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The Role of the IGF System in Cancer Growth and Metastasis: Overview and Recent Insights

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IGF-I receptor (IGF-IR) signaling and functions are mediated through the activities of a complex molecular network of positive (*e.g.*, type I IGF) and negative (*e.g.*, the type II IGF receptor, IGF-IIR) effectors. Under normal physiological conditions, the balance between the expression and activities of these molecules is tightly controlled. Changes in this delicate balance (*e.g.*, overexpression of one effector) may trigger a cascade of molecular events that can ultimately lead to malignancy. In recent years, evidence has been mounting that the IGF axis may be involved in human cancer progression and can be targeted for therapeutic intervention. Here we review old and more recent evidence on the role the IGF system in malignancy and highlight experimental and clinical studies that provide novel insights into the complex mechanisms that contribute to its oncogenic potential. Controversies arising from conflicting evidence on the relevance of IGF-IR and its ligands to human cancer are discussed. Our review highlights the importance of viewing the IGF axis as a complex multifactorial system and shows that changes in the expression levels of any one component of the axis, in a given malignancy, should be interpreted with caution and viewed in a wider context that takes into account the expression levels, state of activation, accessibility, and functionality of other interacting components. Because IGF targeting for anticancer therapy is rapidly becoming a clinical reality, an understanding of this complexity is timely because it is likely to have an impact on the design, mode of action, and clinical outcomes of newly developed drugs. (*Endocrine Reviews* 28: 20–47, 2007)

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Abbreviations: AP-1, Adaptor protein; CDK, cyclin-dependent kinase; DMBA, dimethylbenz[α]anthracene; ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, EGF receptor; ER, estrogen receptor; HIF-1a, hypoxia-inducible factor 1a; IGFBP, IGF binding protein; IGF-IR, IGF-I receptor; IGF-IIR, IGF-II receptor; IR, insulin receptor; IRS, IR substrate; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; LOI, loss of imprinting; MDM2, mouse double minute 2; MDR, multiple drug resistance; MEF, mouse embryo fibroblast; MMP, matrix metalloproteinase; PC, proprotein convertase; PI-3K, phosphatidylinositol 3-kinase; PIN, prostate intraepithelial neoplasia; PSA, prostate-specific antigen; PTEN, phosphatase and tensin homologous on chromosome 10; PTP-1B, protein tyrosine phosphatase 1B; RACK1, receptor for activated C kinases; sCLU, secretory clusterin; SH, Src homology; SHP-2, tyrosine phosphatase Src-homology 2-containing phosphotyrosine phosphatase-2; SHPS-1, Src homology 2 domain containing protein tyrosine phosphatase substrate-1; STAT, signal transducers and activators of transcription; TRAMP, transgenic adenocarcinoma of mouse prostate; uPA, urokinase plasminogen activator; uPAR, uPA receptor; VEGF, vascular endothelial growth factor; WT, Wilms' tumor.

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I. Introduction

CELL CYCLE PROGRESSION and cellular proliferation are regulated by a complex network of intrinsic factors and external stimuli. The very early events that rescue cells from cell cycle arrest are mediated through signals transmitted by a group of peptides, collectively known as growth factors. These molecules can be classified into two subgroups, namely the "competence" factors, such as the platelet-derived growth factor that enable cells to enter into the G_1 phase, and the "progression" factors, such as the IGFs that are required for progression from G_1 into the S phase and, ultimately, cell division (1, 2).

Overexpression of growth factors and/or their receptors is a common event in malignancy and provides the underlying mechanisms for one of the hallmarks of cancer, namely uncontrolled proliferation (3). Growth autonomy can occur when both growth factors and their receptors are overexpressed in the same cell, or when cells express constitutively activated receptors and lose their ligand dependency for growth. Overexpression of growth factor receptors in the absence of up-regulated ligand expression may, on the other hand, heighten tumor cell sensitivity and response to the appropriate ligand(s) and, thereby, regulate their growth in different microenvironments (4, 5).

II. The IGF System

The IGF system consists of two ligands, IGF-I and IGF-II; three cell-membrane receptors, IGF-I receptor (IGF-IR), insulin receptor (IR), and IGF-II receptor (IGF-IIR); and six high-affinity IGF binding proteins, IGFBP-1 through -6.

IGF-IR is a type 2 tyrosine kinase receptor that shares a 60% homology at the amino acid sequence level with the IR. The human IGF-IR cDNA contains an open reading frame of 4101 nucleotides that encode a protein of 1367 amino acids. The IGF-IR is synthesized as a single chain pre-propeptide with a 30-amino acid signal peptide that is cleaved after translation. The propeptide is then glycosylated, dimerized, and transported to the Golgi where it is processed at a furin cleavage site to yield α- and β-subunits (6). These subunits, through disulfide bonds, form a tetramer (β–α–α–β) that is transported to the plasma membrane (7). It has been shown that N-linked glycosylation of IGF-IR is required for its translocation to the cell surface (8, 9) and that this, in turn, may be rate-limited by the availability of dolichyl phosphate in the endoplasmic reticulum (8). For example, in MDA231 breast cancer cells, low cell-surface IGF-IR expression levels were linked to a low rate of *de novo* dolichyl phosphate synthesis and could be increased when additional dolichyl phosphate was provided to the cells (10). It has also been shown that the depletion of mevalonic acid in melanoma SK-MEL-2 cells reduced dolichyl phosphate biosynthesis and that this, in turn, reduced N-linked glycosylation and IGF-IR cell surface expression levels, causing growth arrest (9).

The mature cell membrane-bound IGF-IR consists of two 130- to 135-kDa α-chains and two 90- to 95-kDa β-chains, with several α–α and α–β disulfide bridges (11). The α-subunits, which are entirely extracellular, form the ligand-binding domain (12) that binds one ligand molecule. IGF-I and IGF-II share a 62% homology in amino acid sequence, and there is a 40% homology between the IGFs and proinsulin (13). Several lines of evidence suggest that the binding sites for IGF-I and IGF-II on the receptor may be distinct (14, 15). Recent receptor binding affinity assays, using a recombinant, high-affinity form of the IGF-IR, and studies based on the surface plasmon resonance technology revealed a difference of 4-fold in the affinities of IGF-I (4.45 nm) and IGF-II (17.8 nm) for the recombinant human IGF-IR, and this agreed closely with cell-based assays (16). However, ligand binding affinities may vary with cell type and specific experimental conditions. For instance, in cultured adult bovine chromaffin cells, IGF-IR bound IGF-I and IGF-II with identical affinities (Kd ~ 1 nM) (17). It is of interest that, in human fetus and adult sera, IGF-II concentrations are 5- and 3.5-fold higher, respectively, than IGF-I levels (18). Interestingly, IGF-II can also bind to the insulin receptor subtype A (IR-A), with an affinity similar to that of insulin. IR-A is more mitogenic than subtype B (19, 20), the latter having a more metabolic function. IR-A is expressed in certain tumors, such as mammary cancers, and the IGF-II/IR-A interaction may play a role in cancer growth (for a more detailed discussion, see *Section X.F*). In addition, hybrid heterodimeric receptors consisting of insulin and IGF-I receptor subunits may form and could play a role in receptor signaling in normal and abnormal tissues. In a striking example, one study that examined eight human breast cancer cell lines and 39 human breast cancer specimens found that the hybrid receptor content exceeded the IGF-IR content in over 75% of the specimens. In the human breast cancer cell line MDA-MB157, these hybrid receptors were autophosphorylated in response to IGF-I, and this response exceeded IGF-IR autophosphorylation and led to increased proliferation, suggesting that the hybrid receptors were the major mediators of IGF signaling in these cells (21).

IGF-IR signaling is transmitted by an intracellular domain consisting of a binding site for phosphorylated substrates at tyrosine residue 950; a tyrosine kinase domain that contains an ATP-binding site at lysine 1003 and three critical tyrosines at positions 1131, 1135 and 1136; and a C-terminal domain containing several tyrosines and serines, such as tyrosines 1250, 1251, and 1316 and serines 1280–1283 that are phosphorylated and play a role in IGF-IR signaling. The contributions that these C-terminal domain amino acids make to IGF-IR function in normal and malignant cells are not fully understood and are the subject of active investigation in several laboratories, including our own (22–24). IGF-II and, with a much lower affinity, IGF-I can also bind to a second receptor, namely, IGF-IIR, which is identical to the cation-independent mannose-6-phosphate receptor and serves as a scavenger receptor (25).

The physiological activities of the IGFs are modulated by their association with the IGFBPs. These comprise a struc-

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turally related superfamily of secreted proteins consisting of BP1–6 that bind the IGFs with different affinities (*e.g.*, IGFBP-6 binds IGF-II with a 20- to 100-fold higher affinity than IGF-I) and several related proteins that bind ligand with lower affinities (26, 27). The IGFBPs regulate the biological accessibility and activity of the IGFs in several ways, which can be summarized as follows. They transport IGFs from the circulation to peripheral tissues (*e.g.*, IGFBP-1, -2, and -4), maintain a reservoir of IGFs in the circulation (a function principally of IGFBP-3), potentiate or inhibit IGF action, and mediate IGF-independent biological effects (for review, see Ref. 28).

III. Signal Transduction by the IGF-IR

Upon ligand binding, the intrinsic tyrosine kinase of the IGF-IR is activated, and this results in autophosphorylation of tyrosines on the intracellular portion of the β-subunit, including tyrosine residues in the juxtamembrane and C-terminal domains. Once phosphorylated, tyrosine 950 in the juxtamembrane domain can serve as a docking site for several receptor substrates, including the insulin receptor substrates (IRS) 1–4 and Shc (for reviews, see Refs. 29 and 30). These substrates can initiate phosphorylation cascades that serve to transmit the IGF-IR signal. Phosphorylated IRS-1 can activate the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI-3K), leading to activation of several downstream substrates, including the p70 S6 kinase and protein kinase B (Akt) (31). Akt phosphorylation, in turn, enhances protein synthesis through mTOR activation and triggers the anti-apoptotic effects of IGF-IR through phosphorylation and inactivation of Bad (32). IRS-1 has also been implicated in signaling by other receptor systems, including those from other growth factors (33), cytokines (34), and integrins (35), and may therefore be involved in crosstalk between different signaling systems. In parallel to PI-3K-driven signaling, recruitment of Grb2/SOS by phosphorylated IRS-1 or Shc leads to recruitment of Ras and activation of the Raf-1/MEK/ERK pathway and downstream nuclear factors, resulting in the induction of cellular proliferation (36, 37) (summarized in Fig. 1).

In some cell types, the IGF-IR can also directly phosphorylate the Janus kinases (JAK)-1 and -2 that are involved in cytokine-mediated signaling, and JAK proteins may, in turn, phosphorylate IRS-1 (38). Phosphorylation of JAK proteins can lead to phosphorylation/activation of signal transducers and activators of transcription (STAT) proteins. STAT-3 (39) and STAT-3 activation, in particular, may be essential for the transforming activity of IGF-IR (40). Other effectors downstream of IGF-IR activation include Src (41), the pp125 focal adhesion kinase that is directly phosphorylated by IGF-IR (42), and the proto-oncogenes c-Crk II and CrkL (43, 44) that can link IGF-IR to integrin-mediated signaling and the cytoskeleton through p130 Cas and paxillin, thereby regulating cell shape and motility (42, 45). Because intracellular calcium levels increase in response to IGF-I, phospholipase C-γ is also thought to be indirectly involved through its products inositol 1,4,5-triphosphate and 1,2 diacylglycerol (24). This was also demonstrated by the fact that IGF could not rescue Plcg1 null ($Plcg1^{-/-}$) mouse embryonic fibroblasts (MEFs) from anoikis-induced apoptosis, but the response could be restored by reexpression of phospholipase C-γ in these cells (46). The relative importance of these pathways and the effectors engaged in signal transduction by IGF-IR are probably cell context-dependent and remain to be fully elucidated.

IV. IGF-IR and the Cell Cycle

The IGF-IR can regulate cell-cycle progression through control of several cycle checkpoints. It can facilitate G_0-G_1 transition through activation of p70 S6K, leading to phosphorylation of the S6 ribosomal protein and an increased ribosomal pool necessary for entry into the cycle (47). It can promote G_1-S transition by increasing cyclin D1 and CDK4 gene expression, leading to retinoblastoma protein phosphorylation, release of the transcription factor E2F, and synthesis of cyclin E (48, 49). The IGF-IR-induced increase in cyclin D1 synthesis can be mediated through several alternative mechanisms. It may be transcriptionally regulated through the ERK pathway (50), or it may be mediated through increased mRNA stability in a PI-3K/Akt-dependent manner (51). PI-3K/Akt signaling can also increase cyclin D1 levels through enhanced mTOR-mediated protein translation and inhibition of glycogen synthase kinase-3-mediated cyclin D1 phosphorylation (51–53). In addition, IGF-IR can also down-regulate the transcription of the cyclin-dependent kinase (CDK) inhibitor (CDKI) $p27^{KIP1}$ or alter its processing and nuclear localization (54) through a PI-3K/Akt and phosphatase and tensin homologous on chromosome 10 (PTEN)-dependent mechanism (see diagram in Fig. 2). The IGF-IR may also exert a regulatory role at the G_2-M transition, possibly by increasing cyclins A and B and cdc2 synthesis (55). Taken together, the data suggest that the IGF-IR/IGF axis can positively regulate cell-cycle progression at several phases, but its major direct effect is probably exerted at the G_1-S interface, and this is mediated through the PI-3K/Akt and/or ERK pathways.

V. Post-Ligand Binding Receptor Processing and the Regulation of IGF-IR Activity

A. Role of phosphatases

IGF-I stimulates the phosphorylation of the membrane scaffolding protein Src homology 2 domain-containing protein tyrosine phosphatase substrate-1 (SHPS-1). Phosphorylated SHPS-1 subsequently recruits the Src homology 2 domain tyrosine phosphatase (SHP-2) to the phosphorylated IGF-IR (56). SHP-2 can have both positive and negative effects on IGF-IR signaling. The dephosphorylation of IGF-IR by SHP-2 attenuates PI-3K-mediated IGF-IR signaling, as has also been shown for the IR (57). In contrast, data suggest that MAPK signaling may actually be enhanced by SHPS-1 phosphorylation and SHP-2 recruitment (58) through Shc/Grb2 (59).

One of the molecules that can associate with SHP-2, Src, and the p85 subunit of PI-3K is the receptor for activated C

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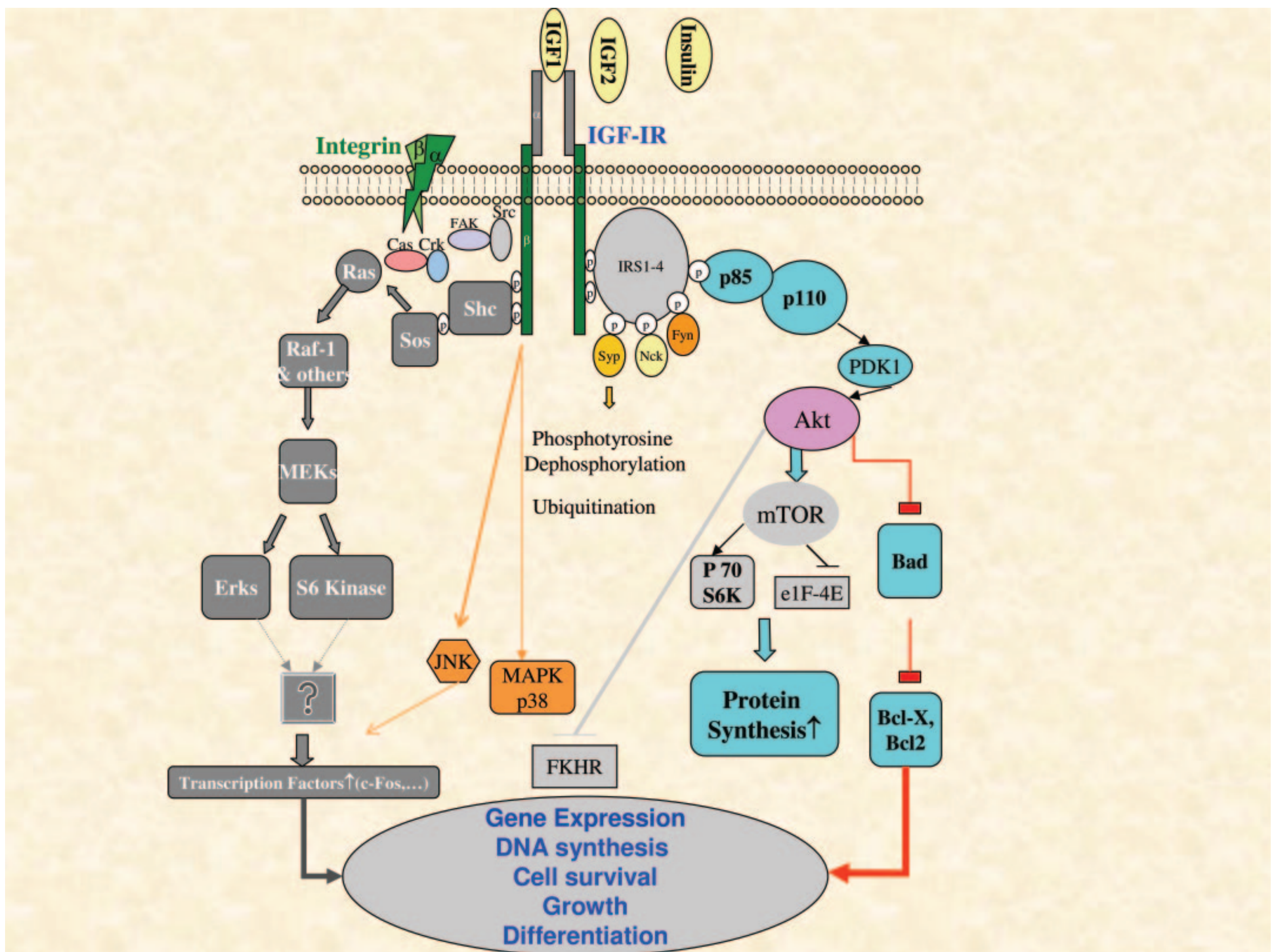

FIG. 1. Signal transduction pathways of the ligand-activated IGF-IR. A schematic representation of the major signaling pathways than can be activated by the autophosphorylated IGF-IR. The cell context, ligand concentrations, and crosstalk with other signaling systems affect the type and strength of the signal and the biological outcome.

kinases (RACK1), a homolog of the β-subunit of heterotrimeric G proteins. Recently, it has been shown that RACK1 also interacts with IGF-IR (60, 61) in a tyrosine kinase activity and receptor autophosphorylation-independent manner and that this requires an intact serine 1248 in the C terminus of the receptor. Interestingly, overexpression of RACK1 has a negative effect on the activation of the PI-3K pathway, but a positive effect on the activation of the MAPK and c-Jun N-terminal kinase (JNK) pathways (61), as was also shown for IGF-IR-associated SHP-2.

Protein tyrosine phosphatase 1B (PTP-1B), a 50-kDa non-transmembrane tyrosine phosphatase, is localized predominantly in the endoplasmic reticulum with its phosphatase domain oriented toward the cytoplasm (62). PTP-1B can dephosphorylate both IGF-IR (63) and IRS-1 (64), and, indeed, in MEFs derived from PTP-1B-deficient mice, IGF-I-induced IGF-IR autophosphorylation and kinase activity were higher than in controls (63). IGF-IR may, in turn, inhibit PTP-1B activity, perhaps through a negative feedback loop. Such a mechanism has been described for the IR. The IR can inactivate PTP-1B by inducing a burst of intracellular hydrogen peroxide. The IR may also suppress serine phosphorylation and thereby inactivate PTP-1B (65).

B. Role of $\alpha v\beta 3$

The integrin $\alpha v\beta 3$ plays an important role in IGF-IR signaling and its biological functions (66). Maile *et al.* (67) showed that $\alpha v\beta 3$ signaling changes the subcellular localization of SHP-2 in a way that decreases its access to phosphorylated IGF-IR, thereby prolonging IGF-IR signaling. Ling *et al.* (68) identified β3 as the subunit that recruits SHP-2 and prevents its association with IGF-IR. In smooth muscle cells, echistatin, a distintegrin that blocks $\alpha v\beta 3$ ligand binding, reduced receptor phosphorylation, cellular migration, and DNA synthesis in response to IGF-I (67). In some cells, IGF-I can induce a potent JNK response (69). JNK, in turn, can mediate serine phosphorylation of IRS-1 and thereby attenuate IGF-IR signaling (70).

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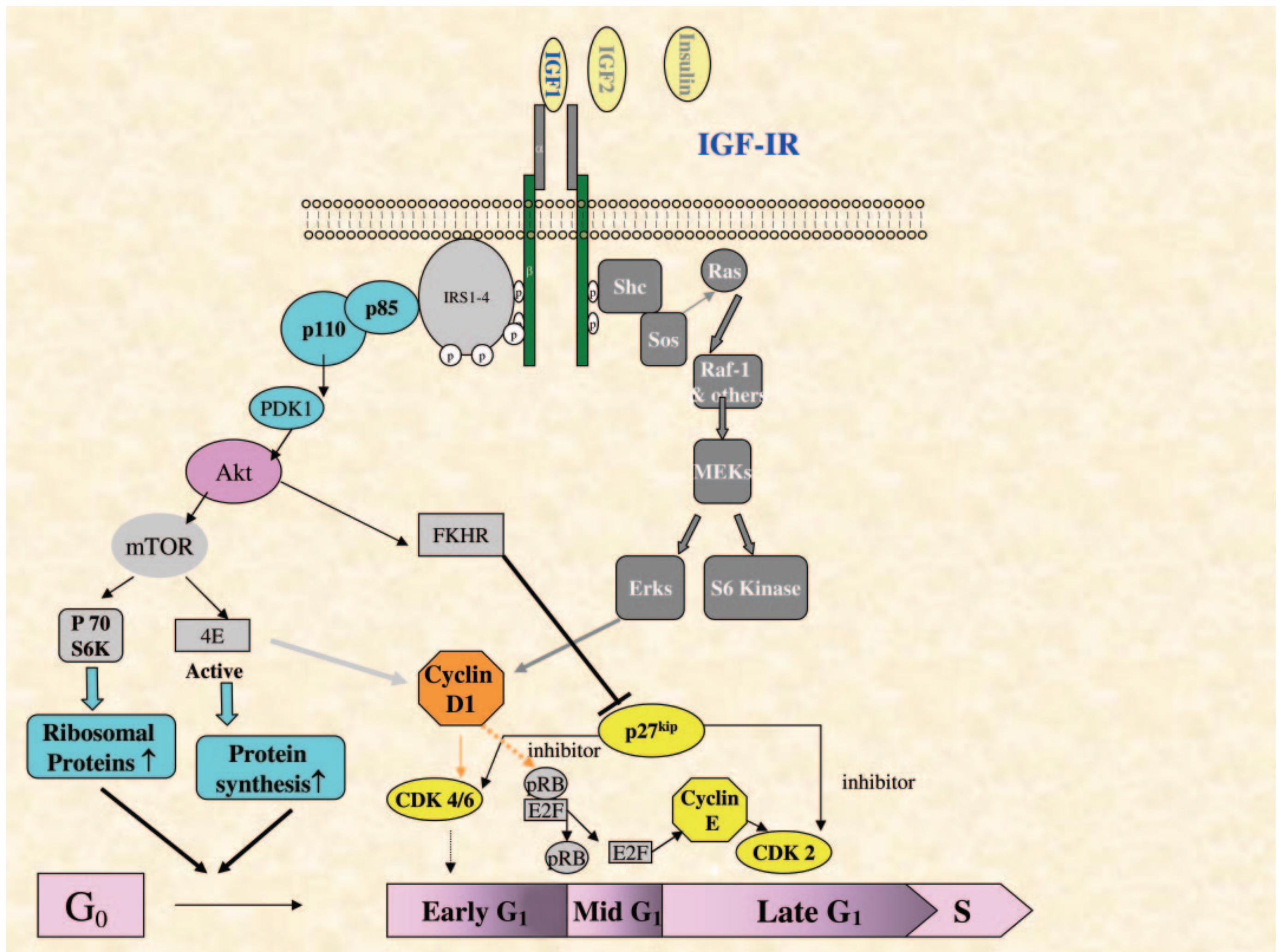

FIG. 2. The role of the IGF-IR in cell-cycle progression. The IGF-IR can affect cell-cycle progression through control of several cycle checkpoints, but its major direct regulatory effect is probably exerted at the G_1-S interface, via the PI-3K/Akt and/or ERK pathways.

