-R expression in normal and pathologic states. Expression of the IGF-I-R gene is regulated by both positive and negative factors. The first group comprises a number of growth factors and oncogenic agents that positively affect cell division (mitogenic agents), whereas the second group includes negative modulators of cell growth such as tumor suppressors.

Growth factors that have been shown to stimulate transcription of the IGF-I-R gene include basic FGF and PDGF [55–57]. bFGF, for example, has been shown to increase receptor binding and mRNA levels, and this effect has been mapped to a region of the proximal IGF-I-R promoter localized between nucleotides -476 and -188 in the 5′-flanking region of the gene. Likewise, PDGF increased the activity of the IGF-I-R promoter via an $\sim$100-bp promoter fragment located immediately upstream of the transcription start site. Since this region has a canonical c-*myc* binding site, and since PDGF induces c-*myc*, the effect of PDGF on IGF-I-R expression may be mediated by c-*myc*. Interestingly, upregulation of the IGF-I-R by bFGF and PDGF is consistent with the hypothesis that the main role of these competence factors is to generate enough IGF-I and IGF-I-R to induce the growth response [58, 59]. In contrast, expression of the IGF-I-R gene is negatively regulated by the local concentrations of IGF-I [55, 60].

IGF-I-R gene expression is induced by steroid hormones in addition to peptide growth factors. Treatment of MCF-7 cells and normal breast xenografts with estradiol increased IGF-I-R mRNA levels two- to threefold, whereas progesterone treatment decreased these levels by $\sim$50% [61]. These results indicate that augmenting the concentration of the IGF-I-R, and thereby increasing the responsiveness of the organ to the circulating or locally produced IGFs, is a potential mechanism by which estradiol stimulates cellular proliferation.

The IGF-I-R promoter is also targeted by multiple oncogenes. Constitutive overexpression of the protooncogene c-*myb* in Balb/c-3T3 cells has been shown to abrogate the requirement for IGF-I in the growing media. This effect of c-*myb* was associated with an increase in the levels of both IGF-I and IGF-I-R mRNAs [62, 63]. Another oncogene known to stimulate IGF-I-R promoter activity is the hepatitis B virus X (HBx) protein. In hepatocellular carcinoma-derived cell lines containing HBx protein, endogenous levels of the IGF-I-R mRNA were increased approximately fivefold compared with controls [64]. The implication of these findings is that HBx may play a role in the etiology of hepatocellular carcinoma by stimulating the expression of the IGF-I-R gene.

In addition to controlling transcription of the IGF-I-R gene, oncogenes can also affect IGF-I-R action by nontranscriptional mechanisms. For instance, transformation of human cells by $pp60^{src}$, the product of the *src* oncogene of the Rous sarcoma virus, results in constitutive phosphorylation of the receptor β-subunit, whereas addition of IGF-I further increases the level of phosphorylation. $pp60^{src}$ thus induces the ligand-independent phosphorylation and activation of the IGF-I-R, thereby subjecting the cell to a constitutively mitogenic signal [65, 66].

Tumor suppressors, a family of negative growth regulators, have been linked to the development of a wide variety of human cancers, including breast, colon and lung cancer [67–69]. Due to the central role of the IGF-I-R in cell cycle progression and transformation, it has been postulated that a potential mechanism by which the postmitotic, fully differentiated cell is kept out of the cell cycle may involve the constitutive inhibition of the IGF-I-R gene by wild-type tumor suppressors [70].

WT1 is a tumor suppressor whose inactivation has been linked to the pathogenesis of a subset of Wilms' tumors, a pediatric kidney neoplasia [71]. WT1, via its zinc-finger DNA binding domain, has been shown to bind to specific cis elements in the IGF-I-R promoter region, and to suppress the activity of transfected promoter fragments as well as the endogenous levels of IGF-I-R mRNA [72–74]. Loss of WT1 activity in Wilms' tumor and related malignancies (resulting from chromosomal deletions, missense or nonsense mutations, translocations or alternative splicing) may result in transcriptional derepression of the IGF-I-R gene. Activation of the overexpressed receptor by circulating or locally produced IGFs may be a key step in the biology of Wilms' tumor.