C. Intracellular processing of the IGF-IR/IGF complex

The signaling of receptor tyrosine kinases is tightly linked to, and regulated by, receptor endocytosis and trafficking. Emerging evidence suggests that IGF-IR internalization and signaling are regulated by mechanisms both akin to, and distinct from, those of other receptor kinases. For example, similar to other tyrosine kinase receptors such as the epidermal growth factor (EGF) receptor (EGFR), the mitogenic responses of IGF-IR were shown to be regulated by adaptor protein-2 (AP-2)-dependent endocytosis (71, 72), and two potential "linkers" of IGF-IR to α-adaptin of the AP-2 complex, EHD1 and SNAP29, have recently been identified (71). In addition, ligand-induced IGF-IR recruitment into lipid raft caveolae has also been documented (73–75).

Once in the endosomal compartment, ligand dissociation and proteolysis can also exert a regulatory role on signaling (76, 77). Although the proteolytic enzymes that degrade internalized IGF-I or IGF-II have not been fully characterized, recent work by the Brodt group (77) implicated an E-64-sensitive cysteine proteinase in this process, and this was confirmed in a recent report that documented limited proteolytic cleavage of IGF-I at its C-terminal D domain by cathepsin B (78). In addition, recent studies by the Brodt group (79) suggest that, in intact tumor cells, cathepsin L also participates in this process.

D. Receptor ubiquitination

The ubiquitin system also plays a role in the processing of internalized IGF-IR. Recent studies with cultured cells have implicated two E3 ligases, namely, Nedd4 and mouse double minute 2 (MDM2), in IGF-IR ubiquitination and processing. Nedd4 binds IGF-IR through the adaptor protein Grb10. Studies in MEFs overexpressing Nedd4 and IGF-IR have shown that the Grb10/Nedd4/IGF-IR complex drives ligand-dependent ubiquitination of the internalized IGF-IR. In these cells, ubiquitination was shown to occur at the plasma membrane, probably before the formation of endocytic vesicles (80). Interestingly, mice with a disruption in the maternal Grb10 allele had embryo and placental overgrowth and were 30% overweight compared with wild-type controls, identifying Grb10 as a potent growth inhibitor (81).

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Another member of the E3 ligase family involved in IGF-IR degradation is the MDM2 proto-oncoprotein, a RING finger ubiquitin ligase that is transcriptionally regulated by IGF-I (82). The MDM2 protein binds to the IGF-IR β-subunit through the adaptor β-arrestin (83), thereby recruiting ubiquitin to the IGF-IR and initiating its degradation (84).

E. Role of IGFBP-3

IGF-I up-regulates IGFBP-3 at the transcriptional and/or posttranscriptional levels (85). The cell context determines the final effect of IGFBP-3 on the cell and whether it acts as an inhibitor or potentiator of IGF functions. For instance, in both normal and transformed mammary epithelial cells, IGFBP-3 potentiates the mitogenic effects of IGF-I (86). However, in other cancer cells such as the human breast carcinoma cell line MCF-7, IGFBP-3 activates a phosphotyrosine phosphatase that dephosphorylates IGF-IR, thereby disrupting signaling. This inhibitory effect is independent of IGF-I binding (87). In several non-small cell lung cancer cell lines, IGFBP-3 has been shown to act as a potent inhibitor of IGF-IR signaling by interfering with both the PI-3K/Akt and MAPK signaling pathways, causing growth arrest and inducing apoptosis (88).

VI. The Evidence for the Involvement of IGF in Human Malignancy

The IGF-IR and its ligands play a critical role during embryogenesis and development in human and animal species. During postnatal development and longitudinal growth, many GH functions are mediated via IGF-I. During puberty, elevated sex steroid levels (particularly estrogens) stimulate GH production, leading to activation of the GH/IGF-I axis (89). Although serum IGF-I levels decline progressively after puberty, significant levels of circulating IGF-II are detectable throughout adult life. IGF-IR mRNA levels also decline after puberty, but remain high in some tissues such as the brain and kidney. However, increased expression of IGF-I, IGF-II, IGF-IR, or combinations thereof have been documented in various malignancies including glioblastomas, neuroblastomas, meningiomas (90), medulloblastomas (91), carcinomas of the breast (reviewed in Refs. 92 and 93), malignancies of the gastrointestinal tract, such as colorectal and pancreatic carcinomas, and ovarian cancer. Summarized in Table 1 are different studies that examined IGF-I and IGF-II levels in clinical specimens obtained from different tumor types. These data show that whereas a correlation between IGF-I/IGF-II expression levels and tumor progression could be consistently documented in some malignancies (*e.g.*, colorectal, hepatocellular, and pancreatic carcinomas), no consistent correlation was seen in others (*e.g.*, breast cancer). Moreover, in some cases, conflicting results were obtained in different studies that analyzed the same types of cancers (*e.g.*, gliomas). These discrepancies may have been due to differences in the methods used for the analyses or to other variables such as tissue preservation methods and sample size (see Table 1 and references therein). Taken as a whole, however, these studies suggest that the IGFs can play a paracrine and/or autocrine role in promoting tumor growth *in situ* during tumor progression but that these roles may vary depending on the tissue of origin. These studies also highlight the fact that studies based on direct analysis of tissue specimens should be interpreted with caution.

Evidence from recent epidemiological studies is also consistent with the conclusion that IGFs play a role in tumor development. These studies have shown that high concentrations of serum IGFs (when the highest quartile is compared with the lowest, within the normal range) and/or lower levels of IGFBPs are associated with increased risk for several cancers, including premenopausal breast carcinoma (94–96), prostate carcinoma (97, 98), lung cancer (99), colorectal carcinoma (100–102), endometrial cancer (103), and bladder cancer (104), supporting a possible paracrine role for IGFs in tumor development. A very recent report identified the loss of imprinting (LOI) of the IGF-II gene, frequently observed in the colonic mucosa of colorectal carcinoma patients, as an individual risk factor for developing colorectal carcinoma. Using a DNA-based blood test, the same genetic alteration was also found in peripheral lymphocytes of the patients (105). In addition, an increased incidence of precancerous colonic adenoma (large or tubulovillous/villous) (106) and cervical squamous intraepithelial lesions (107) has been linked to higher levels of circulating IGF-I, suggesting that the IGF system may play a role in the early stages of transformation and carcinogenesis.

Although *in vitro* studies and animal models provide compelling evidence in support of the functional importance of the IGF-IR in cancer cell growth (see *Section VIII*), the clinical and prognostic significance of IGF-IR expression levels to human malignancies remains unclear. This is particularly true for breast cancer where conflicting data exist. For example, when IGF-IR expression was recently evaluated in 210 formalin-fixed, paraffin-embedded primary breast cancers using immunohistochemistry, IGF-IR overexpression was observed in 43.8% of the tumors, but it had no correlation with prognosis, tumor size, nodal status, histological grade, hormone receptor status, or human EGF2 status (108). In contrast, however, other studies using breast cancer specimens identified IGF-IR as a potential prognostic factor in this disease. For example, in a study of 35 cases of benign breast disease, 43% of the lesions were IGF-IR-positive, suggesting that the increase in IGF-IR could be a marker of progression (109). A study of a larger cohort that included 126 breast cancer samples revealed that 39% were IGF-IR-positive, a significant correlation between IGF-IR expression and estrogen receptor (ER) status was noted, and the expression of IGF-IR did correlate with disease-free survival (110). This study suggested that patients with ER-negative but IGF-IR-positive tumors have a worse prognosis than patients with ER- and IGF-IR-negative tumors. Another study of 150 cases of primary breast carcinoma revealed that 47% of the tumors had increased levels of IGF-IR, and this was correlated with lower nuclear grade, positive ER and/or progesterone status (111).

In prostate cancer, the evidence for the involvement of IGF-IR in disease progression is also controversial. For example, when expression of IGF-I, IGF-II, and their receptors was examined in 23 paired benign and neoplastic prostate specimens, no correlation was found between their expres-

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TABLE 1. IGF-I and IGF-II expression levels in human cancer specimens

Tumor type	Method of detection	Summary of the findings	Ref.
Glioma	Slot blot and Northern blot analysis	IGF-I mRNA was increased 1.1- to 4.0-fold in glioma and anaplastic astrocytoma specimens as compared to normal brain tissue. IGF-II expression was increased 5- to 50- fold in 4 of 4 gliomas.	309
Glioma	Immunohistochemistry	IGF-I immunoreactivity was found in 6 of 9 gliomas.	310
Astrocytoma	*In situ* hybridization and immunohistochemistry	IGF-I and IGF-II were expressed in 10 of 10 human astrocytoma specimens.	311
Meningioma		IGF-II was expressed in 3 meningiomas.	
Astrocytoma	Immunohistochemistry	IGF-I immunoreactivity in 39 astrocytomas showed positive correlation with histopathological grade.	312
Astrocytoma	Northern blot	No expression of IGF-II was found in 5 astrocytomas and 2 glioblastomas.	313
Glioblastoma			
Glioma	Northern blot	No expression of IGF-II was found in 9 of 9 gliomas.	314
Meningioma		IGF-II mRNA was found in 4 of 4 meningiomas.	
Meningioma	Northern blot	IGF-II was expressed in 2 of 2 meningiomas.	315
Meningioma	Northern blot	IGF-II and IGFBP-2 expression was found in all meningiomas. High IGF-II/IGFBP-2 ratio was associated with poor prognosis and death.	310
Meningioma	cDNA microarray	IGF-II expression was increased in meningiomas.	90
Meduloblastoma	*In situ* hybridization	IGF-I was significantly increased in 14 of 14 tumor specimens whereas IGF-II showed variable but significant expression in 9 of 14 samples. No relationship could be established between clinical parameters and IGF-II mRNA expression levels.	316
Colon cancer	Northern blot	IGF-I mRNA was elevated 3- to 5-fold in 4 of 20 colon cancer specimens. IGF-II mRNA was increased 10- to 50-fold in 8 of 20 colon cancer specimens.	317
Colon cancer	Northern blot	IGF-II mRNA was increased 2- to 800-fold in 7 of 21 colon cancer specimens.	318
Colon cancer	Immunohistochemistry	IGF-II mRNA was detected in 68 of 92 colon cancer specimens. A positive correlation was found between IGF-II expression and tumor grade.	319
Colon cancer	RNase protection assay	IGF-II mRNA was increased 40-fold in 6 of 6 colon cancer specimens. IGF-II protein levels were increased 2-fold.	320
Colon cancer	Oligonucleotide arrays and real time PCR	IGF-II expression was significantly increased in 139 primary tumors as compared to normal mucosae. However, they were significantly lower in liver metastases than in colorectal tumors or normal mucosae.	321
Gastric cancer	*In situ* hybridization	IGF-I mRNA expression was detected in 3 of 3 surgical specimens of gastric cancer.	322
Gastrinomas	RT-PCR	IGF-I expression was increased over 254-fold in 89% of the samples. Increased IGF-I levels correlated with increased tumor growth, aggressiveness, and liver metastasis.	323
Pancreatic cancer	*In situ* hybridization	IGF-I and IGF-II levels were increased 32-fold in 12 of 12 pancreatic cancer specimens.	324
Hepatoma	Northern blot	Low IGF-I expression was found in 7 of 7 hepatoma specimens. IGF-II expression was found in 4 of 7 specimens.	325
Liver cancer	Northern blot	IGF-II mRNA was increased 40- to 100-fold in 9 of 40 liver cancer surgical specimens.	326
Hepatocellular carcinoma	*In situ* hybridization and immunohistochemistry	IGF-II mRNA was increased in hepatocellular carcinoma specimens.	327
Hepatocellular carcinoma	Immunohistochemistry	IGF-II expression was detected in 9 of 15 hepatitis B-positive patients and 10 of 39 hepatitis B-negative patients.	328
Hepatocellular carcinoma	DNA microarray	Increased IGF-II expression was correlated with advanced tumor stage and poor prognosis.	329
Lung cancer	RIA	IGF-I concentration in cancerous tissue extracts was increased 3-fold as compared to normal lung tissue.	330
Tyroid cancer	RIA	IGF-I concentrations in cancerous tyroid extracts, was increased as compared to normal tyroid tissue.	331
Breast cancer	*In situ* hybridization	IGF-I mRNA was detected in 12 of 20 breast cancer surgical specimens but was limited to the stromal element.	332
Breast cancer	*In situ* hybridization	IGF-I and IGF-II mRNA were detected only in stromal cells and were not detected in either the normal or malignant epithelial cells. In 1 of 10 specimens IGF-II expression was detected in malignant epithelial cells.	333
Breast cancer	*In situ* hybridization	IGF-II mRNA was detected in 7 of 11 breast cancer surgical specimens but was limited to the stromal element.	334

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TABLE 1. *Continued*

Tumor type	Method of detection	Summary of the findings	Ref.
Breast cancer	Real time PCR	IGF-I mRNA level was lower in the cancerous tissue than non-neoplastic breast tissue in 31 specimens.	286
Ovarian cancer	RNase protection assay	IGF-I mRNA was detected in 7 of 7 ovarian cancer surgical specimens.	335
Ovarian cancer	Real time PCR	IGF-II expression increased 300-fold in ovarian cancer as compared to normal tissue. High IGF-II gene expression was associated with high grade and advanced disease stage and was an independent predictor of poor survival in patients with epithelial ovarian cancer.	125
Endometrial cancer	Immunohistochemistry	IGF-I immunoreactivity was increased 7- to 20-fold and IGFBP-1 was decreased 3-fold in endometrial specimens.	336
Prostate cancer	*In situ* hybridization	IGF-II expression was increased by 30% in prostate adenocarcinoma as compared to benign epithelial cells.	113
Prostate cancer	Immunohistochemistry	IGF-I and IGF-II expressions were higher in high-grade as compared to low-grade tumor areas in 56 prostate cancer specimens.	337
Seminoma	Microarray and real time PCR	IGF-I expression did not differ significantly between normal testicular tissue and seminoma. IGF-II was down-regulated and did not seem to be involved in growth regulation of seminomas.	338
Osteosarcoma	PCR	IGF-I and IGF-II expression was detected in all surgical osteosarcoma specimens.	339
Gallbladder cancer	Immunohistochemistry	IGF-I and IGF-II immunoreactivity was detected in 55 of 57 primary tumors and in 17 of 18 metastases. No association was found with tumor stage, grade, or prognosis.	121
Renal cell carcinoma	Tissue microarray	IGF-I was detected in 110 of 134 clear cell, 8 of 22 chromophobes, and 3 of 20 renal cell carcinomas. All tumors lacked IGF-II. Expression of IGF-I was not related to tumor stage, grade, or prognosis.	340

sion and tumor grade, stage, perineural invasion, or extraprostatic involvement (112). In another study that analyzed benign epithelium, high-grade prostate intraepithelial neoplasia (PIN), and adenocarcinomas, it was found that IGF-IR mRNA was decreased by 42% in PIN and by 35% in cancer cells compared with benign tissue, suggesting that IGF-IR expression levels were inversely correlated with progression (113). However, these findings were challenged based on another study that demonstrated a significant up-regulation of IGF-IR mRNA and protein expression in primary prostate cancers and bone metastases, compared with benign prostatic epithelium (114).

A role for the IGF system in cancer metastasis has recently been documented in several human cancers. For example, Barozzi *et al.* (115) recently studied a panel of markers, including TGF-α, EGFR, matrix metalloproteinase (MMP)-2, IGF-II, vascular endothelial growth factor (VEGF), and microvessel density in human colorectal cancer specimens as potential predictors of colorectal carcinoma metastasis, and they found that only the overexpression of TGF-α, MMP-2, and IGF-II was predictive of liver metastasis. This is consistent with another study by Hakam *et al.* (116) that showed a stepwise increase in the expression of IGF-IR during progression from colonic adenomas toward primary colorectal adenocarcinomas and metastases. Although moderate to strong cytoplasmic immunostaining was observed in over 90% of the adenocarcinomas and metastases, only a faint cytoplasmic stain was seen in the majority of adenomas. Strong IGF-IR positivity correlated with the stage of the disease (116). Although these data suggest that IGF-IR expression increases during the progression of this disease, it is important to note that a recent study based on tissue microarray analysis, performed on 86 colorectal carcinoma metastases, revealed that IGF-IR and p53 expression levels were not significantly associated with long-term survival (117). Xie *et al.* (118) compared IGF-IR expression in human synovial sarcomas with different levels of lung metastases and found that overexpression of IGF-IR was significantly linked to gain of a metastatic phenotype in this malignancy. All-Ericsson *et al.* (119) found a significant correlation between high IGF-IR expression levels and the risk of liver metastasis and death in uveal melanoma patients. Similar findings were reported in studies of gastric cancer where it was found that IGF-IR overexpression in the primary tumor correlated with increased lymph node metastasis (120). Moreover, an analysis of gallbladder carcinomas revealed IGF-IR expression in 52 of 55 primary tumors and in all 17 corresponding metastases (121).

The link between IGF and metastasis may be the basis for the identification of IGF and IGF-IR as predictors of poor outcome in some other types of cancer. For example, a Kaplan-Meier analysis suggested that patients with IGF-IR-positive renal clear cell carcinoma (CC-RCC) had a significantly decreased cancer-related survival rate relative to those with IGF-IR-negative CC-RCC (122, 123). For adrenocortical tumors, a high risk of postsurgical recurrence is generally predicted in cases with regional invasion or distant metastases. Results of a recent prospective study involving 114 patients identified IGF-II overexpression as a reliable prognostic marker in this disease, suggesting that it can be used to identify patients with a high risk of recurrence (124). In a multivariate analysis with 109 epithelial ovarian cancer patients, the relative IGF-II expression level was found to be an

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independent predictor of poor survival that was associated with high-grade, advanced-stage disease (125).

More recent findings have shown that IGF-I/IGFBP ratios may actually be better predictors of disease progression than either one of these factors independently. For example, in 51 patients that underwent radical cystectomy for bladder cancer, plasma IGF-I and IGFBP-3 levels, when measured separately, were not significantly different from those in healthy subjects. However, lower preoperative plasma levels of IGFBP-3 in these patients, when adjusted for serum IGF-I levels, were found to be associated with a higher incidence of lymph node metastases and a poorer clinical outcome (126). Similarly, among a group of markers analyzed, including serum prostate-specific antigen (PSA), IGF-I/PSA ratios, and TNM staging, IGFBP-3/PSA ratios were identified as the only significant variable for relapse-free survival and an independent predictor of survival in advanced prostate cancer (127).

When viewed collectively, these studies show that the significance of IGF and IGF-IR expression levels *per se* as indicators of tumor stage or predicators of disease outcome defies simple generalization and may be highly tumor-type specific. However, as discussed extensively below, the relevance of the IGF axis to cancer progression cannot be fully appreciated by analysis of the expression levels of the IGF-IR and its ligands alone because activation of the signaling pathway may occur through alternate mechanisms that bypass the requirement for receptor and/or ligand up-regulation.

VII. Role of the IGF Axis in Metastasis: Insight into Molecular Mechanisms

Cancer metastasis is a multistep process driven by complex molecular interactions between the disseminating cancer cell and its changing microenvironment. Among the rate-limiting steps regulating this process are tumor-induced neovascularization (angiogenesis and lymphangiogenesis), extracellular matrix (ECM) degradation, cell movement through tissue barriers, including entry into and exit from blood vessels, and survival/proliferation within new, otherwise hostile, organ microenvironments (128). As summarized in *Section VII*, the IGF axis can play a regulatory role at each of these intersections, thereby propelling the process forward.

A. Role of the IGF axis in angiogenesis and lymphangiogenesis

One of the major rate-limiting factors in tumor growth is the availability of nutrients and oxygen. Expanding tumors can overcome the lack of a nutrient supply and hypoxic conditions by inducing a process of neovascularization, *i.e.*, angiogenesis. Newly formed vessels also provide the tumor with access to the circulation that is essential for hematogenous metastasis.

Although hypoxia is a major trigger for tumor-dependent angiogenesis, the IGFs and insulin may actually play an early role in this process, preceding and/or augmenting the hypoxic stimulus. In cultured cells, IGF-I and IGF-II can induce the expression of hypoxia-inducible factor 1α (HIF-1α) (129), and this can lead to the formation of the HIF-1α/arylhydrocarbon receptor nuclear translocator complex. This complex is involved in transcriptional regulation of hypoxia response element-containing genes such as VEGF (130), a major tumor-derived angiogenic factor. In contrast to hypoxia, which can induce HIF-1α expression by inhibiting its ubiquitination and degradation, IGF-I can induce HIF-1α protein synthesis directly, via the MAPK and PI-3K pathways (131). In turn, HIF-1α is required for IGF-II, IGFBP-2, and IGFBP-3 synthesis (129).

The IGF system is involved in angiogenesis through several other mechanisms. IGF-I and IGF-II can induce angiogenesis by stimulating the migration and morphological differentiation of endothelial cells (132, 133). IGF-I is transported via a paracellular route across the vascular endothelial cell lining and binds to the subendothelial ECM, where it may play a role in endothelial cell survival and stability (134). The direct effect of IGF on vascular endothelial cells, and thereby angiogenesis, has recently been confirmed using mice with a vascular endothelial insulin receptor knockout (VENIRKO) or a vascular endothelial IGF receptor knockout (VENIFARKO). The significant reduction in oxygen-induced retinal neovascularization in these mice, relative to controls, implicated both insulin and IGF-I signaling in retinal neovascularization and the regulation of the vascular mediators VEGF, endothelial nitric oxide synthase, and endothelin-1 (135).