A particular case in which WT1 has been shown to be disrupted is the case of the desmoplastic small, round cell tumor (DSRCT), a very aggressive abdominal tumor. DSRCT is characterized by a recurrent translocation [t(11;22)(p13;q12)] that joins the N-terminal (activation) domain of EWS1 (the ubiquitously expressed Ewings' sarcoma gene) to the C-terminal (DNA binding) domain of WT1 [75–77]. Pathologic fusion of EWS to WT1 has been shown to abrogate the tumor suppressor function of WT1 and to generate an oncogenic chimeric protein capable of binding and activating the IGF-I-R promoter [78]. This gain-of-function event constitutes a novel paradigm in oncogenesis.

Likewise, p53, which is the most frequently mutated tumor suppressor, is capable of suppressing the activity of the IGF-I-R promoter, as well as endogenous levels of IGF-I-R mRNA. In contrast, tumor-derived, mutant versions of p53 significantly stimulated promoter activity [79, 80]. It is reasonable to speculate that part of the

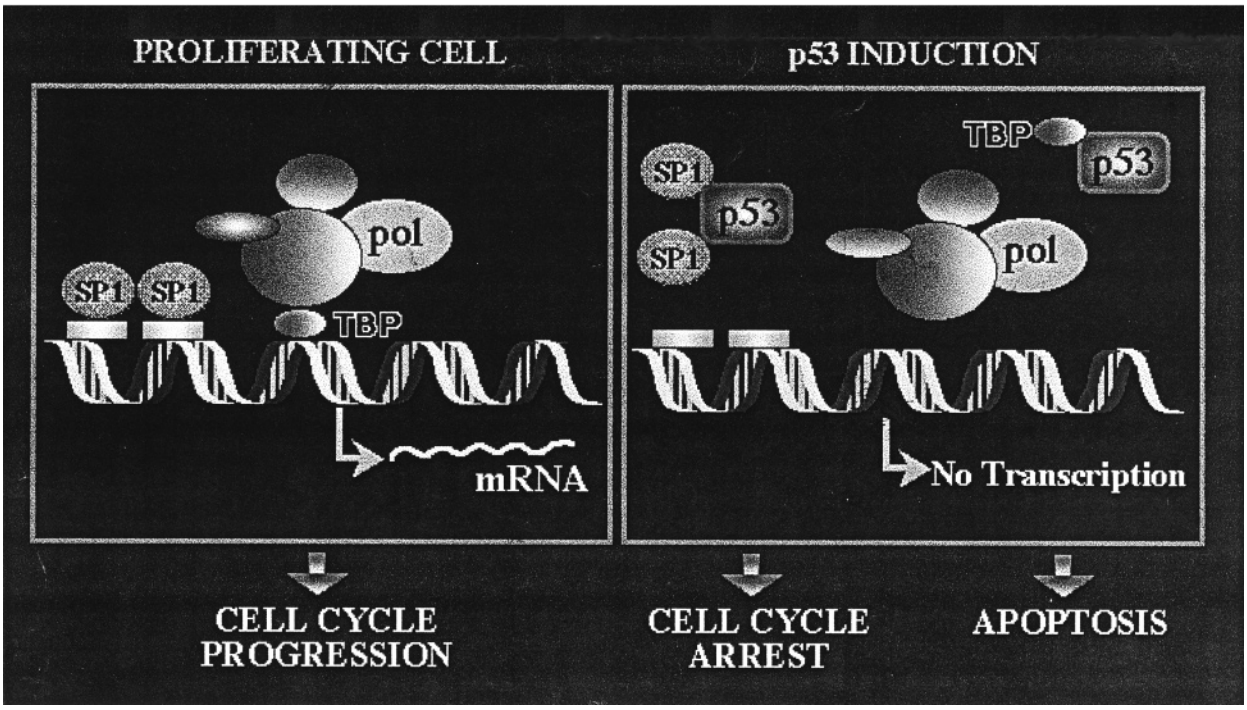

Figure 2. Model for p53 suppression of IGF-I receptor promoter. The IGF-I-R promoter contains multiple binding sites for transcription factor SP1, a zinc-finger nuclear protein that strongly stimulates transcription from a specific group of RNA-polymerase II-dependent promoters in vertebrates. In addition, transcription from the IGF-I-R gene seems to depend on the binding of TBP (the TATA-binding protein) to the 'initiator' region, with ensuing assembling of a functional transcription initiation complex that includes RNA polymerase-II (pol). p53, which is usually induced following cellular insults such as DNA damage, can bind both SP1 and TBP, thus precluding these proteins from binding specifically to the IGF-I-R promoter region. Consequently, transcription from the IGF-I-R gene is impaired, and as a result, cell cycle arrest or apoptosis may occur.

effects of p53 on cell cycle arrest and apoptosis are mediated via suppression of the IGF-I-R promoter [fig. 2]. This may therefore imply that lack of suppression by mutant p53 in tumors may facilitate expansion of a malignant population of cells. Importantly, additional components of the IGF system are regulated by p53. That is, transcription of the IGF-II gene is similarly reduced by wild-type p53 [81], whereas IGFBP3 (which usually functions as an inhibitor of IGF action) is stimulated by p53 [82]. In conclusion, p53 controls the IGF signaling system by regulating expression of ligands, receptors and binding proteins.