Moreover, IGF synthesis is regulated by hypoxic conditions. For example, in newborn rats, hypoxia increased IGF-I mRNA synthesis in the heart and increased both IGF-I and IGF-IR mRNA in the lungs (136). The induction of IGF-II by hypoxia has been documented in the human hepatocellular carcinoma HepG2 cell line (137). The contribution of hypoxia to the up-regulation of IGF-II has been linked to its dual effect on the transcription factor Egr-1 and the Wilms' tumor (WT) 1 suppressor gene. Hypoxia induces the expression and activity of Egr-1, which in turn activates the IGF-II promoter. On the other hand, the expression of WT1, a repressor of IGF-II expression, is reduced under hypoxic conditions (138). In NIH 3T3 cells transfected with the luciferase reporter gene under the control of the IGF-IR promoter, hypoxia, acidic pH, and low-glucose conditions that are typically found in the microenvironment of solid tumors were found to activate the IGF-IR promoter and induce IGF-IR synthesis (139). Thus, the crosstalk between hypoxia and the IGF system, two inducers of angiogenesis, appears to be reciprocal.

The IGF system can cooperate with other tyrosine kinase receptors such as the EGFR in the induction of angiogenesis. This was recently demonstrated in NIH-3T3 cells expressing a truncated, constitutively active form of the EGFR. Under normoxic conditions, VEGF expression in these cells was comparable to control cells expressing an intact receptor, but it increased by up to 6-fold under hypoxic conditions. In the presence of IGF, however, VEGF expression in cells expressing the mutant EGFR was high under normoxic or hypoxic conditions, suggesting that hypoxia (0.1% O_2) did not have an additive effect on VEGF production in the presence of IGF-I (140).

In addition to neovascularization, tumor cells can also

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induce the formation of new lymphatic vessels, a process known as lymphangiogenesis. The major angiogenic factors mediating this process are VEGF-C (141) and VEGF-D (142). These factors bind to the VEGFR-3, also known as *fms*-like tyrosine kinase 4 (Flt-4) (see review in Ref. 143). In several human cancers, increased expression of VEGF-C in the primary tumors was shown to correlate with regional lymph node metastasis. VEGF-C may recruit preexisting lymphatic vessels that may then become directly involved in tumor cell chemotaxis, intralymphatic intravasation, and distal dissemination (144). Recent studies by the Brodt group (145) identified the IGF-IR as a positive regulator of VEGF-C production and lymphatic metastasis. The PI-3K signaling pathway was implicated in this induction. A more recent study in an experimental animal model confirmed the role of the IGFs in lymphatic metastasis and showed that, in addition to the induction of VEGF-C, lymphangiogenesis may also be driven by a direct activation of the IGF-IR on lymphatic endothelial cells, resulting in increased motility and proliferation (146).

B. The role of the IGF axis in tumor invasion

1. Regulation of MMP expression and activity. The Zn^{2+}-dependent MMPs play a major role in ECM remodeling and tissue homeostasis under normal physiological conditions. They have been identified as the major molecular mediators of ECM degradation and tumor invasion in malignant progression and metastasis (see review in Ref. 147). In particular, the type IV collagenases MMP-2 and MMP-9 and the membrane type 1 MMP (MMP-14) have been implicated in ECM degradation and cellular invasion that are associated with both tumor-dependent angiogenesis and tumor invasion. The IGF system plays a role in the regulation of several of the MMPs and can, thereby, promote tumor invasion. Several groups, including our own, identified IGF-IR as a promoter of MMP-2 synthesis and tumor invasion, as measured *in vitro* in Matrigel (148–151). Down- or up-modulation of IGF-IR expression altered MMP-2 expression levels, invasion, and metastasis in a Lewis lung carcinoma model of liver metastasis (150). Using IGF-IR mutants with tyrosine-alanine substitutions in the kinase and C-terminal domains, it was found that signals from both regions of the receptor were required for MMP-2 induction and tumor invasion (152). Subsequently, it was shown that the IGF-IR can exert a dual regulatory effect on MMP-2 production, up-regulating it when the PI-3K signaling pathway is optimally activated, but reducing it under conditions that preferentially activate the MAPK pathway. This may partially explain an apparent dichotomy in the role of IGF in MMP-2 regulation, because both positive and negative regulatory effects have been reported (148).

The MMPs are generally produced as inactive proenzymes and require activation through proteolytic processing (147). MMP-14 is a major proteolytic activator of pro-MMP-2, and has recently been identified as another member of the MMP family that is subject to regulation by the IGF-IR, via PI-3K/Akt/mTOR signaling (153). Thus, the IGF system can optimize ECM degradation and invasion by coordinately promoting MMP-2 production and its activation.

IGF-I has also been implicated in the regulation of a second collagenase, namely MMP-9. Mira *et al.* (154) have shown that in the breast carcinoma MCF-7 cell line, IGF-I significantly increased cell surface-associated MMP-9 activity and enhanced cell migration through vitronectin. However, in this model, the increased production of MMP-9 did not appear to correlate with the invasive phenotype of MCF-7 cells.

It should be noted that, whereas positive regulation of metalloproteinases by IGF-I has been well documented in tumorigenic cells, this may not be the case for normal cells or tumor cells with a nonaggressive phenotype. For instance, in mesangial kidney cells obtained from nonobese, diabetic mice, IGF-I decreased MMP-2 expression and activity, and this was thought to contribute to reduced turnover of ECM and the development of glomerulosclerosis, shortly after the onset of diabetes (155). In mouse skeletal myoblasts, IGF-I did not induce MMP-2 expression or activity (156). Yoon and Hurta (149) reported that IGF-I could induce MMP-2 production in metastatic H-ras transformed C3 cells, but not in the parental 10T1/2 cells or H-ras transformed cells that form benign tumors *in vivo*. A lack of a direct regulatory link has also been reported for the poorly metastatic MDA-MB-231 human breast cancer cells that express IGF-IR as well as MMP-2 and MMP-9 (157). Taken together, the studies suggest that tumor progression through the accumulation of genetic alterations, a hallmark of cancer, may enable an IGF-mediated regulation of MMP-2 (possibly through the release of a negative regulation of PI-3K signaling), and that the crosstalk between the two systems is reciprocal. Indeed, in cells where IGF-IR regulates MMP expression, MMPs in turn, can increase IGF bioavailability by degrading IGFP-3 (158) and IGFBP-5 (159). This regulatory loop was recently demonstrated in the DU-145 androgen-independent human prostate adenocarcinoma cell line. When these cells were transfected with an MMP-9 antisense DNA, cell proliferation was markedly reduced, and this reduction could be reversed by the addition of IGF-I. The reduction in cell proliferation in this system was linked to reduced IGFBP-3 proteolysis and decreased IGF-IR-mediated signaling (160). In agreement with these observations is the finding that tissue inhibitor of metalloproteinases-1, an inhibitor of MMP-mediated proteolysis, blocks IGFBP-3 proteolysis and can reduce IGF-IR signaling (161).

Finally, several recent studies have implicated the matrilysin MMP-7, an enzyme frequently up-regulated during malignant progression (162), in proteolytic processing of all six IGFBPs, identifying it as an enhancer of IGF-IR phosphorylation and signaling (162–164).

2. Regulation of the uPAR/uPA system. The urokinase plasminogen activator (uPA) receptor (uPAR)/uPA system also plays a major role in ECM proteolysis and tumor invasion. Secreted uPA binds to its cell membrane-associated receptor and converts serum plasminogen to plasmin, a broad-spectrum serum protease that can degrade basement membrane proteins. In addition, tumor and endothelial cell-derived uPA can also promote angiogenesis. IGF-IR was shown to regulate uPA expression in breast cancer cells (157) and both uPA and uPAR in pancreatic cancer cells (165). In the latter study, it was shown that the IGF-I-dependent migration and

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invasion of human pancreatic carcinoma L3.6 pl cells were uPA/uPAR-driven, because they could be blocked by antibodies directed to either uPA or uPAR. The role of IGF-IR in uPA regulation was also demonstrated when murine mammary carcinoma EMT6 cells were transfected with an antisense IGF-IR construct, which led to a reduction in uPA expression (166).

IGF can up-regulate uPA transcription directly through the AP-1 and Ets sites in the uPA promoter in a MAPK- and PI-3K-dependent manner (167). Alternatively, as was recently shown in a rhabdomyosarcoma cell line, IGF-I and -II can increase binding of single-chain uPA to cell-surface uPAR, possibly through the posttranscriptional modification of the uPAR by a mechanism yet to be defined (168).

C. Role of IGF in tumor survival and growth in secondary sites

Metastasis to secondary organs is not an efficient process (169). The majority of tumor cells invading a secondary site either die or cannot proliferate in the foreign microenvironment. The ability of tumor cells to colonize secondary sites is determined by a combination of tumor and host-dependent factors. Site selectivity of metastasis is thought to reflect a complementarity between tumor cell properties and unique favorable growth conditions in the target organ. Work by Brodt *et al.*, using variants of the Lewis lung carcinoma, identified IGF-I as the major factor promoting the growth of highly metastatic tumor cells in the mouse liver. Site-directed mutagenesis showed that tyrosines in the kinase and C-terminal domains of the IGF-IR were involved in promoting liver metastasis in this model (152). Moreover, highly metastatic H-59 cells engineered to express a soluble IGF-IR molecule (IGF-IR^{933}), consisting of the entire extracellular domain of the receptor, had a significantly diminished potential to form liver metastases (Fig. 3), directly implicating paracrine, hepatic IGF-I in liver metastasis in this model (170).

A validation of these findings in an animal model of colon adenocarcinoma was recently provided by the LeRoith group (171). In this study, murine colon 38 adenocarcinoma tissue fragments were orthotopically implanted onto the cecum of mice with a liver-specific IGF-I deficiency (LID mice), in which serum IGF-I levels were reduced to 25% of control levels. Mice were then divided into two groups; one group was injected with IGF-I twice daily for 6 wk, and the other received saline injections. IGF-I treatment significantly increased the growth of "primary" cecal tumors, as well as liver metastasis, compared with the saline-injected controls. VEGF expression and vessel abundance in the cecal tumors were shown to be dependent on serum IGF-I levels (171).

Several other lines of evidence support the conclusion that the IGF system plays a role in the regulation of metastases formation in the liver. For example, in hepatic resections of colorectal carcinoma patients, immunohistochemical analyses have shown that the invasive margins of liver metastases were highly positive for IGF-II and IGF-IR. Overexpression of IGF-II mRNA and protein was demonstrated in the normal liver adjacent to the tumor, suggesting that a paracrine interaction may exist between the tumor cells and the adjacent normal stroma (172).

As alluded to earlier, the IGF system regulates lymphangiogenesis. New lymphatic vessels recruited to the tu-

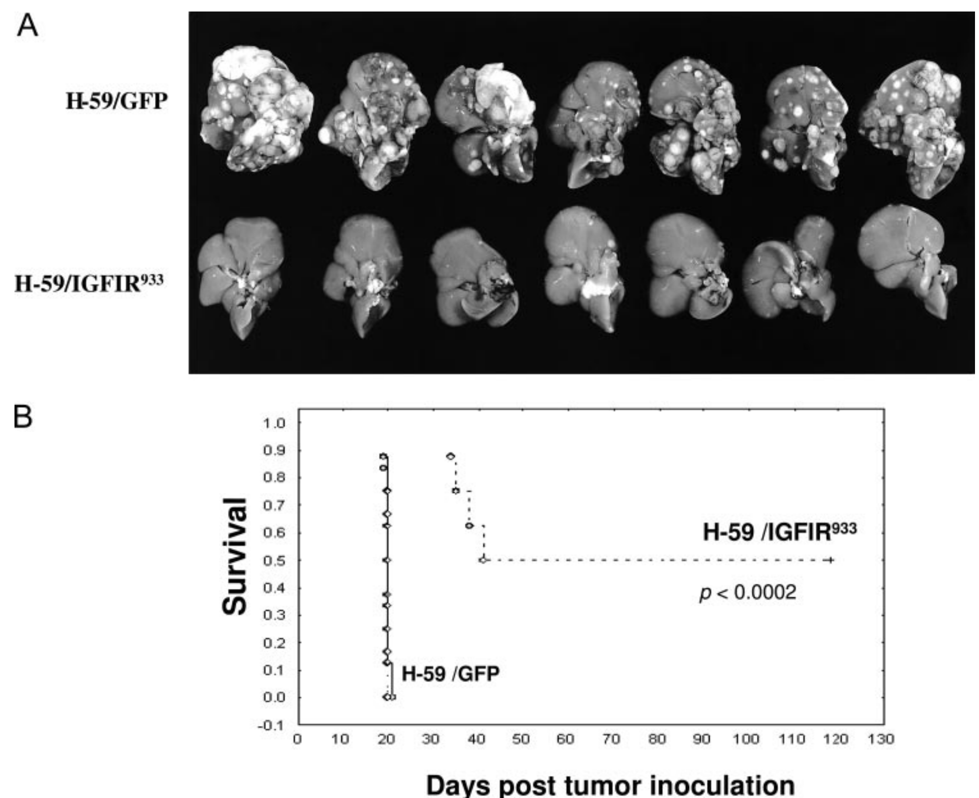

FIG. 3. The soluble IGF-IR^{933} blocks hepatic metastasis of carcinoma cells, resulting in increased animal survival. H-59 carcinoma cells were transduced with retroviral particles expressing (or not, H-59/GFP) sIGF-IR^{933}, and 10^5 cells were injected into syngeneic mice by the intrasplenic/portal route (A). Liver metastases were enumerated 14 d later. Animal survival (experiment shown in panel B) was monitored for up to 180 d. [Adapted from A. A. Samani *et al.*: *Cancer Res* 64:3380–3385, 2004 (170).]

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mor serve as a route of tumor dissemination to the draining lymph nodes and the circulation. In a recent study, the Brodt group has shown that lymphatic stromal cell-derived IGF-I and EGF promote the growth of human and rat breast carcinoma cells, suggesting that IGF may also be involved in tumor cell survival and growth in this organ (173).

The IGF-IR and its ligands may also promote growth of metastatic cancer cells in the bone, the preferred site of metastases for different cancers, including prostate carcinoma (174). The IGFs are among the more abundant growth factors in bone tissue and are synthesized by various bone cells, including osteoblasts and bone endothelial cells (175, 176). Osteoblasts also produce multiple IGFBPs (177). A recent study has shown that medium conditioned by the PA-III prostate carcinoma cells could degrade IGFBPs produced by osteoblastic cells, and uPA was identified as the proteolytic enzyme involved (178). This proteolytic activity could potentially increase the bioavailability of IGFs at the site of the invading tumor, with a resulting increase in the growth of both metastatic carcinoma cells (paracrine effect) and osteoblasts (autocrine effect), eventually leading to the osteoblastic reaction characteristic of bone-metastatic prostate cancers (179). In another study, the injection of MDA-Pca-2b human prostate carcinoma cells into the bone of severe combined immunodeficient mice was recently shown to induce *de novo* bone formation within 2 wk of the injection. Coculture of primary mouse osteoblasts derived from the same mice with MDA PCa 2b cells induced DNA synthesis in prostate carcinoma cells, and this was coincidental with IGFBP-3 downregulation in these cells (180). It is highly relevant to note that preoperative plasma IGFBP-3 levels were found to be reduced in patients with prostate cancer metastases and were identified as an independent predictor of tumor progression after surgery, presumably because of an association with occult metastatic disease present at the time of radical prostatectomy (181). Findings relevant to the role of IGF in prostate carcinoma bone metastasis were recently obtained when it was shown that the growth of MDA PCa 2b tumors in human bone fragments that were preimplanted in severe combined immunodeficient mice was drastically reduced after injection of a rat antihuman IGF-I/IGF-II antibody (KM1468) (64).

VIII. Animal Models Confirm the Importance of the IGF System in Cancer Growth and Metastasis

Compelling evidence for the role of the IGF system in cancer initiation, progression, and metastasis has also been obtained from the study of animal models, particularly from genetically altered mice. For example, in the transgenic adenocarcinoma of mouse prostate (TRAMP) model, selective overexpression of the human IGF-I DNA in basal epithelial cells of the prostate resulted in overexpression of IGF-IR in these cells and the spontaneous and stepwise development of the early stages of prostate carcinoma (182). The forced overexpression of IGF-I in the epidermal cells of HK1.IGF-I transgenic mice hypersensitized these cells to tumor induction by promoters such as 12-*O*-tetradecanoylphorbol-13-acetate. Additionally, the exposure of the epidermis to tumor promoters in these mice resulted in both a reduction in the latent period and a dramatic increase in the number of tumors per mouse, compared with nontransgenic controls (183). In epidermal cells derived from 12-*O*-tetradecanoylphorbol-13-acetate-treated HK1.IGF-I mice, both the IGF-IR and EGFR were constitutively activated. Recently, overexpression of human IGF-I with the bovine keratin 5 (BK5) promoter (BK5.IGF-I transgenic mice) was shown to induce persistent epidermal hyperplasia and lead to spontaneous skin tumor formation in a PI-3K/Akt dependent manner (184).

The tumor-promoting effect of transgenic IGF may be independent of its binding to the IGFBPs. This was demonstrated in mice that overexpress the transgene des-IGF-I, an IGF-I analog with low affinity for IGFBP that retains a high binding affinity for IGF-IR. In WAP-DES mice, there was a marked increase in the incidence of mammary adenocarcinomas, with 53% of the mice developing tumors by 23 months of age (compared with no tumors in the controls). Interestingly, when these mice were mated with mice transgenic for a mutant form of p53 (p53172R-H), the incidence of mammary tumors in the WAP-DES/p53 bitransgenic offspring was similar to that seen in WAP-DES mice, but 2- to 3-fold higher than that in p53172R-H females (185), suggesting that the IGF system can provide the "second hit" needed for malignant transformation after loss of tumor suppressor expression.

In another study by Carboni *et al.* (186), mice were engineered to express a novel human $CD8_{\alpha}$-IGF-IR fusion protein under the control of the mouse mammary tumor virus promoter. The extracellular $CD8_{\alpha}$ portion of this protein could homodimerize through disulfide bond formation, constitutively activating the intracellular IGF-IR portion in a ligand-independent manner. These mice developed salivary and mammary adenocarcinomas as early as 6 wk after birth, with a palpable mammary mass at 8 wk of age. The salivary gland tumors were associated with scattered lymphatic vessels and invaded the adjacent striated muscle. The mammary adenomas and adenocarcinomas were multifocal and seen within adjacent lobules (186).

Supporting evidence for the role of IGF in the development of mammary and prostate carcinomas was obtained from a study using Noble rats implanted sc for 1 yr with testosterone and estradiol capsules. In these rats, a stepwise tumor progression from hyperplasia/dysplasia to carcinoma *in situ* and adenocarcinoma was observed. All 62 treated rats developed prostatic hyperplasia within 2 months, 51 rats showed dysplastic features, and 24 rats had microscopic tumors that fully developed into macroscopic adenocarcinomas within 7 months after treatment. Immunohistochemical analysis of the prostate glands confirmed that the exposure to increased hormone levels caused increased IGF-I production in prostate stromal cells and that this, in turn, caused increased prostatic epithelial cell proliferation in a paracrine manner. VEGF overexpression was detectable during the dysplastic and carcinomatous phases. Eventually, the malignant epithelial cells were able to produce IGF-I and promote their own growth in an autocrine manner (187). This paracrine/autocrine switch was coincidental with the progression to a malignant phenotype. Interestingly, similar to

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findings in the TRAMP model (above), IGF-IR was weakly expressed in glandular epithelial cells of the normal prostate, but its expression was strongly increased in hyperplastic, dysplastic, and neoplastic epithelial tumor cells.

Increased, IGF-induced tumor incidence is not specific for glandular hormone-dependent malignancies. In an IGF-II transgenic model, transgene overexpression in the lung epithelium induced lung tumors with morphological characteristics of human pulmonary adenocarcinoma in 69% of mice older than 18 months. Analysis of tumor sections revealed that downstream mediators of IGF-IR signaling such as Erk1/2, were hyperphosphorylated in these cells. This model validated earlier clinical studies that identified increased levels of IGF-II as a risk factor for human pulmonary adenocarcinoma (188).

Compelling evidence for the potential role of the IGF system in malignant progression in the pancreas was recently obtained using transgenic RIP1-Tag2 mice that overexpress the SV40-T antigen under the control of an insulin promoter. These mice have focally up-regulated IGF-II expression in the pancreatic islet β-cells, causing the formation of hyperproliferative/dysplastic β-cell lesions. The role of IGF-II in this process was confirmed by crossing these mice to IGF-II$^{-/-}$ null mice. The double transgenic RIP1-Tag2/IGF-II$^{-/-}$ mice developed markedly smaller tumors than RIP1-Tag2 mice, and this was linked to a 5-fold increase in β-cell apoptosis (189). To assess whether the low IGF-IR levels expressed in the β-cells limited the progression of hyperplastic foci to malignant/invasive lesions in these mice, the RIP1-Tag2 mice were crossed with RIP7-IGF-IR mice that overexpress human IGF-IR under the control of the insulin promoter. The overexpression of both IGF-II and the IGF-IR in the offspring resulted in accelerated β-cell tumor formation, with the rapid development of lymph-node metastases (190). This study confirmed previous observations based on genetically altered tumor cell lines, and provided strong *in vivo* evidence that IGF-IR can drive the acquisition of an invasive/metastatic phenotype by tumor cells.

In human embryonic development, IGF-II expression is maternally imprinted under the control of the upstream, untranslated *H19* gene. Deletion of the *H19* gene results in a biallelic expression (LOI) of IGF-II. To test the effect of IGF-II overproduction on the development of colon tumors, Sakatani *et al.* (191) engineered a novel mouse model by crossing a male Apc$^{+/Min}$ *Min* mouse carrying a mutation in the *Apc* gene with a female H19$^{+/-}$ mouse. The H19$^{-/+}$ progeny had a 2-fold increase in IGF-II expression levels and developed twice as many adenomas as control H19$^{+/+}$ mice in both the small intestine and the colon. Interestingly, H19$^{-/+}$ mice also had longer intestinal crypts, independently of *Apc* status. This increase did not result from increased cell proliferation or decreased apoptosis in the crypt and may have been due to a shift in the ratio of undifferentiated to differentiated epithelial cells in the mucosa, as suggested by the increased expression of stem cell markers such as musashi1 in these crypts. These findings may be highly relevant to the human disease, because an analysis of routine colonoscopy biopsies recently revealed that 90–100% of patients with LOI in their colonic specimens also exhibited abundant expression of musashi1 and altered colon epithelial maturation. Together, these observations suggest that loss of IGF-II imprinting triggers a dedifferentiation in colon mucosal cells, and this sets the stage for a tumor initiation process.