Interactions between wild-type and mutant forms of these transcription factors are very complicated and may involve additional DNA-binding and non-DNA-binding interacting proteins. It is likely that a finely tuned interplay between these stimulatory and inhibitory factors ultimately determines the level of expression of the IGF-I-R gene and the proliferative status of the cell. A clear understanding of these interactions will prove important in our attempts to target the IGF-I-R as a potential therapeutic approach

Future directions

The IGF system of ligands, receptors and binding proteins is undoubtedly a major player in normal cellular growth and differentiation. These elements also play important roles in aberrant growth as seen in neoplastic disorders. Whereas the IGFs and the IGF-I-R have not been shown to be, by themselves, oncogenic, evidence has evolved that strongly suggest that they may enhance proliferation of preneoplastic and neoplastic cells. Attacking this ubiquitous system of growth factors for adjunct therapies in cancer patients is therefore an obvious new and hopefully fruitful direction of research.

Acknowledgments. H.W. is the recipient of a Guastalla Fellowship, the Rashi Foundation, Israel. Its work is supported by grants from the Israel Ministry of Health, the Israel Cancer Association, the U.S.-Israel Binational Science Foundation, the Israel Academy of Sciences, the Israel Cancer Research Fund (New York), the Fogarty International Center (NIH, USA) and the Recanati Foundation.

1 Rosenfeld R. G. and Roberts C. T. Jr (1999) The IGF System: Molecular Biology, Physiology and Clinical Applications. Humana Press, Totowa, NJ
2 Roberts C. T. Jr, Brown A. L., Graham D. E., Seelig S., Berry S., Gabbay K. H. et al. (1986) J. Biol. Chem. **261:** 10025–10028
3 Adamo M. L., Ben-Hur H., Roberts C. T. Jr and LeRoith D. (1991) Regulation of start site usage in the two leader exons of the rat insulin-like growth factor I gene by development, fasting and diabetes. Mol. Endocrinol. **5:** 1677–1686
4 Salmon W. D. and Daughaday W. H. (1957) A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. J. Lab. Clin. Med. **49:** 825–836
5 D'Ercole A. J., Applewhite G. T. and Underwood L. E. (1980) Evidence that somatomedin is synthesized by multiple tissues in the fetus. Dev. Biol. **75:** 315–322
6 Yakar S., Liu J. L., Stannard B., Butler A., Accili D., Sauer B. et al. (1999) Normal growth and development in the absence of hepatic insulin-like growth factor I. Proc. Natl. Acad. Sci. USA **96:** 7324–7329
7 Morrione A., Valentinis B., Xu S., Yumet G., Louvi A., Efstratiadis A. et al. (1997) Insulin-like growth factor II stimulates cell proliferation through the insulin receptor. Proc. Natl. Acad. Sci. USA **94:** 3777–3782
8 Nissley S. P. (1999) Type 2 IGF receptor-mediated events. In: The IGF System: Molecular Biology, Physiology and Clinical Applications, pp. 165–197, Rosenfeld R. G. and Roberts C. T. Jr (eds), Humana Press, Totowa, NJ
9 Kiess W. (1999) Molecular biology of the IGF-II/mannose-6-phosphate receptor. In: The IGF System: Molecular Biology, Physiology and Clinical Applications, pp. 89–109, Rosenfeld R. G. and Roberts C. T. Jr (eds), Humana Press, Totowa, NJ
10 Siddle K. and Soos M. A. (1999) Alternative IGF-related receptors. In: The IGF System: Molecular Biology, Physiology and Clinical Applications, pp. 199–225, Rosenfeld R. G. and Roberts C. T. Jr (eds), Humana Press, Totowa, NJ
11 LeRoith D., Werner H., Beitner-Johnson D. and Roberts C. T. Jr (1995) Molecular and cellular aspects of the insulin-like growth factor I receptor. Endocrine Rev. **16:** 143–163
12 Shier P. and Watt V. M. (1989) Primary structure of a putative receptor for a ligand of the insulin family. J. Biol. Chem. **264:** 14605–14608
13 Zhang B. and Roth R. A. (1992) The insulin receptor-related receptor. Tissue expression, ligand binding specificity and signaling capabilities. J. Biol. Chem. **267:** 18320–18328
14 Soos M. A., Field C. E. and Siddle K. (1993) Purified hybrid insulin/insulin-like growth factor-I receptors bind insulin-like growth factor-I, but not insulin, with high affinity. Biochem. J. **290:** 419–425
15 Kasuya J., Paz B., Maddux B. A., Goldfine I. D., Hefta S. A. and Fujita-Yamaguchi Y. (1993) Characterization of human placental insulin-like growth factor-I/insulin hybrid receptors by protein microsequencing and purification. Biochemistry **32:** 13531–13536
16 Frattali A. L. and Pessin J. E. (1993) Relationship between α subunit ligand occupancy and β subunit autophosphorylation in insulin/insulin-like growth factor-I hybrid receptors. J. Biol. Chem. **268:** 7393–7400
17 Bailyes E. M., Nave B. T., Soos M. A., Orr S. R., Hayward A. C. and Siddle K. (1997) Insulin receptor/IGF-I receptor hybrids are widely distributed in mammalian tissues: quantification of individual receptor species by selective immunoprecipitation and immunoblotting. Biochem. J. **327:** 209–215
18 Baxter R. C., Binoux M. A., Clemmons D. R., Conover C. A., Drop S. L. S., Holly J. M. P. et al. (1998) Recomendations for nomenclature of the insulin-like growth factor binding protein superfamily. Endocrinology **139:** 4036
19 Hwa V., Oh Y., Burren C. P., Choi W.-K., Graham D. L., Ingermann A. et al. (1999) The IGF binding protein superfamily. In: The IGF System: Molecular Biology, Physiology and Clinical Applications, pp. 315–327, Rosenfeld R. G. and Roberts C. T. Jr (eds), Humana Press, Totowa, NJ
20 Ullrich A., Gray A., Tam A. W., Yang-Feng T., Tsubowka M., Collins C. et al. (1986) Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests determinants that define functional specificity. EMBO J. **5:** 2503–2512
21 Myers M. G. Jr, Backer J. M., Sun X.-J., Shoelson S. E., Hu P., Schlessinger J. et al. (1992) IRS-1 activates the phosphatidylinositor 3′-kinase by associating with the src homology 2 domains of p85. Proc. Natl. Acad. Sci. USA **89:** 10350–10354
22 Myers M. G. Jr, Sun X.-J., Cheatham B., Jachna B. R., Glasheen E. M., Backer J. M. et al. (1993) IRS-1 is a common element in insulin and insulin-like growth factor-I signaling to the phosphatidylinositol 3′-kinase. Endocrinology **132:** 1421–1430
23 White M. F. and Kahn C. R. (1994) The insulin signaling system. J. Biol. Chem. **269:** 1–4
24 Beitner-Johnson D. and LeRoith D. (1995) Insulin-like growth factor-I stimulates tyrosine phosphorylation of endogenous c-Crk. J. Biol. Chem. **270:** 5187–5190
25 Beitner-Johnson D., Blakesley V. A., Shen-Orr Z., Jimenez M., Stannard B., Wang L.-M. et al. (1996) The proto-oncogene product c-Crk associates with insulin receptor substrate-1 and 4PS: modulation by insulin growth factor-I (IGF-I) and enhanced IGF-I signaling. J. Biol. Chem. **271:** 9287–9290
26 Baker J., Liu J.-P., Robertson E. J. and Efstratiadis A. (1993) Role of insulin-like growth factors in embryonic and postnatal growth. Cell **75:** 73–82
27 DeChiara T. M., Efstratiadis A. and Robertson E. J. (1990) A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. Nature **345:** 78–80
28 Powell-Braxton L., Hollingshead P., Warburton C., Dowd M., Pitts-Meek S., Dalton D. et al. (1993) IGF-I is required for normal embryonic growth in mice. Genes Dev. **7:** 2609–2617
29 Liu J.-P., Baker J., Perkins A. S., Robertson E. J. and Estratiadis A. (1993) Mice carrying null mutations of the genes encoding insulin-like growth factor I (*Igf-1*) and type 1 IGF receptor (*Igf1r*). Cell **75:** 59–72
30 Bondy C. A., Werner H., Roberts C. T. Jr and LeRoith D. (1990) Cellular pattern of insulin-like growth factor I (IGF-I) and type I IGF receptor gene expression in early organogenesis: comparison with IGF-II gene expression. Mol. Endocrinol. **4:** 1386–1398
31 Werner H., Woloschak M., Adamo M., Shen-Orr Z., Roberts C. T. Jr and LeRoith D. (1989) Developmental regulation of the rat insulin-like growth factor I receptor gene. Proc. Natl. Acad. Sci. USA **86:** 7451–7455
32 Baserga R. and Rubin R. (1993) Cell cycle and growth control. Crit. Rev. Eukaryot. Gene Expr. **3:** 47–61
33 Accili D., Drago J., Lee E. J., Johnson M. D., Cool M. H., Salvatore P. et al. (1996) Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. Nat. Genet. **12:** 106–109
34 Wang Z.-Q., Fung M. R., Barlow D. P. and Wagner E. F. (1994) Regulation of embryonic growth and lysosomal targeting by the imprinted Igf2/Mpr gene. Nature **372:** 464–467
35 Ludwig T., Eggenschwiler J., Fisher P., D'Ercole A. J., Davenport M. L. and Efstratiadis A. (1996) Mouse mutants lacking the type 2 IGF receptor (IGF2R) are rescued from perinatal lethality in Igf2 and Igf1r null backgrounds. Dev. Biol. **177:** 517–535
36 Roback E. W., Barakat A. J., Dev V. G., Mbikay M., Chreitien M. and Butler M. G. (1991) An infant with deletion of the distal long arm of chromosome 15 (q26.1-qter) and loss of insulin-like growth factor I receptor gene. Am. J. Med. Genet. **38:** 74–79
37 Siebler T., Lopazynski W., Terry C. L., Casella S. J., Munson P., De Leon D. et al. (1995) Insulin-like growth factor I receptor expression and function in fibroblasts from two patients with deletion of the distal long arm of chromosome 15. J. Clin. Endocrinol. Metab. **80:** 3447–3457