If overexpression or constitutive activation of positive effectors of the IGF system promotes cancer, it follows that down-regulation of these molecules should have the opposing effect, namely, inhibition of tumor growth. Indeed, down-regulation of IGF-I or overexpression of IGFBP-1 has been associated with reduced tumorigenicity in animal models. In Lit/lit mice that have a defect in GH production and, therefore, have a significant reduction in IGF-I expression in all tissues, a reduction in the growth of xenotransplanted human MCF-7 cells was observed (192). Likewise, dw/dw dwarf mice that are deficient in GH/IGF-I were shown to be resistant to dimethylbenz[α]anthracene (DMBA)-induced carcinogenesis. In transgenic mice that express a GH antagonist and have decreased circulating IGF-I levels, a reduced incidence of mammary tumors relative to controls was observed after exposure to the carcinogen DMBA (193). However, it should be pointed out that in these models, the involvement of other factors affected by reduced GH levels cannot be ruled out. In LID mice with a 75% decrease in circulating IGF-I levels (see above), there was a decrease in the incidence of mammary tumors induced with DMBA or the SV40-large T antigen transgene (194). Further evidence for the involvement of the IGF axis in carcinogenesis came from transgenic C57BL/6 mice overexpressing an inducible, human IGFBP-1 cDNA under the control of the mouse metallothionin promoter (195). In control mice, a single ip injection of diethylnitrosamine induced the formation of preneoplastic hepatic lesions that generally overexpressed both IGF-I and IGF-II. However, when IGFBP-1 gene expression was induced by adding $ZnSO_4$ to the drinking water, hepatocarcinogenesis was inhibited (196), suggesting that IGFBP-1 could effectively interfere with carcinogenesis, presumably by blocking IGF-I functions.

A reduction in serum IGF-I levels can also be achieved through a calorie-restricted diet. When IGF-I was infused into calorie-restricted, p53-deficient mice, the anticarcinogenic effect of calorie restriction was abolished (197). Similar results were obtained when GH or IGF-I was administered to calorie-restricted Fischer rats bearing mononuclear cell leukemia (198). The role of IGF-I in the obesity-linked cancer risk is, however, still unclear. It is thought that in obese individuals, hyperinsulinemia may lead to increased IGF-I and decreased IGFBP-1 levels, resulting in an overall increase in IGF bioavailability and in enhanced IGF-IR and/or IR-A activation. In a study conducted by Yakar *et al.* (199), it was recently found that obese animals have an increased incidence of transplanted tumors, and that this was linked to higher systemic levels of insulin and IGF-I. In the same study, it was also shown that, in the presence of sera from obese mice, anchorage-independent growth of tumor cells, as measured in semisolid agar, was significantly augmented relative to controls, suggesting that factors present in the sera of obese mice can promote several parameters of tumorigenicity (199). However, it should be emphasized that obesity can have multiple physiological effects on the mice and cause up-regulation of multiple serum proteins. The interpretation of these findings, particularly the elucidation of the role of

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IGF-I in tumor promotion in these mice, will therefore require further analysis.

IX. The IGF System Confers Radioresistance and Multiple Drug Resistance

The IGF axis may affect the clinical course of malignant disease not only by promoting tumor progression and the acquisition of an aggressive phenotype, but also by providing escape mechanisms from conventional treatment regimens. For the majority of cancers, radio- and chemotherapy are still the treatments of choice. Often, however, tumors develop resistance to these treatments and eventually recur with a more aggressive phenotype. Recent studies suggest that the IGF-IR may play a role in protection from DNA-damaging agents such as chemotherapeutic drugs, UVB and ionizing radiation (200–205). This acquired drug resistance may involve IGF-IR-mediated cell rescue from apoptosis via the PI-3K pathway and/or protection from drug-induced cytostatic effects through activation of the MAPK pathway (201). In addition, the IGF system may play a role in conferring a multiple drug resistance (MDR) phenotype on the cancer cells through the induction of MDR-related genes such as mdr-1, c-H-ras, and MnSOD (206).

IGFs can also interfere with the normal function of p53, a suppressor gene that can trigger cell growth arrest and/or apoptosis in cells with radiation-induced DNA damage. In a recent study, Heron-Milhavet and LeRoith (82) have shown that, in IGF-IR-overexpressing NIH-3T3 cells that are exposed to 4-nitroquinoline-1-oxide-induced DNA damage, IGF-IR can up-regulate MDM2 mRNA synthesis through p38 MAPK activation, but cannot increase the MDM2-p19^{ARF} association. Free, unbound MDM2 translocates to the nucleus, where it can ubiquitinate p53, thereby targeting it to the cytoplasm for proteasomal degradation (82) and reducing its apoptotic effects (207).

Finally, a recent study suggests that IGF-IR may also provide radio-resistance through up-regulation of secretory clusterin (sCLU), a protein that plays a role in breast and colon cancer cell survival. A recent report by Criswell *et al.* (208) implicated IGF-I in the irradiation-induced up-regulation of sCLU in MCF-7 breast carcinoma cells. It was shown that after irradiation, MCF-7 cells overexpress IGF-I, and this results in the phosphorylation/activation of IGF-IR in an autocrine fashion, leading to MAPK signaling, transactivation of egr-1, and transcriptional activation of the sCLU gene. Irradiation-induced sCLU expression in these cells could be abrogated by adding IGFBP-3 or a tyrosine kinase inhibitor (AG1024) (208).

In view of the role of the IGF axis in conferring drug and radiation resistance onto malignant cells, receptor and ligand expression levels may provide useful criteria for the selection of a preferred treatment strategy. For example, for patients with locally advanced squamous cell carcinoma of the head and neck that were enrolled in a phase III, combined radiotherapy and chemotherapy trial, loss of heterozygosity for the IGF-IIR was associated with a 3-fold reduction in the 5-yr relapse-free survival and locoregional control in the group that was treated with radiotherapy alone. Interestingly, these patients benefited most from concurrent chemotherapy (209).

X. Multiple Molecular Mechanisms Can Contribute to the Changes in IGF/IGF-IR Expression and/or Function That Underlie Malignant Progression

Under normal physiological conditions, the IGF system is tightly regulated. The evidence detailed above clearly demonstrates that changes in any of the components of this system may disrupt this equilibrium and consequently trigger and drive malignant progression.

Although IGF-IR and IGF overexpression was recognized early on as a major promoter of tumor progression, it has become clear in recent years that the IGF system can also be altered through diverse molecular mechanisms that do not directly affect receptor or ligand expression levels. These changes can occur via genetic and epigenetic changes in gene expression, perturbed protein synthesis, and posttranslational modifications of different proteins that affect IGF-IR receptor function. They may result in enhancement of pro-malignant effectors such as the IGF ligands, or they may inhibit the production and/or activities of proteins with tumor suppressor functions such as the IGF-IIR, leading to augmented cell growth and tumorigenesis. A review of these diverse mechanisms that alter the function of the IGF system is provided below. A summary can be found in Table 2.

A. Overexpression of IGF-II through loss of imprinting

In human adults, IGF-II is the predominant endocrine IGF ligand, with plasma levels 3- to 7-fold higher than IGF-I (210). In humans, the IGF-II gene is normally maternally imprinted (210). However, several reports on bi-allelic expression (LOI) of the IGF-II gene in different tumors suggest that the dysregulation of IGF-II imprinting may contribute to tumorigenesis. LOI has been reported in colorectal carcinomas (105), Wilms tumor (211), juvenile nasopharyngeal angiofibromas (212), and childhood acute lymphoblastic leukemia (213). In cells that express both parental IGF-II alleles, the

TABLE 2. Multiple molecular mechanisms can alter IGF/IGF-IR expression and/or function and contribute to malignant progression

Mechanism	Effect
LOI	IGF-II overexpression
Gene amplification	IGF-IR overexpression
Overexpression of PCs	Overexpression of multiple effectors
Overexpression of AP-2 transcription factors	Overexpression of multiple effectors
Altered glycosylation of the IGF-IR	Increased ligand binding affinity
Altered IGFBP synthesis or proteolysis	Increased bioavailability of ligands
Constitutive activation of downstream effectors of the IGF-IR	Ligand-independent signaling
Loss of tumor suppressor genes	Overexpression of multiple effectors Reduced degradation of IGF-IR Downregulation of IGFBPs

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increase in IGF-II production may be the major mechanism promoting cancer development. A recent report on a mouse model of IGF-II LOI and overexpression (described in detail above) supports the role of IGF-II as a tumor initiator in intestinal cancers (191).

B. IGF-IR gene amplification

Gene amplification is a common mechanism for overexpression of proto-oncogenes associated with cellular transformation and tumor progression. IGF-IR gene amplification has been reported in malignant melanoma (214), primary breast cancers (215), and in pancreatic adenocarcinoma where an amplification of band 15q26 was observed (216). However, as discussed in detail above, the frequency of IGF-IR overexpression in human malignancies and its functional relevance to tumor development are the subject of conflicting reports and controversy.

C. Overexpression of the IGFs and IGF-IR through deregulated expression of proprotein convertases

Overexpression of convertases of the proprotein family has been documented in several cancers (217). To date, seven dibasic, specific mammalian proprotein convertases (PCs) have been identified, including the ubiquitously expressed furin, PC1/PC3, PC2, PC4, PACE4, PC5/PC6, and PC7/LPC/PC8. These convertases mediate the processing of multiple polypeptide precursors. In addition to the ubiquitously expressed furin that has been implicated in the processing of multiple proteins of the IGF axis, including IR, IGF-IR, and IGFs (218, 219), several other convertases have been implicated in tissue-specific processing of polypeptide precursors. These include PC5A for IGF-IR (220); PC6A and LPC for IGF-I (218); PACE4, PC6A, PC6B, and LPC for IGF-II (221); and PACE4 for IR (222). In addition, PACE4 and PC6 are also involved in the processing of stromelysin-3 (MMP-11 or Str-3) and membrane type 1-MMP (223), respectively. Inhibition of furin by stable expression of the α1-antitrypsin Portland (PDX) cDNA in two astrocytoma cell lines inhibited their tumorigenesis (224). In human colon carcinoma HT-29 cells treated in a similar manner, the ability of exogenous IGF-I to activate IGF-IR was suppressed, and this resulted in reduced angiogenesis and suppressed tumor growth following sc implantation (220). These studies demonstrate that enhanced expression of convertases may be an additional mechanism for increasing the activity of the IGF system. This effect can be direct, or it may involve enhanced MMP production that, in turn, can increase IGF bioavailability through IGFBP degradation.

D. Enhanced IGF and IGF-IR expression mediated through overexpression of the AP-2 transcription factors

The AP-2 transcription factors are required for normal growth and morphogenesis during mammalian development. Potential AP-2 binding sites have been identified in the promoters of both the IGF and IGF-IR genes (225, 226). Therefore, the overexpression of AP-2 factors may contribute to the enhanced production and activity of the IGF system. For instance, a significant association between increased expression of AP-2γ, a factor frequently amplified in breast cancer cell lines and breast carcinoma (227), and the increased expression of IGF-IR was documented in breast carcinoma specimens (228), whereas in rhabdomyosarcoma cells, an increase in AP-2-dependent IGF-II mRNA expression relative to normal human adult skeletal muscle was reported (229).

E. Posttranslational modification of IGF-IR alters binding affinities

Malignant cells frequently display aberrant protein glycosylation that can result in altered protein function (230). Altered glycosylation of the IGF-IR has been reported in several tumor systems. For example, in glioma cell lines established from different human brain tumors of glial origin (astrocytoma grades III and IV), expression of a 130-kDa IGF-IR α-subunit that binds IGF-I and IGF-II with equal affinities has been reported (231), possibly providing a mechanism for increased utilization of IGF-II by these cells. A 105-kDa form of the β-subunit was identified in two murine neuroblastoma cell lines. Glycopeptidase F digestion confirmed that the increased molecular mass was due to altered N-linked complex-type glycosylation in the extracellular domain of this subunit (232). Interestingly, the same pattern has been reported for rat fetal skeletal muscle IGF-IRβ. During fetal development and for 2 wk postnatally, a 105-kDa β-chain is predominant and it is subsequently replaced by the 95-kDa form. This fetal receptor, unlike the mature IGF-IR, can be autophosphorylated in the presence of low physiological concentrations of either insulin or IGF-I (233). The expression of this altered β-chain could therefore enable a response to low concentrations of IGF, providing them with a growth advantage.

F. Overexpression of the insulin receptor (IR)-A

The IR can be expressed in two isoforms (IR-A and IR-B) that differ by the presence or absence of 12 amino acids due to alternative splicing of exon 11. As alluded to earlier, IR-A, unlike IR-B, can bind IGF-II with the same affinity as insulin, and this can induce a mitogenic response (20) (reviewed in Ref. 234). Interestingly, Pandini *et al.* (235) recently demonstrated that the binding of IGF-II or insulin to this receptor in cultured cells, even within the same cell context, can have distinct outcomes and induce different gene expression profiles. In this model, cell cycle progression, DNA synthesis, and angiogenesis were preferentially induced by IGF-II. In another model, the differential effects of IGF-II and insulin were compared in leiomyosarcoma cells deficient in IGF-IR. Although IGF-I had no effect on these cells, IGF-II was more potent than insulin in activating the Shc/ERK pathway and stimulating cell migration, whereas insulin was more potent in activating the PI-3K/Akt pathway and rescuing cells from apoptosis (236).

Under normal physiological conditions, IR-A is expressed in fetal cells. However, increased expression of IR-A has been reported in human breast, colon, and lung tumor specimens (20) and on primary cultures and cell lines derived from

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human tumors such as ovarian and thyroid carcinoma cells (234). Because IGF-II is the predominant circulating IGF in human adults, this added mechanism for response to IGF-II can clearly provide the cells with a growth advantage.

G. Reduced expression of IGFBPs can promote cancer progression

In many cancer cell lines, IGFBP-3 was shown to have an inhibitory effect on IGF functions. For example, in human MCF-7 breast cancer cells, IGFBP-3 activates a phosphotyrosine phosphatase that dephosphorylates IGF-IR (87), attenuating its functions. IGFBP-3 can induce apoptosis in an IGF-I-dependent and -independent manner. Reduced IGFBP-3 levels can, therefore, affect cell growth in multiple ways, such as increasing IGF bioavailability, attenuating an inhibitory effect on the IGF system through down-regulation of phosphatase activity, and reducing IGF-independent growth inhibitory effects. Indeed, lower IGFBP-3 levels were linked to increased risk and poor prognosis in many cancers (237, 238). Recently, hypermethylation of the IGFBP-3 promoter has been identified as one of the mechanisms down-regulating IGFBP-3 synthesis and resulting in potentiation of the IGF response in non-small cell lung cancer. In this malignancy, IGFBP-3 promoter hypermethylation was found to be a frequent event, strongly associated with poor prognosis, and the only independent predictor of disease-free survival in stage I disease (239, 240).

Recently, a link was identified between IGFBP-3 and cathepsin L, a cysteine proteinase that is frequently overexpressed and secreted in transformed and malignant cells and that has been implicated in tumor invasion and metastasis (77, 241). In MEF derived from cathepsin L-deficient mice, an accumulation of IGFBP-3 in the conditioned medium was revealed, suggesting that increased IGF bioavailability may be one mechanism by which secreted cathepsin L may enhance the invasive/metastatic phenotype (242).

The mechanisms that control IGFBP synthesis are complex, and the role of IGF in their synthesis appears to be variable and cell type-specific. For example, in primary chondrocytes, IGF-I was shown to increase IGFBP-3 and IGFBP-5 synthesis, and MAPK and PI-3K signaling were implicated (243). In contrast, in the SNU-484 human gastric carcinoma cell line, IGFBP-3 synthesis was down-regulated in a PI-3K-dependent manner (244). Similarly, IGF can also have divergent effects on IGFBP processing and was shown to inhibit (245) or promote (246) IGFBP-5 proteolysis, apparently in a cell type-specific manner.

Insulin also affects IGFBP synthesis. It is the principal regulator of hepatic IGFBP-1 production (247), rapidly decreases plasma IGFBP-1 levels in response to nutritional intake (248), and can down-regulate IGFBP-3 levels (249). Because obesity is frequently associated with chronic hyperinsulinemia, this effect of insulin on IGFBP synthesis may provide one possible mechanism underlying the link observed between obesity and increased risk for several cancers, such as breast and ovarian carcinomas (250).

H. Overexpression and/or constitutive activation of downstream effectors conveying the IGF signal increase IGF-mediated functions

The IRSs are a family of adaptor proteins that can link the IGF-IR to downstream signal transducers, regulating cellular growth. The four members of the IRS family, IRS-1 to -4, share a high degree of homology. IRS-1 is the most extensively studied of these proteins. This 165- to 195-kDa molecule does not contain Src homology (SH)2 or SH3 domains, but can bind to the IGF-IR β-subunit through a PTB (pTyr-binding) domain. It contains at least 20 potential tyrosine phosphorylation sites and can act as a multisite "docking" protein associating with multiple downstream signaling proteins, including the PI-3K, Syp, Fyn, Nck, and Grb-2, through their SH2 domains (30).

IRS-1 is constitutively activated in various solid tumors, including breast cancers, leiomyomas, Wilms tumor, rhabdomyosarcomas, liposarcomas, leiomyosarcomas, and adrenal cortical carcinomas (251). Overexpression of IRS-1 in MCF-7 breast carcinoma cells was shown to be inversely correlated with the requirement for estrogen (252). In some cancers, overexpression or constitutive activation of IRS-2 seems to be more important than that of IRS-1 for malignant progression. For example, abundant IRS-2 expression was found in ductal carcinoma of the pancreas (253, 254). When IRS phosphorylation status in bone-metastasizing human breast carcinoma MDA-231-BO cells was compared with parental, nonmetastatic MDA-MB-231 cells, IRS-2 (and not IRS-1) was found to be preferentially phosphorylated, in response to IGF-I (255). Recent studies, based on murine models of diethylnitrosamine and SV40 large T antigen-induced hepatocarcinogenesis, as well as on analyses of human hepatocellular carcinoma specimens and Hep3B hepatoma cell lines, identified the coordinated up-regulation of IGF-II, IRS-1, and IRS-2 as one of the common early events in hepatocellular carcinogenesis (256).

Constitutive phosphorylation/activation of ERK1/2 is another mechanism that may reduce tumor cell dependency on exogenous IGFs or on IGF-IR expression. For instance, primary melanoma cells do not produce IGF-I and depend on exogenous ligand for growth (through the MAPK pathway) and β-catenin stabilization (through the PI-3K pathway). However, metastatic melanoma cells become independent of IGF for these functions due to constitutive activation of the MAPK pathway (257).

I. Loss of tumor suppressor genes enhances IGF expression and/or function

Changes in IGF expression and function in cancerous cells may occur as a result of loss or altered expression of several suppressor genes known to regulate different proteins of the IGF axis. Some of these changes are listed below.

1. p53. The p53 protein is a nuclear transcription factor that is activated in response to genotoxic stress and hypoxia. Activation of p53 triggers cell cycle arrest and apoptosis through DNA-specific transcriptional activation, transcriptional repression, and protein-protein interactions. Wild-type p53 can exert a suppressive effect on the IGF axis by

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up-regulating IGFBP-3 (258) and down-regulating the expression of IGF-IR (259), IR (260), IGF-II (261–263), and IRS-3 (264). However, in over 50% of all human cancers, p53 is mutated, and this can alter the IGF system by reducing IGFBP-3 synthesis while simultaneously increasing expression of positive effectors of the IGF system, such as IGF-IR. Mutated p53 can induce expression of the positive mediators of IGF signaling through the release of a repressive mechanism or through gain of function. For example, a mutation in codon 249 (p53mt249) that can be induced by aflatoxin B1 results in up-regulation of IGF-II synthesis through increased formation of transcriptional complexes that bind to the IGF-II promoter (261). This mechanism is thought to contribute to hepatocarcinogenesis. Indeed, transfection of p53-negative Hep3B hepatoma cells with p53mt249 increased IGF-IR and IGF-II expression (265). p53 mutants can also up-regulate IGF-IR by recruiting the ubiquitin ligase MDM2, thereby reducing IGF-IR ubiquitination and increasing intracellular IGF-IR levels (84). IGF-IR, in turn, can regulate MDM2 expression levels and thereby increase wild-type p53 ubiquitination (82).

2. WT1. The Wilms tumor suppressor gene *wt1* encodes a zinc-finger, DNA-binding protein that functions as a transcriptional repressor. WT1 binds to multiple sites on the IGF-IR and IGF-II promoters, inhibiting transcription. In Wilms tumors, however, mutations/deletions in WT1 result in overexpression of IGF-IR (266, 267) and IGF-II (268). An inverse correlation between functional WT1 levels and the expression of IGF-II and IGF-IR has also been documented in breast cancer (269).

3. PTEN. The tumor suppressor and lipid phosphatase PTEN dephosphorylates phosphatidylinositol 3,4,5-triphosphate, thereby blocking activation of Akt, a major transducer of the IGF-IR signal. In malignant cells, the *PTEN* gene is frequently mutated, leading to increased Akt activation. Recently, it was shown that IGF-II can both initiate and "turn off" Akt-mediated IGF-IR signaling in mammary gland epithelial cells. When the inguinal mammary glands were injected with IGF-II, it was observed that, in addition to Akt activation, PTEN expression was up-regulated. This, in turn, caused a reduction in Akt phosphorylation and an increase in the degradation of cyclin D1 (a substrate downstream of Akt) (188). Transfection of MEF with a luciferase-tagged PTEN promoter construct revealed that IGF-II up-regulates PTEN transcription via egr-1 (188). Conversely, we and others have shown that PTEN can down-regulate IGF-IR, IGF-I, and IGF-II expression and functions and up-regulate IGFBP-3 expression in a wide range of human and mouse cancer cell lines including prostate, gastric, and lung carcinoma cell lines (153, 244, 270, 271). Thus, loss of PTEN function can enhance IGF signaling by several different mechanisms; it can increase IGF-IR-induced PI-3K signaling, increase the expression of IGF-IR and/or its ligands, and reduce the expression of IGFBP-3.

4. The IGF-IIR. The IGF-IIR binds IGF-II with a Kd of 0.5 nM and IGF-I and insulin with 10 times and 10^5 times lower affinities, respectively (17). Although the IGF-IIR was initially thought to potentiate the growth-promoting effect of IGF-II (272, 273), recent evidence identified the IGF-IIR as a negative regulator of IGF signaling. Compelling evidence for this role was obtained from mice with a double deficiency in the *IGF-IR* and *IGF-IIR* genes. Mice with an isolated *IGF-IR* deficiency invariably died within minutes of birth but could be rescued if they carried a second, null mutation in the *IGF-IIR* gene. It has been postulated that deletion of *IGF-IIR* may increase the bioavailability of IGF-II for the insulin receptor, thereby providing an alternative growth mechanism for the embryos (274).