38 Peoples R., Milatovich A. and Francke U. (1995) Hemizygosity at the insulin-like growth factor I receptor (IGF1R) locus and growth failure in the ring chromosome 15 syndrome. Cytogenet. Cell Genet. **70:** 228–234

39 Woods K. A., Camacho-Hubner C., Savage M. O. and Clark A. J. L. (1996) Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. New Engl. J. Med. **335:** 1363–1367

40 Werner H. and LeRoith D. (1996) The role of the insulin-like growth factor system in human cancer. Adv. Cancer Res. **68:** 183–223

41 Baserga R. (1998) The IGF-I receptor in normal and abnormal growth. In: Hormones and Growth Factors in Development and Neoplasia, pp. 269–287, Dickson R. and Salomon D. S. (eds), Wiley-Liss, New York

42 Russell W. E., Van Wyk J. J. and Pledger W. J. (1984) Inhibition of the mitogenic effect of plasma by a monoclonal antibody to somatomedin C. Proc. Natl. Acad. Sci. USA **81:** 2389–2393

43 Chan J. M., Stampfer M. J., Giovannucci E., Gann P. H., Ma J., Wilkinson P. et al. (1998) Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science **279:** 563–566

44 Hankinson S. E., Willett W. C., Colditz G. A., Hunter D. J., Michaud D. S., Deroo B. et al. (1998) Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet **351:** 1393–1396

45 Baserga R., Sell C., Porcu P. and Rubini M. (1994) The role of the IGF-I receptor in the growth and transformation of mammalian cells. Cell Proliferation **27:** 63–71

46 Morrione A., DeAngelis T. and Baserga R. (1995) Failure of the bovine papillomavirus to transform mouse embryo fibroblasts with a targeted disruption of the insulin-like growth factor I receptor gene. J. Virol. **69:** 5300–5303

47 Sell C., Dumenil G., Deveaud C., Miura M., Coppola D., DeAngelis T. et al. (1994) Effect of a null mutation of the insulin-like growth factor I receptor gene on growth and transformation of mouse embryo fibroblasts. Mol. Cell. Biol. **14:** 3604–3612