Frequent mutations, loss of heterozygosity, and microsatellite instability that affect the IGF-IIR gene have been reported in several primary human tumors (275–278). Overexpression of a dominant-negative IGF-IIR that could not bind IGF-II in human prostate cancer LNCaP cells induced a higher and more rapid proliferation index compared with mock-transfected cells. Conversely, forced expression of IGF-IIR in PC-3 human prostate cancer cells resulted in decreased proliferation (279). Similarly, it was recently shown that overexpression of IGF-IIR in a mouse mammary tumor cell line had an antiproliferative effect on the cells, inhibiting the formation of mammary tumors *in vivo* (280). It should be noted that, in addition to its role in the binding and targeting IGF-II for lysosomal degradation, IGF-IIR could regulate cellular growth by several other mechanisms. It can activate TGF-β1, a potent growth inhibitor for most cell types (281), and it can affect tumor susceptibility to the host immune system. In a recent study, the IGF-IIR was shown to play a key role in the intracellular transport of the cytotoxic T cell-derived granzyme B, an apoptosis-inducing serine proteinase (282). Taken together, these studies identify the IGF-IIR as a functional "tumor suppressor" (283), and its loss can therefore provide an alternative mechanism for enhancement of tumor cell growth.

XI. Controversies and Conflicting Evidence on the Role of IGF-IR in Malignancy

Although it has been generally recognized that the IGF axis promotes tumor progression, driving the process toward the acquisition of an aggressive phenotype, the evidence regarding the role of the IGF-IR itself and the clinical significance of increased IGF-IR expression levels have been the subject of some conflicting reports and controversy (as also discussed in *Section VI*). For example, studies with the TRAMP mice model of prostate carcinoma revealed an increase in the expression of prostate-specific IGF-I during prostate cancer progression, but the expression levels of IGF-IR and IGF-IIR were unaltered in primary prostate cancers and dramatically decreased in metastatic lesions (172). These findings were in fact in agreement with clinical data obtained by Tennant *et al.* (113) showing a 42% reduction in IGF-IR positivity (as assessed by immunohistochemistry), and a concomitant 30% increase in IGF-II expression in malignant, compared with benign epithelium. These findings are at variance with results of other clinical studies that showed an incremental increase in the expression of IGF-IR, IRS-1, IGF-I, and IGF-II during tumor progression from early PIN to metastatic lesions (114, 284). Interestingly, a similar

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discrepancy can also be found in results from breast cancer studies. Although some studies identified an overexpression of IGF-IR and its ligands in human breast carcinomas, others reported that expression of IGF-IR and IRS-1 were inversely correlated with high proliferation rates in dedifferentiated breast cancers (285). More recently, Chong *et al.* (286) found no difference in IGF-IR expression levels when breast cancer and the adjacent non-neoplastic tissue were compared in 20 breast cancer specimens.

Other studies relevant in this context are those that identified IGF-IR as a negative regulator of cell motility. This, for example, was shown for MCF-7 cells where receptor overexpression was reported to stimulate cell aggregation (287), whereas reduced expression was shown to increase motility (288), suggesting that reduced IGF-IR expression may actually promote the metastatic properties of some tumor cells. These studies are, however, at variance with other observations using the same (289) or other (152, 290) tumor models, suggesting that variations in assay conditions and/or the particular cell context may determine the outcome of altered IGF-IR expression levels. It is important to note in this context that in all animal studies reported to date (without exception), where IGF-IR or its ligands were targeted directly, a loss of tumorigenicity and/or metastatic ability was observed.

The apparent discrepancies between results of laboratory and clinical studies may be due to the obvious differences between investigations based on cell and animal models and those dependent on analysis of human specimens. Whereas in the former, molecular effectors of the IGF axis can be forcibly overexpressed or depleted under experimental conditions where other variables (*e.g.*, genetic background, host immune parameters, exposure to environmental factors, other oncogenic triggers, *etc.*) are controlled, in the analysis of clinical specimens, multiple tumor-specific and host factors may alter the actual contribution of the IGF-IR and its ligand(s) to malignant progression, and their contribution may therefore remain unappreciated. Furthermore, the conflicting reports on the significance of altered IGF-IR expression to malignant progression may also be reconciled when the IGF signaling system is viewed as a whole, and the expression levels and state of activation of different signaling effectors are all taken into account. Namely, it should be recognized that a reduction in IGF-IR expression *per se* may not always reflect and/or translate into a decline in IGF signaling. Several observations support this conclusion. For example, Knowlden *et al.* (291) observed that in breast carcinoma cell lines, MCF-7 and T47D, comparable levels of IGF-IR phosphorylation could be seen in response to IGF-I, despite significantly higher receptor levels in the former. These findings suggest that the levels of phosphorylated (*i.e.*, activated) IGF-IR or downstream signaling effectors in tumor specimens may be a more reliable indicator of IGF function and its contribution to disease progression than the number of receptors *per se*. Indeed, Del Valle *et al.* (292) found that in 10 of 17 biopsies of human medulloblastoma, the levels of phosphorylated IGF-IR were significantly higher than in control cerebellar tissues. In addition, studies with an animal model of leiomyoma (the Eker rat) revealed that the progression of normal uterine cells to leiomyomas was accompanied by a reduction in IGF-IR expression levels with a concomitant 7.5-fold increase in IGF-I expression and a 5.7-fold increase in the levels of phosphorylated IRS-1 (293).

XII. Targeting the IGF System for Cancer Therapy

The central role that the IGF system plays in initiating and promoting tumor progression makes it an attractive target for cancer therapy. Various strategies have been used to target components of this system in established animal and human tumor cell lines (*in vitro* studies) and in animal models of cancer (*in vivo* studies), and some of these strategies may be advancing to clinical use (294). Among them are down-regulation of IGF-IR by antisense oligonucleotides (295), antisense RNA (296, 297), small interfering RNA (298), triple helix-forming oligodeoxynucleotides (299), single-chain antibody (300), fully humanized anti-IGF-IR monoclonal antibodies (301, 302), and specific kinase inhibitors (303–305). IGF-I was targeted by similar strategies including IGF-I peptide analogs (306), antisense oligonucleotides (299), and triple helix-expressing vectors (307). Overexpression of IGF-IIR (281) and IGFBP-3 (88) were used to decrease the bioavailability of IGF-II and IGF-I, respectively. The Brodt group (170) recently described a novel soluble IGF-IR molecule, namely IGF-IR^{933}, that consists of the entire extracellular domain of the receptor. It was shown that IGF-IR^{933} can neutralize the effects of exogenous IGF-I and significantly diminish liver metastasis formation by highly invasive Lewis lung carcinoma H-59 cells, markedly increasing long-term animal survival (Ref. 170 and Fig. 3). Thus far, the evidence *in vitro* suggests that one of the humanized antireceptor antibodies is internalized and can down-regulate IGF-IR levels, resulting in a profound inhibition of receptor signaling (294), but no effect on the IR functions. The small molecule IGF-IR tyrosine kinase inhibitors also have a profound effect on IGF-IR activity and tumor cell growth *in vitro* and *in vivo* in a range of tumor models; however, their potential cross-reactivity with the IR system needs to be further evaluated (294).

The available data suggest that targeting of the IGF system *in vivo* may inhibit cancer progression and/or cause cancer regression directly by inducing apoptosis and cell growth arrest. In addition to these direct effects, it has been shown that inhibition of IGF action could increase the efficacy of other therapeutic modalities, such as radiotherapy (308).

XIII. Concluding Remarks

The evidence reviewed above shows that the role of the IGF system in the development and progression of malignant disease is complex and multifactorial. Although data based on cellular and animal models have demonstrated a causal link between receptor and/or ligand overexpression and malignancy, the evidence for a functional involvement of IGF-IR and its ligands in human malignancies is not as clear-cut. This may be the result of the obvious limitations that cell- and animal-based systems have as models for the human disease. It is also likely, however, that in addition to IGF-IR and ligand overexpression, more indirect mechanisms for

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engaging the IGF system, some of which may actually bypass the requirement for receptor overexpression, are at play, and they can be equally effective in promoting human malignancy. This complexity and intertumoral variability are likely to hinder progress toward developing IGF-targeted therapeutics with global anticancer efficacy. Similar to the experience with other biology-based therapies, effective targeting of the IGF system may require a customized approach in which tumor profiling guides the selection of the appropriate drugs.

Acknowledgments

We attempted to cite a wide, representative, and comprehensive selection of relevant studies. However, in a review of this magnitude, inadvertent omissions are practically unavoidable. Where they occurred, these omissions do not reflect a judgment on the quality or importance of the work. We apologize in advance to the respective authors.

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References

1. **Pardee AB** 1989 G1 events and regulation of cell proliferation. Science 246:603–608
2. **Cross M, Dexter TM** 1991 Growth factors in development, transformation, and tumorigenesis. Cell 64:271–280
3. **Hanahan D, Weinberg RA** 2000 The hallmarks of cancer. Cell 100:57–70
4. **Yarden Y, Ullrich A** 1988 Growth factor receptor tyrosine kinases. Annu Rev Biochem 57:443–478
5. **Pawson T** 1995 Protein modules and signalling networks. Nature 373:573–580
6. **Ullrich A, Gray A, Tam AW, Yang-Feng T, Tsubokawa M, Collins C, Henzel W, Le Bon T, Kathuria S, Chen E, Jacobs S, Francke U, Ramachandrau J, Fujita-Yamaguchi Y** 1986 Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. EMBO J 5:2503–2512
7. **Jansson M, Hallen D, Koho H, Andersson G, Berghard L, Heidrich J, Nyberg E, Uhlen M, Kordel J, Nilsson B** 1997 Characterization of ligand binding of a soluble human insulin-like growth factor I receptor variant suggests a ligand-induced conformational change. J Biol Chem 272:8189–8197
8. **Dricu A, Wang M, Hjertman M, Malec M, Blegen H, Wejde J, Carlberg M, Larsson O** 1997 Mevalonate-regulated mechanisms in cell growth control: role of dolichyl phosphate in expression of the insulin-like growth factor-1 receptor (IGF-1R) in comparison to Ras prenylation and expression of c-myc. Glycobiology 7:625–633
9. **Carlberg M, Dricu A, Blegen H, Wang M, Hjertman M, Zickert P, Hoog A, Larsson O** 1996 Mevalonic acid is limiting for N-linked glycosylation and translocation of the insulin-like growth factor-1 receptor to the cell surface. Evidence for a new link between 3-hydroxy-3-methylglutaryl-coenzyme a reductase and cell growth. J Biol Chem 271:17453–17462
10. **Dricu A, Kanter L, Wang M, Nilsson G, Hjertman M, Wejde J, Larsson O** 1999 Expression of the insulin-like growth factor 1 receptor (IGF-1R) in breast cancer cells: evidence for a regulatory role of dolichyl phosphate in the transition from an intracellular to an extracellular IGF-1 pathway. Glycobiology 9:571–579
11. **Massague J, Czech MP** 1982 The subunit structures of two distinct receptors for insulin-like growth factors I and II and their relationship to the insulin receptor. J Biol Chem 257:5038–5045
12. **Garrett TP, McKern NM, Lou M, Frenkel MJ, Bentley JD, Lovrecz GO, Elleman TC, Cosgrove LJ, Ward CW** 1998 Crystal structure of the first three domains of the type-1 insulin-like growth factor receptor. Nature 394:395–399
13. **Furstenberger G, Senn HJ** 2002 Insulin-like growth factors and cancer. Lancet Oncol 3:298–302
14. **Steele-Perkins G, Roth RA** 1990 Monoclonal antibody alpha IR-3 inhibits the ability of insulin-like growth factor II to stimulate a signal from the type I receptor without inhibiting its binding. Biochem Biophys Res Commun 171:1244–1251
15. **Germain-Lee EL, Janicot M, Lammers R, Ullrich A, Casella SJ** 1992 Expression of a type I insulin-like growth factor receptor with low affinity for insulin-like growth factor II. Biochem J 281(Pt 2):413–417
16. **Forbes BE, Hartfield PJ, McNeil KA, Surinya KH, Milner SJ, Cosgrove LJ, Wallace JC** 2002 Characteristics of binding of insulin-like growth factor (IGF)-I and IGF-II analogues to the type 1 IGF receptor determined by BIAcore analysis. Eur J Biochem 269:961–968
17. **Danielsen A, Larsen E, Gammeltoft S** 1990 Chromaffin cells express two types of insulin-like growth factor receptors. Brain Res 518:95–100
18. **Bennett A, Wilson DM, Liu F, Nagashima R, Rosenfeld RG, Hintz RL** 1983 Levels of insulin-like growth factors I and II in human cord blood. J Clin Endocrinol Metab 57:609–612
19. **Sciacca L, Costantino A, Pandini G, Mineo R, Frasca F, Scalia P, Sbraccia P, Goldfine ID, Vigneri R, Belfiore A** 1999 Insulin receptor activation by IGF-II in breast cancers: evidence for a new autocrine/paracrine mechanism. Oncogene 18:2471–2479
20. **Frasca F, Pandini G, Scalia P, Sciacca L, Mineo R, Costantino A, Goldfine ID, Belfiore A, Vigneri R** 1999 Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. Mol Cell Biol 19:3278–3288
21. **Pandini G, Vigneri R, Costantino A, Frasca F, Ippolito A, Fujita-Yamaguchi Y, Siddle K, Goldfine ID, Belfiore A** 1999 Insulin and insulin-like growth factor-I (IGF-I) receptor overexpression in breast cancers leads to insulin/IGF-I hybrid receptor overexpression: evidence for a second mechanism of IGF-I signaling. Clin Cancer Res 5:1935–1944
22. **LeRoith D, Clemmons D, Nissley P, Rechler MM** 1992 NIH conference. Insulin-like growth factors in health and disease. Ann Intern Med 116:854–862
23. **Baserga R, Hongo A, Rubini M, Prisco M, Valentinis B** 1997 The IGF-I receptor in cell growth, transformation and apoptosis. Biochim Biophys Acta 1332:F105–F126
24. **Rubin R, Baserga R** 1995 Insulin-like growth factor-I receptor. Its role in cell proliferation, apoptosis, and tumorigenicity. Lab Invest 73:311–331
25. **Moschos SJ, Mantzoros CS** 2002 The role of the IGF system in cancer: from basic to clinical studies and clinical applications. Oncology 63:317–332
26. **Clemmons DR** 1998 Role of insulin-like growth factor binding proteins in controlling IGF actions. Mol Cell Endocrinol 140:19–24
27. **Rosenfeld RG, Hwa V, Wilson L, Lopez-Bermejo A, Buckway C, Burren C, Choi WK, Devi G, Ingermann A, Graham D, Minniti G, Spagnoli A, Oh Y** 1999 The insulin-like growth factor binding protein superfamily: new perspectives. Pediatrics 104(4 Pt 2):1018–1021
28. **Jones JI, Clemmons DR** 1995 Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 16:3–34
29. **Butler AA, Yakar S, Gewolb IH, Karas M, Okubo Y, LeRoith D**

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1998 Insulin-like growth factor-I receptor signal transduction: at the interface between physiology and cell biology. Comp Biochem Physiol B Biochem Mol Biol 121:19–26
30. **Samani AA, Brodt P** 2001 The receptor for the type I insulin-like growth factor and its ligands regulate multiple cellular functions that impact on metastasis. Surg Oncol Clin N Am 10:289–312, viii
31. **Giorgetti S, Ballotti R, Kowalski-Chauvel A, Tartare S, Van Obberghen E** 1993 The insulin and insulin-like growth factor-I receptor substrate IRS-1 associates with and activates phosphatidylinositol 3-kinase in vitro. J Biol Chem 268:7358–7364
32. **Petley T, Graff K, Jiang W, Yang H, Florini J** 1999 Variation among cell types in the signaling pathways by which IGF-I stimulates specific cellular responses. Horm Metab Res 31:70–76
33. **Argetsinger LS, Hsu GW, Myers Jr MG, Billestrup N, White MF, Carter-Su C** 1995 Growth hormone, interferon-γ, and leukemia inhibitory factor promoted tyrosyl phosphorylation of insulin receptor substrate-1. J Biol Chem 270:14685–14692
34. **Myers Jr MG, Grammer TC, Wang LM, Sun XJ, Pierce JH, Blenis J, White MF** 1994 Insulin receptor substrate-1 mediates phosphatidylinositol 3′-kinase and p70S6k signaling during insulin, insulin-like growth factor-1, and interleukin-4 stimulation. J Biol Chem 269:28783–28789
35. **Vuori K, Ruoslahti E** 1994 Association of insulin receptor substrate-1 with integrins. Science 266:1576–1578
36. **Hermanto U, Zong CS, Wang LH** 2000 Inhibition of mitogen-activated protein kinase kinase selectively inhibits cell proliferation in human breast cancer cells displaying enhanced insulin-like growth factor I-mediated mitogen-activated protein kinase activation. Cell Growth Differ 11:655–664
37. **Grey A, Chen Q, Xu X, Callon K, Cornish J** 2003 Parallel phosphatidylinositol-3 kinase and p42/44 mitogen-activated protein kinase signaling pathways subserve the mitogenic and antiapoptotic actions of insulin-like growth factor I in osteoblastic cells. Endocrinology 144:4886–4893
38. **Gual P, Baron V, Lequoy V, Van Obberghen E** 1998 Interaction of Janus kinases JAK-1 and JAK-2 with the insulin receptor and the insulin-like growth factor-1 receptor. Endocrinology 139:884–893
39. **Zong CS, Chan J, Levy DE, Horvath C, Sadowski HB, Wang LH** 2000 Mechanism of STAT3 activation by insulin-like growth factor I receptor. J Biol Chem 275:15099–15105
40. **Zong CS, Zeng L, Jiang Y, Sadowski HB, Wang LH** 1998 Stat3 plays an important role in oncogenic Ros- and insulin-like growth factor I receptor-induced anchorage-independent growth. J Biol Chem 273:28065–28072
41. **Sekharam M, Nasir A, Kaiser HE, Coppola D** 2003 Insulin-like growth factor 1 receptor activates c-SRC and modifies transformation and motility of colon cancer in vitro. Anticancer Res 23:1517–1524
42. **Baron V, Calleja V, Ferrari P, Alengrin F, Van Obberghen E** 1998 p125Fak focal adhesion kinase is a substrate for the insulin and insulin-like growth factor-I tyrosine kinase receptors. J Biol Chem 273:7162–7168
43. **Koval AP, Karas M, Zick Y, LeRoith D** 1998 Interplay of the proto-oncogene proteins CrkL and CrkII in insulin-like growth factor-I receptor-mediated signal transduction. J Biol Chem 273:14780–14787
44. **Beitner-Johnson D, LeRoith D** 1995 Insulin-like growth factor-I stimulates tyrosine phosphorylation of endogenous c-Crk. J Biol Chem 270:5187–5190
45. **Casamassima A, Rozengurt E** 1998 Insulin-like growth factor I stimulates tyrosine phosphorylation of p130(Cas), focal adhesion kinase, and paxillin. Role of phosphatidylinositol 3′-kinase and formation of a p130(Cas).Crk complex. J Biol Chem 273:26149–26156
46. **Chattopadhyay A, Carpenter G** 2002 PLC-γ1 is required for IGF-I protection from cell death induced by loss of extracellular matrix adhesion. J Cell Sci 115:2233–2239
47. **Dupont J, Pierre A, Froment P, Moreau C** 2003 The insulin-like growth factor axis in cell cycle progression. Horm Metab Res 35:740–750
48. **Rosenthal SM, Cheng ZQ** 1995 Opposing early and late effects of insulin-like growth factor I on differentiation and the cell cycle regulatory retinoblastoma protein in skeletal myoblasts. Proc Natl Acad Sci USA 92:10307–10311
49. **Dupont J, Karas M, LeRoith D** 2000 The potentiation of estrogen on insulin-like growth factor I action in MCF-7 human breast cancer cells includes cell cycle components. J Biol Chem 275:35893–35901
50. **Lavoie JN, L'Allemain G, Brunet A, Muller R, Pouyssegur J** 1996 Cyclin D1 expression is regulated positively by the p42/p44MAPK and negatively by the p38/HOGMAPK pathway. J Biol Chem 271:20608–20616
51. **Hamelers IH, van Schaik RF, Sipkema J, Sussenbach JS, Steenbergh PH** 2002 Insulin-like growth factor I triggers nuclear accumulation of cyclin D1 in MCF-7S breast cancer cells. J Biol Chem 277:47645–47652
52. **Diehl JA, Cheng M, Roussel MF, Sherr CJ** 1998 Glycogen synthase kinase-3β regulates cyclin D1 proteolysis and subcellular localization. Genes Dev 12:3499–3511
53. **Muise-Helmericks RC, Grimes HL, Bellacosa A, Malstrom SE, Tsichlis PN, Rosen N** 1998 Cyclin D expression is controlled post-transcriptionally via a phosphatidylinositol 3-kinase/Akt-dependent pathway. J Biol Chem 273:29864–29872
54. **Coats S, Flanagan WM, Nourse J, Roberts JM** 1996 Requirement of p27Kip1 for restriction point control of the fibroblast cell cycle. Science 272:877–880
55. **Furlanetto RW, Harwell SE, Frick KK** 1994 Insulin-like growth factor-I induces cyclin-D1 expression in MG63 human osteosarcoma cells in vitro. Mol Endocrinol 8:510–517
56. **Maile LA, Clemmons DR** 2002 Regulation of insulin-like growth factor I receptor dephosphorylation by SHPS-1 and the tyrosine phosphatase SHP-2. J Biol Chem 277:8955–8960
57. **Myers Jr MG, Mendez R, Shi P, Pierce JH, Rhoads R, White MF** 1998 The COOH-terminal tyrosine phosphorylation sites on IRS-1 bind SHP-2 and negatively regulate insulin signaling. J Biol Chem 273:26908–26914
58. **Maile LA, Badley-Clarke J, Clemmons DR** 2003 The association between integrin-associated protein and SHPS-1 regulates insulin-like growth factor-I receptor signaling in vascular smooth muscle cells. Mol Biol Cell 14:3519–3528
59. **Ling Y, Maile LA, Lieskovska J, Badley-Clarke J, Clemmons DR** 2005 Role of SHPS-1 in the regulation of insulin-like growth factor I-stimulated Shc and mitogen-activated protein kinase activation in vascular smooth muscle cells. Mol Biol Cell 16:3353–3364
60. **Hermanto U, Zong CS, Li W, Wang LH** 2002 RACK1, an insulin-like growth factor I (IGF-I) receptor-interacting protein, modulates IGF-I-dependent integrin signaling and promotes cell spreading and contact with extracellular matrix. Mol Cell Biol 22:2345–2365
61. **Kiely PA, Sant A, O'Connor R** 2002 RACK1 is an insulin-like growth factor 1 (IGF-1) receptor-interacting protein that can regulate IGF-1-mediated Akt activation and protection from cell death. J Biol Chem 277:22581–22589
62. **Frangioni JV, Beahm PH, Shifrin V, Jost CA, Neel BG** 1992 The nontransmembrane tyrosine phosphatase PTP-1B localizes to the endoplasmic reticulum via its 35 amino acid C-terminal sequence. Cell 68:545–560
63. **Buckley DA, Cheng A, Kiely PA, Tremblay ML, O'Connor R** 2002 Regulation of insulin-like growth factor type I (IGF-I) receptor kinase activity by protein tyrosine phosphatase 1B (PTP-1B) and enhanced IGF-I-mediated suppression of apoptosis and motility in PTP-1B-deficient fibroblasts. Mol Cell Biol 22:1998–2010
64. **Goldstein BJ, Bittner-Kowalczyk A, White MF, Harbeck M** 2000 Tyrosine dephosphorylation and deactivation of insulin receptor substrate-1 by protein-tyrosine phosphatase 1B. Possible facilitation by the formation of a ternary complex with the Grb2 adaptor protein. J Biol Chem 275:4283–4289
65. **Cheng A, Dube N, Gu F, Tremblay ML** 2002 Coordinated action of protein tyrosine phosphatases in insulin signal transduction. Eur J Biochem 269:1050–1059
66. **Clemmons DR, Maile LA** 2003 Minireview: Integral membrane proteins that function coordinately with the insulin-like growth factor I receptor to regulate intracellular signaling. Endocrinology 144:1664–1670
67. **Maile LA, Clemmons DR** 2002 The αVβ3 integrin regulates insulin-like growth factor I (IGF-I) receptor phosphorylation by altering the rate of recruitment of the Src-homology 2-containing phospho-

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tyrosine phosphatase-2 to the activated IGF-I receptor. Endocrinology 143:4259–4264

68. **Ling Y, Maile LA, Clemmons DR** 2003 Tyrosine phosphorylation of the β3-subunit of the αVβ3 integrin is required for embrane association of the tyrosine phosphatase SHP-2 and its further recruitment to the insulin-like growth factor I receptor. Mol Endocrinol 17:1824–1833
69. **Monno S, Newman MV, Cook M, Lowe Jr WL** 2000 Insulin-like growth factor I activates c-Jun N-terminal kinase in MCF-7 breast cancer cells. Endocrinology 141:544–550
70. **Mamay CL, Mingo-Sion AM, Wolf DM, Molina MD, Van Den Berg CL** 2003 An inhibitory function for JNK in the regulation of IGF-I signaling in breast cancer. Oncogene 22:602–614
71. **Rotem-Yehudar R, Galperin E, Horowitz M** 2001 Association of insulin-like growth factor 1 receptor with EHD1 and SNAP29. J Biol Chem 276:33054–33060
72. **Lin FT, Daaka Y, Lefkowitz RJ** 1998 β-arrestins regulate mitogenic signaling and clathrin-mediated endocytosis of the insulin-like growth factor I receptor. J Biol Chem 273:31640–31643
73. **Maggi D, Biedi C, Segat D, Barbero D, Panetta D, Cordera R** 2002 IGF-I induces caveolin 1 tyrosine phosphorylation and translocation in the lipid rafts. Biochem Biophys Res Commun 295:1085–1089
74. **Biedi C, Panetta D, Segat D, Cordera R, Maggi D** 2003 Specificity of insulin-like growth factor I and insulin on Shc phosphorylation and Grb2 recruitment in caveolae. Endocrinology 144:5497–5503
75. **Huo H, Guo X, Hong S, Jiang M, Liu X, Liao K** 2003 Lipid rafts/caveolae are essential for insulin-like growth factor-1 receptor signaling during 3T3–L1 preadipocyte differentiation induction. J Biol Chem 278:11561–11569
76. **Desbuquois B, Chauvet G, Kouach M, Authier F** 2003 Cell itinerary and metabolic fate of proinsulin in rat liver: in vivo and in vitro studies. Endocrinology 144:5308–5321
77. **Navab R, Chevet E, Authier F, Di Guglielmo GM, Bergeron JJ, Brodt P** 2001 Inhibition of endosomal insulin-like growth factor-I processing by cysteine proteinase inhibitors blocks receptor-mediated functions. J Biol Chem 276:13644–13649
78. **Authier F, Kouach M, Briand G** 2005 Endosomal proteolysis of insulin-like growth factor-I at its C-terminal D-domain by cathepsin B. FEBS Lett 579:4309–4316
79. **Navab R, Pedraza C, Chevet E, Jenna S, Fallavollita L, Samani AA, Zhang D, Brodt P** 2004 Altered IGF-I receptor trafficking and signaling in carcinoma cells expressing reduced cathepsin L levels. Proc Am Soc Cell Biol No. 72
80. **Vecchione A, Marchese A, Henry P, Rotin D, Morrione A** 2003 The Grb10/Nedd4 complex regulates ligand-induced ubiquitination and stability of the insulin-like growth factor I receptor. Mol Cell Biol 23:3363–3372
81. **Charalambous M, Smith FM, Bennett WR, Crew TE, Mackenzie F, Ward A** 2003 Disruption of the imprinted Grb10 gene leads to disproportionate overgrowth by an Igf2-independent mechanism. Proc Natl Acad Sci USA 100:8292–8297
82. **Heron-Milhavet L, LeRoith D** 2002 Insulin-like growth factor I induces MDM2-dependent degradation of p53 via the p38 MAPK pathway in response to DNA damage. J Biol Chem 277:15600–15606
83. **Girnita L, Shenoy SK, Sehat B, Vasilcanu R, Girnita A, Lefkowitz RJ, Larsson O** 2005 {β}-Arrestin is crucial for ubiquitination and down-regulation of the insulin-like growth factor-1 receptor by acting as adaptor for the MDM2 E3 ligase. J Biol Chem 280:24412–24419
84. **Girnita L, Girnita A, Larsson O** 2003 Mdm2-dependent ubiquitination and degradation of the insulin-like growth factor 1 receptor. Proc Natl Acad Sci USA 100:8247–8252
85. **Bale LK, Conover CA** 1992 Regulation of insulin-like growth factor binding protein-3 messenger ribonucleic acid expression by insulin-like growth factor I. Endocrinology 131:608–614
86. **Cohick WS, Wang B, Verma P, Boisclair YR** 2000 Insulin-like growth factor I (IGF-I) and cyclic adenosine 3′,5′-monophosphate regulate IGF-binding protein-3 gene expression by transcriptional and posttranscriptional mechanisms in mammary epithelial cells. Endocrinology 141:4583–4591
87. **Ricort JM, Binoux M** 2002 Insulin-like growth factor-binding protein-3 activates a phosphotyrosine phosphatase. Effects on the insulin-like growth factor signaling pathway. J Biol Chem 277:19448–19454
88. **Lee HY, Chun KH, Liu B, Wiehle SA, Cristiano RJ, Hong WK, Cohen P, Kurie JM** 2002 Insulin-like growth factor binding protein-3 inhibits the growth of non-small cell lung cancer. Cancer Res 62:3530–3537
89. **Christoforidis A, Maniadaki I, Stanhope R** 2005 Growth hormone/insulin-like growth factor-1 axis during puberty. Pediatr Endocrinol Rev 3:5–10
90. **Wrobel G, Roerig P, Kokocinski F, Neben K, Hahn M, Reifenberger G, Lichter P** 2005 Microarray-based gene expression profiling of benign, atypical and anaplastic meningiomas identifies novel genes associated with meningioma progression. Int J Cancer 114:249–256
91. **Hartmann W, Koch A, Brune H, Waha A, Schuller U, Dani I, Denkhaus D, Langmann W, Bode U, Wiestler OD, Schilling K, Pietsch T** 2005 Insulin-like growth factor II is involved in the proliferation control of medulloblastoma and its cerebellar precursor cells. Am J Pathol 166:1153–1162
92. **Perks CM, Holly JM** 2003 The insulin-like growth factor (IGF) family and breast cancer. Breast Dis 18:45–60
93. **Perks CM, Holly JM** 2003 IGFBPs and breast cancer. Breast Dis 17:91–104
94. **Toniolo P, Bruning PF, Akhmedkhanov A, Bonfrer JM, Koenig KL, Lukanova A, Shore RE, Zeleniuch-Jacquotte A** 2000 Serum insulin-like growth factor-I and breast cancer. Int J Cancer 88:828–832
95. **Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, Rosner B, Speizer FE, Pollak M** 1998 Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 351:1393–1396
96. **Allen NE, Roddam AW, Allen DS, Fentiman IS, Dos Santos Silva I, Peto J, Holly JM, Key TJ** 2005 A prospective study of serum insulin-like growth factor-I (IGF-I), IGF-II, IGF-binding protein-3 and breast cancer risk. Br J Cancer 92:1283–1287
97. **Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, Hennekens CH, and Pollak M** 1998 Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science 279:563–566
98. **Stattin P, Bylund A, Rinaldi S, Biessy C, Dechaud H, Stenman UH, Egevad L, Riboli E, Hallmans G, Kaaks R** 2000 Plasma insulin-like growth factor-I, insulin-like growth factor-binding proteins, and prostate cancer risk: a prospective study. J Natl Cancer Inst 92:1910–1917
99. **Yu H, Spitz MR, Mistry J, Gu J, Hong WK, Wu X** 1999 Plasma levels of insulin-like growth factor-I and lung cancer risk: a case-control analysis. J Natl Cancer Inst 91:151–156
100. **Probst-Hensch NM, Yuan JM, Stanczyk FZ, Gao YT, Ross RK, Yu MC** 2001 IGF-1, IGF-2 and IGFBP-3 in prediagnostic serum: association with colorectal cancer in a cohort of Chinese men in Shanghai. Br J Cancer 85:1695–1699
101. **Palmqvist R, Hallmans G, Rinaldi S, Biessy C, Stenling R, Riboli E, Kaaks R** 2002 Plasma insulin-like growth factor 1, insulin-like growth factor binding protein 3, and risk of colorectal cancer: a prospective study in northern Sweden. Gut 50:642–646
102. **Ma J, Pollak MN, Giovannucci E, Chan JM, Tao Y, Hennekens CH, Stampfer MJ** 1999 Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J Natl Cancer Inst 91:620–625
103. **Petridou E, Koukoulomatis P, Alexe DM, Voulgaris Z, Spanos E, Trichopoulos D** 2003 Endometrial cancer and the IGF system: a case-control study in Greece. Oncology 64:341–345
104. **Zhao H, Grossman HB, Spitz MR, Lerner SP, Zhang K, Wu X** 2003 Plasma levels of insulin-like growth factor-1 and binding protein-3, and their association with bladder cancer risk. J Urol 169:714–717
105. **Cui H, Cruz-Correa M, Giardiello FM, Hutcheon DF, Kafonek DR, Brandenburg S, Wu Y, He X, Powe NR, Feinberg AP** 2003 Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. Science 299:1753–1755
106. **Giovannucci E, Pollak MN, Platz EA, Willett WC, Stampfer MJ, Majeed N, Colditz GA, Speizer FE, Hankinson SE** 2000 A prospective study of plasma insulin-like growth factor-1 and binding

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protein-3 and risk of colorectal neoplasia in women. Cancer Epidemiol Biomarkers Prev 9:345–349
107. **Wu X, Tortolero-Luna G, Zhao H, Phatak D, Spitz MR, Follen M** 2003 Serum levels of insulin-like growth factor I and risk of squamous intraepithelial lesions of the cervix. Clin Cancer Res 9:3356–3361
108. **Shimizu C, Hasegawa T, Tani Y, Takahashi F, Takeuchi M, Watanabe T, Ando M, Katsumata N, Fujiwara Y** 2004 Expression of insulin-like growth factor 1 receptor in primary breast cancer: immunohistochemical analysis. Hum Pathol 35:1537–1542
109. **Peyrat JP, Bonneterre J, Laurent JC, Louchez MM, Amrani S, Leroy-Martin B, Vilain MO, Delobelle A, Demaille A** 1988 Presence and characterization of insulin-like growth factor 1 receptors in human benign breast disease. Eur J Cancer Clin Oncol 24:1425–1431
110. **Railo MJ, von Smitten K, Pekonen F** 1994 The prognostic value of insulin-like growth factor-I in breast cancer patients. Results of a follow-up study on 126 patients. Eur J Cancer 30A:307–311
111. **Ueda S, Tsuda H, Sato K, Takeuchi H, Shigekawa T, Matsubara O, Hiraide H, Mochizuki H** 2006 Alternative tyrosine phosphorylation of signaling kinases according to hormone receptor status in breast cancer overexpressing the insulin-like growth factor receptor type 1. Cancer Sci 97:597–604
112. **Figueroa JA, De Raad S, Speights VO, Rinehart JJ** 2001 Gene expression of insulin-like growth factors and receptors in neoplastic prostate tissues: correlation with clinico-pathological parameters. Cancer Invest 19:28–34
113. **Tennant MK, Thrasher JB, Twomey PA, Drivdahl RH, Birnbaum RS, Plymate SR** 1996 Protein and messenger ribonucleic acid (mRNA) for the type 1 insulin-like growth factor (IGF) receptor is decreased and IGF-II mRNA is increased in human prostate carcinoma compared to benign prostate epithelium. J Clin Endocrinol Metab 81:3774–3782
114. **Hellawell GO, Turner GD, Davies DR, Poulsom R, Brewster SF, Macaulay VM** 2002 Expression of the type 1 insulin-like growth factor receptor is up-regulated in primary prostate cancer and commonly persists in metastatic disease. Cancer Res 62:2942–2950
115. **Barozzi C, Ravaioli M, D'Errico A, Grazi GL, Poggioli G, Cavrini G, Mazziotti A, Grigioni WF** 2002 Relevance of biologic markers in colorectal carcinoma: a comparative study of a broad panel. Cancer 94:647–657
116. **Hakam A, Yeatman TJ, Lu L, Mora L, Marcet G, Nicosia SV, Karl RC, Coppola D** 1999 Expression of insulin-like growth factor-1 receptor in human colorectal cancer. Hum Pathol 30:1128–1133
117. **Shiono S, Ishii G, Nagai K, Murata Y, Tsuta K, Nitadori J, Kodama T, Ochiai A** 2006 Immunohistochemical prognostic factors in resected colorectal lung metastases using tissue microarray analysis. Eur J Surg Oncol 32:308–309
118. **Xie Y, Skytting B, Nilsson G, Brodin B, Larsson O** 1999 Expression of insulin-like growth factor-1 receptor in synovial sarcoma: association with an aggressive phenotype. Cancer Res 59:3588–3591
119. **All-Ericsson C, Girnita L, Seregard S, Bartolazzi A, Jager MJ, Larsson O** 2002 Insulin-like growth factor-1 receptor in uveal melanoma: a predictor for metastatic disease and a potential therapeutic target. Invest Ophthalmol Vis Sci 43:1–8
120. **Jiang Y, Wang L, Gong W, Wei D, Le X, Yao J, Ajani J, Abbruzzese JL, Huang S, Xie K** 2004 A high expression level of insulin-like growth factor I receptor is associated with increased expression of transcription factor Sp1 and regional lymph node metastasis of human gastric cancer. Clin Exp Metastasis 21:755–764
121. **Kornprat P, Rehak P, Ruschoff J, Langner C** 2006 Expression of IGF-I, IGF-II, and IGF-IR in gallbladder carcinoma. A systematic analysis including primary and corresponding metastatic tumours. J Clin Pathol 59:202–206
122. **Parker A, Cheville JC, Lohse C, Cerhan JR, Blute ML** 2003 Expression of insulin-like growth factor I receptor and survival in patients with clear cell renal cell carcinoma. J Urol 170:420–424
123. **Parker AS, Cheville JC, Janney CA, Cerhan JR** 2002 High expression levels of insulin-like growth factor-I receptor predict poor survival among women with clear-cell renal cell carcinomas. Hum Pathol 33:801–805
124. **Gicquel C, Bertagna X, Gaston V, Coste J, Louvel A, Baudin E, Bertherat J, Chapuis Y, Duclos JM, Schlumberger M, Plouin PF, Luton JP, Le Bouc Y** 2001 Molecular markers and long-term recurrences in a large cohort of patients with sporadic adrenocortical tumors. Cancer Res 61:6762–6767
125. **Sayer RA, Lancaster JM, Pittman J, Gray J, Whitaker R, Marks JR, Berchuck A** 2005 High insulin-like growth factor-2 (IGF-2) gene expression is an independent predictor of poor survival for patients with advanced stage serous epithelial ovarian cancer. Gynecol Oncol 96:355–361
126. **Shariat SF, Kim J, Nguyen C, Wheeler TM, Lerner SP, Slawin KM** 2003 Correlation of preoperative levels of IGF-I and IGFBP-3 with pathologic parameters and clinical outcome in patients with bladder cancer. Urology 61:359–364
127. **Miyata Y, Sakai H, Hayashi T, Kanetake H** 2003 Serum insulin-like growth factor binding protein-3/prostate-specific antigen ratio is a useful predictive marker in patients with advanced prostate cancer. Prostate 54:125–132
128. **Chambers AF, Groom AC, MacDonald IC** 2002 Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer 2:563–572
129. **Feldser D, Agani F, Iyer NV, Pak B, Ferreira G, Semenza GL** 1999 Reciprocal positive regulation of hypoxia-inducible factor 1α and insulin-like growth factor 2. Cancer Res 59:3915–3918
130. **Zelzer E, Levy Y, Kahana C, Shilo BZ, Rubinstein M, Cohen B** 1998 Insulin induces transcription of target genes through the hypoxia-inducible factor HIF-1α/ARNT. EMBO J 17:5085–5094
131. **Fukuda R, Hirota K, Fan F, Jung YD, Ellis LM, Semenza GL** 2002 Insulin-like growth factor 1 induces hypoxia-inducible factor 1-mediated vascular endothelial growth factor expression, which is dependent on MAP kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells. J Biol Chem 277:38205–38211
132. **Shigematsu S, Yamauchi K, Nakajima K, Iijima S, Aizawa T, Hashizume K** 1999 IGF-1 regulates migration and angiogenesis of human endothelial cells. Endocr J 46 Suppl: S59–S62
133. **Lee OH, Bae SK, Bae MH, Lee YM, Moon EJ, Cha HJ, Kwon YG, Kim KW** 2000 Identification of angiogenic properties of insulin-like growth factor II in in vitro angiogenesis models. Br J Cancer 82: 385–391
134. **Grulich-Henn J, Ritter J, Mesewinkel S, Heinrich U, Bettendorf M, Preissner KT** 2002 Transport of insulin-like growth factor-I across endothelial cell monolayers and its binding to the subendothelial matrix. Exp Clin Endocrinol Diabetes 110:67–73
135. **Kondo T, Vicent D, Suzuma K, Yanagisawa M, King GL, Holzenberger M, Kahn CR** 2003 Knockout of insulin and IGF-1 receptors on vascular endothelial cells protects against retinal neovascularization. J Clin Invest 111:1835–1842
136. **Moromisato DY, Moromisato MY, Zanconato S, Roberts Jr CT** 1996 Effect of hypoxia on lung, heart, and liver insulin-like growth factor-I gene and receptor expression in the newborn rat. Crit Care Med 24:919–924
137. **Kim KW, Bae SK, Lee OH, Bae MH, Lee MJ, Park BC** 1998 Insulin-like growth factor II induced by hypoxia may contribute to angiogenesis of human hepatocellular carcinoma. Cancer Res 58: 348–351
138. **Bae SK, Bae MH, Ahn MY, Son MJ, Lee YM, Bae MK, Lee OH, Park BC, Kim KW** 1999 Egr-1 mediates transcriptional activation of IGF-II gene in response to hypoxia. Cancer Res 59:5989–5994
139. **Peretz S, Kim C, Rockwell S, Baserga R, Glazer PM** 2002 IGF1 receptor expression protects against microenvironmental stress found in the solid tumor. Radiat Res 158:174–180
140. **Clarke K, Smith K, Gullick WJ, Harris AL** 2001 Mutant epidermal growth factor receptor enhances induction of vascular endothelial growth factor by hypoxia and insulin-like growth factor-1 via a PI3 kinase dependent pathway. Br J Cancer 84:1322–1329
141. **Mandriota SJ, Jussila L, Jeltsch M, Compagni A, Baetens D, Prevo R, Banerji S, Huarte J, Montesano R, Jackson DG, Orci L, Alitalo K, Christofori G, Pepper MS** 2001 Vascular endothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis. EMBO J 20:672–682
142. **Stacker SA, Caesar C, Baldwin ME, Thornton GE, Williams RA, Prevo R, Jackson DG, Nishikawa S, Kubo H, Achen MG** 2001 VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. Nat Med 7:186–191
143. **Jussila L, Alitalo K** 2002 Vascular growth factors and lymphangiogenesis. Physiol Rev 82:673–700