48 Sell C., Rubini M., Rubin R., Liu J.-P., Efstratiadis A. and Baserga R. (1993) Simian virus 40 large tumor antigen is unable to transform mouse embryonic fibroblasts lacking type 1 insulin-like growth factor receptor. Proc. Natl. Acad. Sci. USA **90:** 11217–11221

49 Kaleko M., Rutter W. J. and Miller A. D. (1990) Overexpression of the human insulin-like growth factor I receptor promotes ligand-dependent neoplastic transformation. Mol. Cell. Biol. **10:** 464–473

50 Harrington E. A., Bennett M. R., Fanidi A. and Evan G. I. (1994) c-Myc-induced apoptosis in fibroblasts is inhibited by specific cytokines. EMBO J. **13:** 3286–3295

51 Rodriguez-Tarduchy G., Collins M. K. L., Garcia I. and Lopez-Rivas A. (1992) Insulin-like growth factor-I inhibits apoptosis in IL-3-dependent hemopoietic cells. J. Immunol. **149:** 535–540

52 Resnicoff M., Burgaud J.-L., Rotman H. L., Abraham D. and Baserga R. (1995) Correlation between apoptosis, tumorigenesis and levels of insulin-like growth factor I receptors. Cancer Res. **55:** 3739–3741

53 Sell C., Baserga R. and Rubin R. (1995) Insulin-like growth factor I (IGF-I) and the IGF-I receptor prevent etoposide-induced apoptosis. Cancer Res. **55:** 303–306

54 Holly J. M. P., Gunnell D. J. and Davey Smith G. (1999) Growth hormone, IGF-I and cancer. Less intervention to avoid cancer? More intervention to prevent cancer? J. Endocrinol. **162:** 321–330

55 Hernandez-Sanchez C., Werner H., Roberts C. T. Jr, Woo E. J., Hun D. W., Rosenthal S. M. et al. (1997) Differential regulation of IGF-I receptor gene expression by IGF-I and basic fibroblast growth factor. J. Biol. Chem. **272:** 4663–4670

56 Rubini M., Werner H., Gandini E., Roberts C. T. Jr, LeRoith D. and Baserga R. (1994) Platelet-derived growth factor increases the activity of the promoter of the IGF-I receptor gene. Exp. Cell Res. **211:** 374–379

57 Rosenthal S. M., Brown E. J., Brunetti A. and Goldfine I. D. (1991) Fibroblast growth factor inhibits insulin-like growth factor II (IGF-II) gene expression and increases IGF-I receptor abundance in BC3H-1 muscle cells. Mol. Endocrinol. **5:** 678–684

58 Werner H. and LeRoith D. (1997) The insulin-like growth factor-I receptor signaling pathways are important for tumorigenesis and inhibition of apoptosis. Crit. Rev. Oncog. **8:** 71–92

59 Baserga R., Hongo A., Rubini M., Prisco M. and Valentinis B. (1997) The IGF-I receptor in cell growth, transformation and apoptosis. Biochim. Biophys. Acta **1332:** F105–F126

60 Rosenfeld R. G. and Hintz R. L. (1980) Characterization of a specific receptor for somatomedin C (Sm-C) on cultured human lymphoblasts: evidence that Sm-C modulates homologous receptor concentration. Endocrinology **107:** 1841–1848

61 Clarke R. B., Howell A. and Anderson E. (1997) Type 1 insulin-like growth factor receptor gene expression in normal human breast tissue treated with oestrogen and progesterone. Br. J. Cancer **75:** 251–257

62 Reiss K., Ferber A., Travali S., Porcu P., Phillips P. D. and Baserga R. (1991) The protooncogene c-*myb* increases the expression of insulin-like growth factor I and insulin-like growth factor I receptor messenger RNAs by a transcriptional mechanism. Cancer Res. **51:** 5997–6000

63 Travali S., Reiss K., Ferber A., Petralia S., Mercer W. E., Calabreta B. et al. (1991) Constitutively expressed c-*myb* abrogates the requirement for insulinlike growth factor I in 3T3 fibroblasts. Mol. Cell. Biol. **11:** 731–736

64 Kim S. O., Park J. G. and Lee Y. I. (1996) Increased expression of the insulin-like growth factor I (IGF-I) receptor gene in hepatocellular carcinoma cell lines: implications of IGF-I receptor gene activation by hepatitis B virus X gene product. Cancer Res. **56:** 3831–3836