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144. **Nisato RE, Tille JC, Pepper MS** 2003 Lymphangiogenesis and tumor metastasis. Thromb Haemost 90:591–597
145. **Tang Y, Zhang D, Fallavollita L, Brodt P** 2003 Vascular endothelial growth factor C expression and lymph node metastasis are regulated by the type I insulin-like growth factor receptor. Cancer Res 63:1166–1171
146. **Bjorndahl M, Cao R, Nissen LJ, Clasper S, Johnson LA, Xue Y, Zhou Z, Jackson D, Hansen AJ, Cao Y** 2005 Insulin-like growth factors 1 and 2 induce lymphangiogenesis in vivo. Proc Natl Acad Sci USA 102:15593–15598
147. **Zhang D, Samani AA, Brodt P** 2003 The role of the IGF-I receptor in the regulation of matrix metalloproteinases, tumor invasion and metastasis. Horm Metab Res 35:802–808
148. **Zhang D, Bar-Eli M, Meloche S, Brodt P** 2004 Dual regulation of MMP-2 expression by the type 1 insulin-like growth factor receptor: the phosphatidylinositol 3-kinase/Akt and Raf/ERK pathways transmit opposing signals. J Biol Chem 279:19683–19690
149. **Yoon A, Hurta RA** 2001 Insulin like growth factor-1 selectively regulates the expression of matrix metalloproteinase-2 in malignant H-ras transformed cells. Mol Cell Biochem 223:1–6
150. **Long L, Navab R, Brodt P** 1998 Regulation of the Mr 72,000 type IV collagenase by the type I insulin-like growth factor receptor. Cancer Res 58:3243–3247
151. **Grzmil M, Hemmerlein B, Thelen P, Schweyer S, Burfeind P** 2004 Blockade of the type I IGF receptor expression in human prostate cancer cells inhibits proliferation and invasion, up-regulates IGF binding protein-3, and suppresses MMP-2 expression. J Pathol 202:50–59
152. **Brodt P, Fallavollita L, Khatib AM, Samani AA, Zhang D** 2001 Cooperative regulation of the invasive and metastatic phenotypes by different domains of the type I insulin-like growth factor receptor β subunit. J Biol Chem 276:33608–33615
153. **Zhang D, Brodt P** 2003 Type 1 insulin-like growth factor regulates MT1-MMP synthesis and tumor invasion via PI 3-kinase/Akt signaling. Oncogene 22:974–982
154. **Mira E, Manes S, Lacalle RA, Marquez G, Martinez AC** 1999 Insulin-like growth factor I-triggered cell migration and invasion are mediated by matrix metalloproteinase-9. Endocrinology 140: 1657–1664
155. **Lupia E, Elliot SJ, Lenz O, Zheng F, Hattori M, Striker GE, Striker LJ** 1999 IGF-1 decreases collagen degradation in diabetic NOD mesangial cells: implications for diabetic nephropathy. Diabetes 48:1638–1644
156. **Allen DL, Teitelbaum DH, Kurachi K** 2003 Growth factor stimulation of matrix metalloproteinase expression and myoblast migration and invasion in vitro. Am J Physiol Cell Physiol 284:C805–C815
157. **Dunn SE, Torres JV, Nihei N, Barrett JC** 2000 The insulin-like growth factor-1 elevates urokinase-type plasminogen activator-1 in human breast cancer cells: a new avenue for breast cancer therapy. Mol Carcinog 27:10–17
158. **Fowlkes JL, Enghild JJ, Suzuki K, Nagase H** 1994 Matrix metalloproteinases degrade insulin-like growth factor-binding protein-3 in dermal fibroblast cultures. J Biol Chem 269:25742–25746
159. **Thrailkill KM, Quarles LD, Nagase H, Suzuki K, Serra DM, Fowlkes JL** 1995 Characterization of insulin-like growth factor-binding protein 5-degrading proteases produced throughout murine osteoblast differentiation. Endocrinology 136:3527–3533
160. **Manes S, Llorente M, Lacalle RA, Gomez-Mouton C, Kremer L, Mira E, Martinez AC** 1999 The matrix metalloproteinase-9 regulates the insulin-like growth factor-triggered autocrine response in DU-145 carcinoma cells. J Biol Chem 274:6935–6945
161. **Martin DC, Fowlkes JL, Babic B, Khokha R** 1999 Insulin-like growth factor II signaling in neoplastic proliferation is blocked by transgenic expression of the metalloproteinase inhibitor TIMP-1. J Cell Biol 146:881–892
162. **Hemers E, Duval C, McCaig C, Handley M, Dockray GJ, Varro A** 2005 Insulin-like growth factor binding protein-5 is a target of matrix metalloproteinase-7: implications for epithelial-mesenchymal signaling. Cancer Res 65:7363–7369
163. **Nakamura M, Miyamoto S, Maeda H, Ishii G, Hasebe T, Chiba T, Asaka M, Ochiai A** 2005 Matrix metalloproteinase-7 degrades all insulin-like growth factor binding proteins and facilitates insulin-like growth factor bioavailability. Biochem Biophys Res Commun 333:1011–1016
164. **Miyamoto S, Yano K, Sugimoto S, Ishii G, Hasebe T, Endoh Y, Kodama K, Goya M, Chiba T, Ochiai A** 2004 Matrix metalloproteinase-7 facilitates insulin-like growth factor bioavailability through its proteinase activity on insulin-like growth factor binding protein 3. Cancer Res 64:665–671
165. **Bauer TW, Liu W, Fan F, Camp ER, Yang A, Somcio RJ, Bucana CD, Callahan J, Parry GC, Evans DB, Boyd DD, Mazar AP, Ellis LM** 2005 Targeting of urokinase plasminogen activator receptor in human pancreatic carcinoma cells inhibits c-Met- and insulin-like growth factor-I receptor-mediated migration and invasion and orthotopic tumor growth in mice. Cancer Res 65:7775–7781
166. **Chernicky CL, Tan H, Yi L, Loret de Mola JR, Ilan J** 2002 Treatment of murine breast cancer cells with antisense RNA to the type I insulin-like growth factor receptor decreases the level of plasminogen activator transcripts, inhibits cell growth in vitro, and reduces tumorigenesis in vivo. Mol Pathol 55:102–109
167. **Dunn SE, Torres JV, Oh JS, Cykert DM, Barrett JC** 2001 Up-regulation of urokinase-type plasminogen activator by insulin-like growth factor-I depends upon phosphatidylinositol-3 kinase and mitogen-activated protein kinase kinase. Cancer Res 61:1367–1374
168. **Gallicchio MA, Kaun C, Wojta J, Binder B, Bach LA** 2003 Urokinase type plasminogen activator receptor is involved in insulin-like growth factor-induced migration of rhabdomyosarcoma cells in vitro. J Cell Physiol 197:131–138
169. **Chambers AF, Naumov GN, Varghese HJ, Nadkarni KV, MacDonald IC, Groom AC** 2001 Critical steps in hematogenous metastasis: an overview. Surg Oncol Clin N Am 10:243–255, vii
170. **Samani AA, Chevet E, Fallavollita L, Galipeau J, Brodt P** 2004 Loss of tumorigenicity and metastatic potential in carcinoma cells expressing the extracellular domain of the type 1 insulin-like growth factor receptor. Cancer Res 64:3380–3385
171. **Wu Y, Yakar S, Zhao L, Hennighausen L, LeRoith D** 2002 Circulating insulin-like growth factor-I levels regulate colon cancer growth and metastasis. Cancer Res 62:1030–1035
172. **Kaplan PJ, Mohan S, Cohen P, Foster BA, Greenberg NM** 1999 The insulin-like growth factor axis and prostate cancer: lessons from the transgenic adenocarcinoma of mouse prostate (TRAMP) model. Cancer Res 59:2203–2209
173. **LeBedis C, Chen K, Fallavollita L, Boutros T, Brodt P** 2002 Peripheral lymph node stromal cells can promote growth and tumorigenicity of breast carcinoma cells through the release of IGF-I and EGF. Int J Cancer 100:2–8
174. **Bubendorf L, Schopfer A, Wagner U, Sauter G, Moch H, Willi N, Gasser TC, Mihatsch MJ** 2000 Metastatic patterns of prostate cancer: an autopsy study of 1,589 patients. Hum Pathol 31:578–583
175. **Chen Y, Shu H, Ji C, Casinghino S, Kim K, Gundberg CM, Centrella M, McCarthy TL** 1998 Insulin-like growth factor binding proteins localize to discrete cell culture compartments in periosteal and osteoblast cultures from fetal rat bone. J Cell Biochem 71:351–362
176. **Fiorelli G, Orlando C, Benvenuti S, Franceschelli F, Bianchi S, Pioli P, Tanini A, Serio M, Bartucci F, Brandi ML** 1994 Characterization, regulation, and function of specific cell membrane receptors for insulin-like growth factor I on bone endothelial cells. J Bone Miner Res 9:329–337
177. **McCarthy TL, Casinghino S, Centrella M, Canalis E** 1994 Complex pattern of insulin-like growth factor binding protein expression in primary rat osteoblast enriched cultures: regulation by prostaglandin E2, growth hormone, and the insulin-like growth factors. J Cell Physiol 160:163–175
178. **Koutsilieris M, Polychronakos C** 1992 Proteinolytic activity against IGF-binding proteins involved in the paracrine interactions between prostate adenocarcinoma cells and osteoblasts. Anticancer Res 12:905–910
179. **Koeneman KS, Yeung F, Chung LW** 1999 Osteomimetic properties of prostate cancer cells: a hypothesis supporting the predilection of prostate cancer metastasis and growth in the bone environment. Prostate 39:246–261
180. **Fizazi K, Yang J, Peleg S, Sikes CR, Kreimann EL, Daliani D, Olive M, Raymond KA, Janus TJ, Logothetis CJ, Karsenty G, Navone NM** 2003 Prostate cancer cells-osteoblast interaction shifts

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expression of growth/survival-related genes in prostate cancer and reduces expression of osteoprotegerin in osteoblasts. Clin Cancer Res 9:2587–2597
181. **Shariat SF, Lamb DJ, Kattan MW, Nguyen C, Kim J, Beck J, Wheeler TM, Slawin KM** 2002 Association of preoperative plasma levels of insulin-like growth factor I and insulin-like growth factor binding proteins-2 and -3 with prostate cancer invasion, progression, and metastasis. J Clin Oncol 20:833–841
182. **DiGiovanni J, Kiguchi K, Frijhoff A, Wilker E, Bol DK, Beltran L, Moats S, Ramirez A, Jorcano J, Conti C** 2000 Deregulated expression of insulin-like growth factor 1 in prostate epithelium leads to neoplasia in transgenic mice. Proc Natl Acad Sci USA 97:3455–3460
183. **Wilker E, Bol D, Kiguchi K, Rupp T, Beltran L, DiGiovanni J** 1999 Enhancement of susceptibility to diverse skin tumor promoters by activation of the insulin-like growth factor-1 receptor in the epidermis of transgenic mice. Mol Carcinog 25:122–131
184. **Wilker E, Lu J, Rho O, Carbajal S, Beltran L, DiGiovanni J** 2005 Role of PI3K/Akt signaling in insulin-like growth factor-1 (IGF-1) skin tumor promotion. Mol Carcinog 44:137–145
185. **Hadsell DL, Murphy KL, Bonnette SG, Reece N, Laucirica R, Rosen JM** 2000 Cooperative interaction between mutant p53 and des(1–3)IGF-I accelerates mammary tumorigenesis. Oncogene 19: 889–898
186. **Carboni JM, Lee AV, Hadsell DL, Rowley BR, Lee FY, Bol DK, Camuso AE, Gottardis M, Greer AF, Ho CP, Hurlburt W, Li A, Saulnier M, Velaparthi U, Wang C, Wen ML, Westhouse RA, Wittman M, Zimmermann K, Rupnow BA, Wong TW** 2005 Tumor development by transgenic expression of a constitutively active insulin-like growth factor I receptor. Cancer Res 65:3781–3787
187. **Wang YZ, Wong YC** 1998 Sex hormone-induced prostatic carcinogenesis in the noble rat: the role of insulin-like growth factor-I (IGF-I) and vascular endothelial growth factor (VEGF) in the development of prostate cancer. Prostate 35:165–177
188. **Moorehead RA, Hojilla CV, De Belle I, Wood GA, Fata JE, Adamson ED, Watson KL, Edwards DR, Khokha R** 2003 Insulin-like growth factor-II regulates PTEN expression in the mammary gland. J Biol Chem 278:50422–50427
189. **Christofori G, Naik P, Hanahan D** 1995 Deregulation of both imprinted and expressed alleles of the insulin-like growth factor 2 gene during β-cell tumorigenesis. Nat Genet 10:196–201
190. **Lopez T, Hanahan D** 2002 Elevated levels of IGF-1 receptor convey invasive and metastatic capability in a mouse model of pancreatic islet tumorigenesis. Cancer Cell 1:339–353
191. **Sakatani T, Kaneda A, Iacobuzio-Donahue CA, Carter MG, de Boom Witzel S, Okano H, Ko MS, Ohlsson R, Longo DL, Feinberg AP** 2005 Loss of imprinting of Igf2 alters intestinal maturation and tumorigenesis in mice. Science 307:1976–1978
192. **Yang XF, Beamer WG, Huynh H, Pollak M** 1996 Reduced growth of human breast cancer xenografts in hosts homozygous for the lit mutation. Cancer Res 56:1509–1511
193. **Pollak M, Blouin MJ, Zhang JC, Kopchick JJ** 2001 Reduced mammary gland carcinogenesis in transgenic mice expressing a growth hormone antagonist. Br J Cancer 85:428–430
194. **Wu Y, Cui K, Miyoshi K, Hennighausen L, Green JE, Setser J, LeRoith D, Yakar S** 2003 Reduced circulating insulin-like growth factor I levels delay the onset of chemically and genetically induced mammary tumors. Cancer Res 63:4384–4388
195. **Dai Z, Xing Y, Boney CM, Clemmons DR, D'Ercole AJ** 1994 Human insulin-like growth factor-binding protein-1 (hIGFBP-1) in transgenic mice: characterization and insights into the regulation of IGFBP-1 expression. Endocrinology 135:1316–1327
196. **Lu S, Archer MC** 2003 Insulin-like growth factor binding protein-1 over-expression in transgenic mice inhibits hepatic preneoplasia. Mol Carcinog 36:142–146
197. **Dunn SE, Kari FW, French J, Leininger JR, Travlos G, Wilson R, Barrett JC** 1997 Dietary restriction reduces insulin-like growth factor I levels, which modulates apoptosis cell proliferation, and tumor progression in p53-deficient mice. Cancer Res 57:4667–4672
198. **Hursting SD, Switzer BR, French JE, Kari FW** 1993 The growth hormone: insulin-like growth factor 1 axis is a mediator of diet restriction-induced inhibition of mononuclear cell leukemia in Fischer rats. Cancer Res 53:2750–2757
199. **Yakar S, Nunez NP, Pennisi P, Brodt P, Sun H, Fallavollita L, Zhao H, Scavo L, Novosyadlyy R, Kurshan N, Stannard B, East-Palmer J, Smith NC, Perkins SN, Fuchs-Young R, Barrett JC, Hursting SD, LeRoith D,**Increased tumor growth in mice with diet-induced obesity: impact of ovarian hormones. Endocrinology, in press
200. **Gil-Ad I, Shtaif B, Luria D, Karp L, Fridman Y, Weizman A** 1999 Insulin-like-growth-factor-I (IGF-I) antagonizes apoptosis induced by serum deficiency and doxorubicin in neuronal cell culture. Growth Horm IGF Res 9:458–464
201. **Gooch JL, Van Den Berg CL, Yee D** 1999 Insulin-like growth factor (IGF)-I rescues breast cancer cells from chemotherapy-induced cell death–proliferative and anti-apoptotic effects. Breast Cancer Res Treat 56:1–10
202. **Jiang Y, Rom WN, Yie TA, Chi CX, Tchou-Wong KM** 1999 Induction of tumor suppression and glandular differentiation of A549 lung carcinoma cells by dominant-negative IGF-I receptor. Oncogene 18:6071–6077
203. **Kuhn C, Hurwitz SA, Kumar MG, Cotton J, Spandau DF** 1999 Activation of the insulin-like growth factor-1 receptor promotes the survival of human keratinocytes following ultraviolet B irradiation. Int J Cancer 80:431–438
204. **Liu Y, Lehar S, Corvi C, Payne G, O'Connor R** 1998 Expression of the insulin-like growth factor I receptor C terminus as a myristylated protein leads to induction of apoptosis in tumor cells. Cancer Res 58:570–576
205. **Peters JM, Tsark EC, Wiley LM** 1996 Radiosensitive target in the mouse embryo chimera assay: implications that the target involves autocrine growth factor function. Radiat Res 145:722–729
206. **Guo YS, Jin GF, Houston CW, Thompson JC, Townsend Jr CM** 1998 Insulin-like growth factor-I promotes multidrug resistance in MCLM colon cancer cells. J Cell Physiol 175:141–148
207. **Yu D, Watanabe H, Shibuya H, Miura M** 2003 Redundancy of radioresistant signaling pathways originating from insulin-like growth factor I receptor. J Biol Chem 278:6702–6709
208. **Criswell T, Beman M, Araki S, Leskov K, Cataldo E, Mayo LD, Boothman DA** 2005 Delayed activation of insulin-like growth factor-1 receptor/Src/MAPK/Egr-1 signaling regulates clusterin expression, a pro-survival factor. J Biol Chem 280:14212–14221
209. **Jamieson TA, Brizel DM, Killian JK, Oka Y, Jang HS, Fu X, Clough RW, Vollmer RT, Anscher MS, Jirtle RL** 2003 M6P/IGF2R loss of heterozygosity in head and neck cancer associated with poor patient prognosis. BMC Cancer 3:4
210. **Krassas GE, Pontikides N, Kaltsas T, Dumas A, Frystyk J, Chen JW, Flyvbjerg A** 2003 Free and total insulin-like growth factor (IGF)-I, -II, and IGF binding protein-1, -2, and -3 serum levels in patients with active thyroid eye disease. J Clin Endocrinol Metab 88:132–135
211. **Ogawa O, Becroft DM, Morison IM, Eccles MR, Skeen JE, Mauger DC, Reeve AE** 1993 Constitutional relaxation of insulin-like growth factor II gene imprinting associated with Wilms' tumour and gigantism. Nat Genet 5:408–412
212. **Coutinho-Camillo CM, Brentani MM, Butugan O, Torloni H, Nagai MA** 2003 Relaxation of imprinting of IGFII gene in juvenile nasopharyngeal angiofibromas. Diagn Mol Pathol 12:57–62
213. **Vorwerk P, Wex H, Bessert C, Hohmann B, Schmidt U, Mittler U** 2003 Loss of imprinting of IGF-II gene in children with acute lymphoblastic leukemia. Leuk Res 27:807–812
214. **Zhang J, Trent JM, Meltzer PS** 1993 Rapid isolation and characterization of amplified DNA by chromosome microdissection: identification of IGF1R amplification in malignant melanoma. Oncogene 8:2827–2831
215. **Almeida A, Muleris M, Dutrillaux B, Malfoy B** 1994 The insulin-like growth factor I receptor gene is the target for the 15q26 amplicon in breast cancer. Genes Chromosomes Cancer 11:63–65
216. **Armengol G, Knuutila S, Lluis F, Capella G, Miro R, Caballin MR** 2000 DNA copy number changes and evaluation of MYC, IGF1R, and FES amplification in xenografts of pancreatic adenocarcinoma. Cancer Genet Cytogenet 116:133–141
217. **Bassi DE, Fu J, Lopez de Cicco R, Klein-Szanto AJ** 2005 Proprotein convertases: "master switches" in the regulation of tumor growth and progression. Mol Carcinog 44:151–161
218. **Duguay SJ, Milewski WM, Young BD, Nakayama K, Steiner DF**

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1997 Processing of wild-type and mutant proinsulin-like growth factor-IA by subtilisin-related proprotein convertases. J Biol Chem 272:6663–6670
219. **Lehmann M, Andre F, Bellan C, Remacle-Bonnet M, Garrouste F, Parat F, Lissitsky JC, Marvaldi J, Pommier G** 1998 Deficient processing and activity of type I insulin-like growth factor receptor in the furin-deficient LoVo-C5 cells. Endocrinology 139:3763–3771
220. **Khatib AM, Siegfried G, Prat A, Luis J, Chretien M, Metrakos P, Seidah NG** 2001 Inhibition of proprotein convertases is associated with loss of growth and tumorigenicity of HT-29 human colon carcinoma cells: importance of insulin-like growth factor-1 (IGF-1) receptor processing in IGF-1-mediated functions. J Biol Chem 276: 30686–30693
221. **Duguay SJ, Jin Y, Stein J, Duguay AN, Gardner P, Steiner DF** 1998 Post-translational processing of the insulin-like growth factor-2 precursor. Analysis of O-glycosylation and endoproteolysis. J Biol Chem 273:18443–18451
222. **Alarcon C, Cheatham B, Lincoln B, Kahn CR, Siddle K, Rhodes CJ** 1994 A Kex2-related endopeptidase activity present in rat liver specifically processes the insulin proreceptor. Biochem J 301(Pt 1):257–265
223. **Yana I, Weiss SJ** 2000 Regulation of membrane type-1 matrix metalloproteinase activation by proprotein convertases. Mol Biol Cell 11:2387–2401
224. **Mercapide J, Lopez De Cicco R, Bassi DE, Castresana JS, Thomas G, Klein-Szanto AJ** 2002 Inhibition of furin-mediated processing results in suppression of astrocytoma cell growth and invasiveness. Clin Cancer Res 8:1740–1746
225. **Cooke DW, Bankert LA, Roberts Jr CT, LeRoith D, Casella SJ** 1991 Analysis of the human type I insulin-like growth factor receptor promoter region. Biochem Biophys Res Commun 177:1113–1120
226. **Rietveld LE, Koonen-Reemst AM, Sussenbach JS, Holthuizen PE** 1999 Dual role for transcription factor AP-2 in the regulation of the major fetal promoter P3 of the gene for human insulin-like growth factor II. Biochem J 338(Pt 3):799–806
227. **Bosher JM, Totty NF, Hsuan JJ, Williams T, Hurst HC** 1996 A family of AP-2 proteins regulates c-erbB-2 expression in mammary carcinoma. Oncogene 13:1701–1707
228. **Turner BC, Zhang J, Gumbs AA, Maher MG, Kaplan L, Carter D, Glazer PM, Hurst HC, Haffty BG, Williams T** 1998 Expression of AP-2 transcription factors in human breast cancer correlates with the regulation of multiple growth factor signalling pathways. Cancer Res 58:5466–5472
229. **Zhang L, Zhan S, Navid F, Li Q, Choi YH, Kim M, Seth P, Helman LJ** 1998 AP-2 may contribute to IGF-II overexpression in rhabdomyosarcoma. Oncogene 17:1261–1270
230. **Hakomori S** 2002 Glycosylation defining cancer malignancy: new wine in an old bottle. Proc Natl Acad Sci USA 99:10231–10233
231. **Gammeltoft S, Ballotti R, Kowalski A, Westermark B, Van Obberghen E** 1988 Expression of two types of receptor for insulin-like growth factors in human malignant glioma. Cancer Res 48:1233–1237
232. **Ota A, Wilson GL, Leroith D** 1988 Insulin-like growth factor I receptors on mouse neuroblastoma cells. Two β subunits are derived from differences in glycosylation. Eur J Biochem 174:521–530
233. **Alexandrides TK, Smith RJ** 1989 A novel fetal insulin-like growth factor (IGF) I receptor. Mechanism for increased IGF I- and insulin-stimulated tyrosine kinase activity in fetal muscle. J Biol Chem 264:12922–12930
234. **Denley A, Wallace JC, Cosgrove LJ, Forbes BE** 2003 The insulin receptor isoform exon 11- (IR-A) in cancer and other diseases: a review. Horm Metab Res 35:778–785
235. **Pandini G, Conte E, Medico E, Sciacca L, Vigneri R, Belfiore A** 2004 IGF-II binding to insulin receptor isoform A induces a partially different gene expression profile from insulin binding. Ann NY Acad Sci 1028:450–456
236. **Sciacca L, Mineo R, Pandini G, Murabito A, Vigneri R, Belfiore A** 2002 In IGF-I receptor-deficient leiomyosarcoma cells autocrine IGF-II induces cell invasion and protection from apoptosis via the insulin receptor isoform A. Oncogene 21:8240–8250
237. **Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M** 2004 Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 363:1346–1353
238. **Yu H, Rohan T** 2000 Role of the insulin-like growth factor family in cancer development and progression. J Natl Cancer Inst 92: 1472–1489
239. **Chang YS, Wang L, Liu D, Mao L, Hong WK, Khuri FR, Lee HY** 2002 Correlation between insulin-like growth factor-binding protein-3 promoter methylation and prognosis of patients with stage I non-small cell lung cancer. Clin Cancer Res 8:3669–3675
240. **Chang YS, Kong G, Sun S, Liu D, El-Naggar AK, Khuri FR, Hong WK, Lee HY** 2002 Clinical significance of insulin-like growth factor-binding protein-3 expression in stage I non-small cell lung cancer. Clin Cancer Res 8:3796–3802
241. **Brodt P, Reich R, Moroz LA, Chambers AF** 1992 Differences in the repertoires of basement membrane degrading enzymes in two carcinoma sublines with distinct patterns of site-selective metastasis. Biochim Biophys Acta 1139:77–83
242. **Zwad O, Kubler B, Roth W, Scharf JG, Saftig P, Peters C, Braulke T** 2002 Decreased intracellular degradation of insulin-like growth factor binding protein-3 in cathepsin L-deficient fibroblasts. FEBS Lett 510:211–215
243. **Kiepe D, Ciarmatori S, Hoeflich A, Wolf E, Tonshoff B** 2005 Insulin-like growth factor (IGF)-I stimulates cell proliferation and induces IGF binding protein (IGFBP)-3 and IGFBP-5 gene expression in cultured growth plate chondrocytes via distinct signaling pathways. Endocrinology 146:3096–3104
244. **Yi HK, Kim SY, Hwang PH, Kim CY, Yang DH, Oh Y, Lee DY** 2005 Impact of PTEN on the expression of insulin-like growth factors (IGFs) and IGF-binding proteins in human gastric adenocarcinoma cells. Biochem Biophys Res Commun 330:760–767
245. **Sunic D, McNeil JD, Rayner TE, Andress DL, Belford DA** 1998 Regulation of insulin-like growth factor-binding protein-5 by insulin-like growth factor I and interleukin-1α in ovine articular chondrocytes. Endocrinology 139:2356–2362
246. **Loughran G, Huigsloot M, Kiely PA, Smith LM, Floyd S, Ayllon V, O'Connor R** 2005 Gene expression profiles in cells transformed by overexpression of the IGF-I receptor. Oncogene 24:6185–6193
247. **Mason HD, Margara R, Winston RM, Seppala M, Koistinen R, Franks S** 1993 Insulin-like growth factor-I (IGF-I) inhibits production of IGF-binding protein-1 while stimulating estradiol secretion in granulosa cells from normal and polycystic human ovaries. J Clin Endocrinol Metab 76:1275–1279
248. **Conover CA, Lee PD, Kanaley JA, Clarkson JT, Jensen MD** 1992 Insulin regulation of insulin-like growth factor binding protein-1 in obese and nonobese humans. J Clin Endocrinol Metab 74:1355–1360
249. **Chi MM, Schlein AL, Moley KH** 2000 High insulin-like growth factor 1 (IGF-1) and insulin concentrations trigger apoptosis in the mouse blastocyst via down-regulation of the IGF-1 receptor. Endocrinology 141:4784–4792
250. **Kalli KR, Falowo OI, Bale LK, Zschunke MA, Roche PC, Conover CA** 2002 Functional insulin receptors on human epithelial ovarian carcinoma cells: implications for IGF-II mitogenic signaling. Endocrinology 143:3259–3267
251. **Chang Q, Li Y, White MF, Fletcher JA, Xiao S** 2002 Constitutive activation of insulin receptor substrate 1 is a frequent event in human tumors: therapeutic implications. Cancer Res 62:6035–6038
252. **Surmacz E, Burgaud JL** 1995 Overexpression of insulin receptor substrate 1 (IRS-1) in the human breast cancer cell line MCF-7 induces loss of estrogen requirements for growth and transformation. Clin Cancer Res 1:1429–1436
253. **Kornmann M, Maruyama H, Bergmann U, Tangvoranuntakul P, Beger HG, White MF, Korc M** 1998 Enhanced expression of the insulin receptor substrate-2 docking protein in human pancreatic cancer. Cancer Res 58:4250–4254
254. **Bergmann U, Funatomi H, Kornmann M, Beger HG, Korc M** 1996 Increased expression of insulin receptor substrate-1 in human pancreatic cancer. Biochem Biophys Res Commun 220:886–890
255. **Jackson JG, Zhang X, Yoneda T, Yee D** 2001 Regulation of breast cancer cell motility by insulin receptor substrate-2 (IRS-2) in metastatic variants of human breast cancer cell lines. Oncogene 20: 7318–7325
256. **Boissan M, Beurel E, Wendum D, Rey C, Lecluse Y, Housset C, Lacombe ML, Desbois-Mouthon C** 2005 Overexpression of insulin