65 Kozma L. M. and Weber M. J. (1990) Constitutive phosphorylation of the receptor for insulinlike growth factor I in cells transformed by the *src* oncogene. Mol. Cell. Biol. **10:** 3626–3634

66 Peterson J. E., Jelinek T., Kaleko M., Siddle K. and Weber M. J. (1994) C phosphorylation and activation of the IGF-I receptor in *src*-transformed cells. J. Biol. Chem. **269:** 27315–27321

67 Knudson A. G. (1993) Antioncogenes and human cancer. Proc. Natl. Acad. Sci. USA **90:** 10914–10921

68 Marshall C. J. (1991) Tumor suppressor genes. Cell **64:** 313–326

69 Weinberg R. (1993) Tumor suppressor genes. Neuron **11:** 191–196

70 Werner H. (1998) Dysregulation of the type 1 IGF receptor as a paradigm in tumor progression. Mol. Cell. Endocrinol. **141:** 1–5

71 Rauscher F. J. III (1993) The WT1 Wilms tumor gene product: a developmentally regulated transcription factor in the kidney that functions as a tumor suppressor. FASEB J. **7:** 896–903

72 Werner H., Re G. G., Drummond I. A., Sukhatme V. P., Rauscher F. J. III, Sens D. A. et al. (1993) Increased expression of the insulin-like growth factor-I receptor gene, IGFIR, in Wilms' tumor is correlated with modulation of IGFIR promoter activity by the WT1 Wilms' tumor gene product. Proc. Natl. Acad. Sci. USA **90:** 5828–5832

73 Werner H., Rauscher F. J. III, Sukhatme V. P., Drummond I. A., Roberts C. T. Jr and LeRoith D. (1994) Transcriptional repression of the insulin-like growth factor I receptor (IGF-I-R) gene by the tumor suppressor WT1 involves binding to sequences both upstream and downstream of the IGF-I-R gene transcription start site. J. Biol. Chem. **269:** 12577–12582

74 Werner H., Shen-Orr Z., Rauscher F. J. III, Morris J. F., Roberts C. T. Jr and LeRoith D. (1995) Inhibition of cellular proliferation by the Wilms' tumor suppressor WT1 is associ-

ated with suppression of insulin-like growth factor I receptor gene expression. Mol. Cell. Biol. **15:** 3516–3522

75 Rauscher F. J. III, Benjamin L. E., Fredericks W. J. and Morris J. F. (1994) Novel oncogenic mutations in the WT1 Wilms' tumor suppressor gene: a t(11;22) fuses the Ewings' sarcoma gene, EWS1, to WT1 in desmoplastic small round cell tumor. Cold Spring Harbor Symp. Quant. Biol. **59:** 137–146

76 Ladanyi M. and Gerald W. (1994) Fusion of the EWS and WT1 genes in the desmoplastic small round cell tumor. Cancer Res. **54:** 2837–2840

77 Gerald W. L., Rosai J. and Ladanyi M. (1995) Characterization of the genomic breakpoint and chimeric transcripts in the EWS-WT1 gene fusion of desmoplastic small round cell tumor. Proc. Natl. Acad. Sci. USA **92:** 1028–1032

78 Karnieli E., Werner H., Rauscher F. J. III, Benjamin L. E. and LeRoith D. (1996) The IGF-I receptor gene promoter is a molecular target for the Ewings' sarcoma-Wilms' tumor 1 fusion protein. J. Biol. Chem. **271:** 19304–19309

79 Ohlsson C., Kley N., Werner H. and LeRoith D. (1998) p53 regulates IGF-I receptor expression and IGF-I induced tyrosine phosphorylation in an osteosarcoma cell line: interaction between p53 and Sp1. Endocrinology **139:** 1101–1107

80 Werner H., Karnieli E., Rauscher F. J. III and LeRoith D. (1996) Wild type and mutant p53 differentially regulate transcription of the insulin-like growth factor I receptor gene. Proc. Natl. Acad. Sci. USA **93:** 8318–8323

81 Zhang L., Kashanchi F., Zhan Q., Zhan S., Brady J. N., Fornace A. J. et al. (1996) Regulation of insulin-like growth factor II P3 promoter by p53: a potential mechanism for tumorigenesis. Cancer Res. **56:** 1367–1373

82 Buckbinder L., Talbott R., Velasco-Miguel S., Takenaka I., Faha B., Seizinger B. R. et al. (1995) Induction of the growth inhibitor IGF-binding protein 3 by p53. Nature **377:** 1367–1373

..