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receptor substrate-2 in human and murine hepatocellular carcinoma. Am J Pathol 167:869–877
257. **Satyamoorthy K, Li G, Vaidya B, Patel D, Herlyn M** 2001 Insulin-like growth factor-1 induces survival and growth of biologically early melanoma cells through both the mitogen-activated protein kinase and β-catenin pathways. Cancer Res 61:7318–7324
258. **Buckbinder L, Talbott R, Velasco-Miguel S, Takenaka I, Faha B, Seizinger BR, Kley N** 1995 Induction of the growth inhibitor IGF-binding protein 3 by p53. Nature 377:646–649
259. **Werner H, Karnieli E, Rauscher FJ, 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
260. **Webster NJ, Resnik JL, Reichart DB, Strauss B, Haas M, Seely BL** 1996 Repression of the insulin receptor promoter by the tumor suppressor gene product p53: a possible mechanism for receptor overexpression in breast cancer. Cancer Res 56:2781–2788
261. **Lee YI, Lee S, Das GC, Park US, Park SM** 2000 Activation of the insulin-like growth factor II transcription by aflatoxin B1 induced p53 mutant 249 is caused by activation of transcription complexes; implications for a gain-of-function during the formation of hepatocellular carcinoma. Oncogene 19:3717–3726
262. **Zhang L, Kashanchi F, Zhan Q, Zhan S, Brady JN, Fornace AJ, Seth P, Helman LJ** 1996 Regulation of insulin-like growth factor II P3 promotor by p53: a potential mechanism for tumorigenesis. Cancer Res 56:1367–1373
263. **Zhang L, Zhan Q, Zhan S, Kashanchi F, Fornace Jr AJ, Seth P, Helman LJ** 1998 p53 regulates human insulin-like growth factor II gene expression through active P4 promoter in rhabdomyosarcoma cells. DNA Cell Biol 17:125–131
264. **Sciacchitano S, Orecchio A, Lavra L, Misiti S, Giacchini A, Zani M, Danese D, Gurtner A, Soddu S, Di Mario U, Andreoli M** 2002 Cloning of the mouse insulin receptor substrate-3 (mIRS-3) promoter, and its regulation by p53. Mol Endocrinol 16:1577–1589
265. **Lee YI, Han YJ, Lee SY, Park SK, Park YJ, Moon HB, Shin JH, Lee JH** 2003 Activation of insulin-like growth factor II signaling by mutant type p53: physiological implications for potentiation of IGF-II signaling by p53 mutant 249. Mol Cell Endocrinol 203:51–63
266. **Werner H, Shen-Orr Z, Rauscher 3rd FJ, Morris JF, Roberts Jr CT, LeRoith D** 1995 Inhibition of cellular proliferation by the Wilms' tumor suppressor WT1 is associated with suppression of insulin-like growth factor I receptor gene expression. Mol Cell Biol 15:3516–3522
267. **Werner H, Roberts Jr CT, Rauscher 3rd FJ, LeRoith D** 1996 Regulation of insulin-like growth factor I receptor gene expression by the Wilms' tumor suppressor WT1. J Mol Neurosci 7:111–123
268. **Drummond IA, Madden SL, Rohwer-Nutter P, Bell GI, Sukhatme VP, Rauscher 3rd FJ** 1992 Repression of the insulin-like growth factor II gene by the Wilms tumor suppressor WT1. Science 257:674–678
269. **Tajinda K, Carroll J, Roberts Jr CT** 1999 Regulation of insulin-like growth factor I receptor promoter activity by wild-type and mutant versions of the WT1 tumor suppressor. Endocrinology 140:4713–4724
270. **Zhao H, Dupont J, Yakar S, Karas M, LeRoith D** 2004 PTEN inhibits cell proliferation and induces apoptosis by downregulating cell surface IGF-IR expression in prostate cancer cells. Oncogene 23:786–794
271. **Hwang PH, Kim SY, Lee JC, Kim SJ, Yi HK, Lee DY** 2005 PTEN/MMAC1 enhances the growth inhibition by anticancer drugs with downregulation of IGF-II expression in gastric cancer cells. Exp Mol Med 37:391–398
272. **Hari J, Pierce SB, Morgan DO, Sara V, Smith MC, Roth RA** 1987 The receptor for insulin-like growth factor II mediates an insulin-like response. EMBO J 6:3367–3371
273. **Tally M, Li CH, Hall K** 1987 IGF-2 stimulated growth mediated by the somatomedin type 2 receptor. Biochem Biophys Res Commun 148:811–816
274. **Nakae J, Kido Y, Accili D** 2001 Distinct and overlapping functions of insulin and IGF-I receptors. Endocr Rev 22:818–835
275. **Souza RF, Wang S, Thakar M, Smolinski KN, Yin J, Zou TT, Kong D, Abraham JM, Toretsky JA, Meltzer SJ** 1999 Expression of the wild-type insulin-like growth factor II receptor gene suppresses growth and causes death in colorectal carcinoma cells. Oncogene 18:4063–4068
276. **Wang S, Souza RF, Kong D, Yin J, Smolinski KN, Zou TT, Frank T, Young J, Flanders KC, Sugimura H, Abraham JM, Meltzer SJ** 1997 Deficient transforming growth factor-β1 activation and excessive insulin-like growth factor II (IGFII) expression in IGFII receptor-mutant tumors. Cancer Res 57:2543–2546
277. **Yamada T, De Souza AT, Finkelstein S, Jirtle RL** 1997 Loss of the gene encoding mannose 6-phosphate/insulin-like growth factor II receptor is an early event in liver carcinogenesis. Proc Natl Acad Sci USA 94:10351–10355
278. **Xu YQ, Grundy P, Polychronakos C** 1997 Aberrant imprinting of the insulin-like growth factor II receptor gene in Wilms' tumor. Oncogene 14:1041–1046
279. **Schaffer BS, Lin MF, Byrd JC, Park JH, MacDonald RG** 2003 Opposing roles for the insulin-like growth factor (IGF)-II and mannose 6-phosphate (Man-6-P) binding activities of the IGF-II/Man-6-P receptor in the growth of prostate cancer cells. Endocrinology 144:955–966
280. **Li J, Sahagian GG** 2004 Demonstration of tumor suppression by mannose 6-phosphate/insulin-like growth factor 2 receptor. Oncogene 23:9359–9368
281. **O'Gorman DB, Weiss J, Hettiaratchi A, Firth SM, Scott CD** 2002 Insulin-like growth factor-II/mannose 6-phosphate receptor overexpression reduces growth of choriocarcinoma cells in vitro and in vivo. Endocrinology 143:4287–4294
282. **Motyka B, Korbutt G, Pinkoski MJ, Heibein JA, Caputo A, Hobman M, Barry M, Shostak I, Sawchuk T, Holmes CF, Gauldie J, Bleackley RC** 2000 Mannose 6-phosphate/insulin-like growth factor II receptor is a death receptor for granzyme B during cytotoxic T cell-induced apoptosis. Cells 103:491–500
283. **Hankins GR, De Souza AT, Bentley RC, Patel MR, Marks JR, Iglehart JD, Jirtle RL** 1996 M6P/IGF2 receptor: a candidate breast tumor suppressor gene. Oncogene 12:2003–2009
284. **Cardillo MR, Monti S, Di Silverio F, Gentile V, Sciarra F, Toscano V** 2003 Insulin-like growth factor (IGF)-I, IGF-II and IGF type I receptor (IGFR-I) expression in prostatic cancer. Anticancer Res 23:3825–3835
285. **Schnarr B, Strunz K, Ohsam J, Benner A, Wacker J, Mayer D** 2000 Down-regulation of insulin-like growth factor-I receptor and insulin receptor substrate-1 expression in advanced human breast cancer. Int J Cancer 89:506–513
286. **Chong YM, Williams SL, Elkak A, Sharma AK, Mokbel K** 2006 Insulin-like growth factor 1 (IGF-1) and its receptor mRNA levels in breast cancer and adjacent non-neoplastic tissue. Anticancer Res 26:167–173
287. **Mauro L, Bartucci M, Morelli C, Ando S, Surmacz E** 2001 IGF-I receptor-induced cell-cell adhesion of MCF-7 breast cancer cells requires the expression of junction protein ZO-1. J Biol Chem 276:39892–39897
288. **Pennisi PA, Barr V, Nunez NP, Stannard B, Le Roith D** 2002 Reduced expression of insulin-like growth factor I receptors in MCF-7 breast cancer cells leads to a more metastatic phenotype. Cancer Res 62:6529–6537
289. **Lynch L, Vodyanik PI, Boettiger D, Guvakova MA** 2005 Insulin-like growth factor I controls adhesion strength mediated by α5β1 integrins in motile carcinoma cells. Mol Biol Cell 16:51–63
290. **Kim B, van Golen CM, Feldman EL** 2004 Insulin-like growth factor-I signaling in human neuroblastoma cells. Oncogene 23:130–141
291. **Knowlden JM, Hutcheson IR, Barrow D, Gee JM, Nicholson RI** 2005 Insulin-like growth factor-I receptor signaling in tamoxifen-resistant breast cancer: a supporting role to the epidermal growth factor receptor. Endocrinology 146:4609–4618
292. **Del Valle L, Enam S, Lassak A, Wang JY, Croul S, Khalili K, Reiss K** 2002 Insulin-like growth factor I receptor activity in human medulloblastomas. Clin Cancer Res 8:1822–1830
293. **Burroughs KD, Howe SR, Okubo Y, Fuchs-Young R, LeRoith D, Walker CL** 2002 Dysregulation of IGF-I signaling in uterine leiomyoma. J Endocrinol 172:83–93
294. **Hofmann F, Garcia-Echeverria C** 2005 Blocking the insulin-like growth factor-I receptor as a strategy for targeting cancer. Drug Discov Today 10:1041–1047

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295. **Resnicoff M, Coppola D, Sell C, Rubin R, Ferrone S, Baserga R** 1994 Growth inhibition of human melanoma cells in nude mice by antisense strategies to the type 1 insulin-like growth factor receptor. Cancer Res 54:4848–4850
296. **Nakamura K, Hongo A, Kodama J, Miyagi Y, Yoshinouchi M, Kudo T** 2000 Down-regulation of the insulin-like growth factor I receptor by antisense RNA can reverse the transformed phenotype of human cervical cancer cell lines. Cancer Res 60:760–765
297. **Burfeind P, Chernicky CL, Rininsland F, Ilan J** 1996 Antisense RNA to the type I insulin-like growth factor receptor suppresses tumor growth and prevents invasion by rat prostate cancer cells in vivo. Proc Natl Acad Sci USA 93:7263–7268
298. **Bohula EA, Salisbury AJ, Sohail M, Playford MP, Riedemann J, Southern EM, Macaulay VM** 2003 The efficacy of small interfering RNAs targeted to the type 1 insulin-like growth factor receptor (IGF1R) is influenced by secondary structure in the IGF1R transcript. J Biol Chem 278:15991–15997
299. **Rininsland F, Johnson TR, Chernicky CL, Schulze E, Burfeind P, Ilan J** 1997 Suppression of insulin-like growth factor type I receptor by a triple-helix strategy inhibits IGF-I transcription and tumorigenic potential of rat C6 glioblastoma cells. Proc Natl Acad Sci USA 94:5854–5859
300. **Sachdev D, Li SL, Hartell JS, Fujita-Yamaguchi Y, Miller JS, Yee D** 2003 A chimeric humanized single-chain antibody against the type I insulin-like growth factor (IGF) receptor renders breast cancer cells refractory to the mitogenic effects of IGF-I. Cancer Res 63:627–635
301. **Wang Y, Hailey J, Williams D, Lipari P, Malkowski M, Wang X, Xie L, Li G, Saha D, Ling WL, Cannon-Carlson S, Greenberg R, Ramos RA, Shields R, Presta L, Brams P, Bishop WR, Pachter JA** 2005 Inhibition of insulin-like growth factor-I receptor (IGF-IR) signaling and tumor cell growth by a fully human neutralizing anti-IGF-IR antibody. Mol Cancer Ther 4:1214–1221
302. **Cohen BD, Baker DA, Soderstrom C, Tkalcevic G, Rossi AM, Miller PE, Tengowski MW, Wang F, Gualberto A, Beebe JS, Moyer JD** 2005 Combination therapy enhances the inhibition of tumor growth with the fully human anti-type 1 insulin-like growth factor receptor monoclonal antibody CP-751,871. Clin Cancer Res 11:2063–2073
303. **Bell IM, Stirdivant SM, Ahern J, Culberson JC, Darke PL, Dinsmore CJ, Drakas RA, Gallicchio SN, Graham SL, Heimbrook DC, Hall DL, Hua J, Kett NR, Kim AS, Kornienko M, Kuo LC, Munshi SK, Quigley AG, Reid JC, Trotter BW, Waxman LH, Williams TM, Zartman CB** 2005 Biochemical and structural characterization of a novel class of inhibitors of the type 1 insulin-like growth factor and insulin receptor kinases. Biochemistry 44:9430–9440
304. **Scotlandi K, Manara MC, Nicoletti G, Lollini PL, Lukas S, Benini S, Croci S, Perdichizzi S, Zambelli D, Serra M, Garcia-Echeverria C, Hofmann F, Picci P** 2005 Antitumor activity of the insulin-like growth factor-I receptor kinase inhibitor NVP-AEW541 in musculoskeletal tumors. Cancer Res 65:3868–3876
305. **Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Shringarpure R, Akiyama M, Hideshima T, Chauhan D, Joseph M, Libermann TA, Garcia-Echeverria C, Pearson MA, Hofmann F, Anderson KC, Kung AL** 2004 Inhibition of the insulin-like growth factor receptor-1 tyrosine kinase activity as a therapeutic strategy for multiple myeloma, other hematologic malignancies, and solid tumors. Cancer Cell 5:221–230
306. **Pietrzkowski Z, Mulholland G, Gomella L, Jameson BA, Wernicke D, Baserga R** 1993 Inhibition of growth of prostatic cancer cell lines by peptide analogues of insulin-like growth factor 1. Cancer Res 53:1102–1106
307. **Trojan J, Johnson TR, Rudin SD, Ilan J, and Tykocinski ML** 1993 Treatment and prevention of rat glioblastoma by immunogenic C6 cells expressing antisense insulin-like growth factor I RNA. Science 259:94–97
308. **Wen B, Deutsch E, Marangoni E, Frascona V, Maggiorella L, Abdulkarim B, Chavaudra N, Bourhis J** 2001 Tyrphostin AG 1024 modulates radiosensitivity in human breast cancer cells. Br J Cancer 85:2017–2021
309. **Sandberg AC, Engberg C, Lake M, von Holst H, Sara VR** 1988 The expression of insulin-like growth factor I and insulin-like growth factor II genes in the human fetal and adult brain and in glioma. Neurosci Lett 93:114–119
310. **Sandberg-Nordqvist AC, Stahlbom PA, Reinecke M, Collins VP, von Holst H, Sara V** 1993 Characterization of insulin-like growth factor 1 in human primary brain tumors. Cancer Res 53:2475–2478
311. **Antoniades HN, Galanopoulos T, Neville-Golden J, Maxwell M** 1992 Expression of insulin-like growth factors I and II and their receptor mRNAs in primary human astrocytomas and meningiomas; in vivo studies using in situ hybridization and immunocytochemistry. Int J Cancer 50:215–222
312. **Hirano H, Lopes MB, Laws Jr ER, Asakura T, Goto M, Carpenter JE, Karns LR, VandenBerg SR** 1999 Insulin-like growth factor-1 content and pattern of expression correlates with histopathologic grade in diffusely infiltrating astrocytomas. Neuro-oncol 1:109–119
313. **Lichtor T, Kurpakus MA, Gurney ME** 1993 Expression of insulin-like growth factors and their receptors in human meningiomas. J Neurooncol 17:183–190
314. **Hultberg BM, Haselbacher G, Nielsen FC, Wulff BS, Gammeltoft S** 1993 Gene expression of insulin-like growth factor II in human intracranial meningioma. Cancer 72:3282–3286
315. **Lichtor T, Kurpakus MA, Gurney ME** 1991 Differential expression of insulin-like growth factor II in human meningiomas. Neurosurgery 29:405–409; discussion, 409–410
316. **van Doorn J, Gilhuis HJ, Koster JG, Wesseling P, Reddingius RE, Gresnigt MG, Bloemen RJ, van Muijen GNP, van Buul-Offers SC** 2004 Differential patterns of insulin-like growth factor-I and -II mRNA expression in medulloblastoma. Neuropathol Appl Neurobiol 30:503–512
317. **Tricoli JV, Rall LB, Karakousis CP, Herrera L, Petrelli NJ, Bell GI, Shows TB** 1986 Enhanced levels of insulin-like growth factor messenger RNA in human colon carcinomas and liposarcomas. Cancer Res 46:6169–6173
318. **Lambert S, Vivario J, Boniver J, Gol-Winkler R** 1990 Abnormal expression and structural modification of the insulin-like growth-factor-II gene in human colorectal tumors. Int J Cancer 46:405–410
319. **Kawamoto K, Onodera H, Kondo S, Kan S, Ikeuchi D, Maetani S, Imamura M** 1998 Expression of insulin-like growth factor-2 can predict the prognosis of human colorectal cancer patients: correlation with tumor progression, proliferative activity and survival. Oncology 55:242–248
320. **Freier S, Weiss O, Eran M, Flyvbjerg A, Dahan R, Nephesh I, Safra T, Shiloni E, Raz I** 1999 Expression of the insulin-like growth factors and their receptors in adenocarcinoma of the colon. Gut 44:704–708
321. **Li SR, Ng CF, Banerjea A, Ahmed S, Hands R, Powar M, Ogunkolade W, Dorudi S, Bustin SA** 2004 Differential expression patterns of the insulin-like growth factor 2 gene in human colorectal cancer. Tumour Biol 25:62–68
322. **Chung CK, Antoniades HN** 1992 Expression of c-sis/platelet-derived growth factor B, insulin-like growth factor I, and transforming growth factor α messenger RNAs and their respective receptor messenger RNAs in primary human gastric carcinomas: in vivo studies with in situ hybridization and immunocytochemistry. Cancer Res 52:3453–3459
323. **Furukawa M, Raffeld M, Mateo C, Sakamoto A, Moody TW, Ito T, Venzon DJ, Serrano J, Jensen RT** 2005 Increased expression of insulin-like growth factor I and/or its receptor in gastrinomas is associated with low curability, increased growth and development of metastases. Clin Cancer Res 11:3233–3242
324. **Bergmann U, Funatomi H, Yokoyama M, Beger HG, Korc M** 1995 Insulin-like growth factor I overexpression in human pancreatic cancer: evidence for autocrine and paracrine roles. Cancer Res 55:2007–2011
325. **Su TS, Liu WY, Han SH, Jansen M, Yang-Fen TL, P'Eng FK, Chou CK** 1989 Transcripts of the insulin-like growth factors I and II in human hepatoma. Cancer Res 49:1773–1777
326. **Cariani E, Lasserre C, Seurin D, Hamelin B, Kemeny F, Franco D, Czech MP, Ullrich A, Brechot C** 1988 Differential expression of insulin-like growth factor II mRNA in human primary liver cancers, benign liver tumors, and liver cirrhosis. Cancer Res 48:6844–6849
327. **Lamas E, Le Bail B, Housset C, Boucher O, Brechot C** 1991 Localization of insulin-like growth factor-II and hepatitis B virus

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mRNAs and proteins in human hepatocellular carcinomas. Lab Invest 64:98–104
328. **D'Errico A, Grigioni WF, Fiorentino M, Baccarini P, Lamas E, De Mitri S, Gozzetti G, Mancini AM, Brechot C** 1994 Expression of insulin-like growth factor II (IGF-II) in human hepatocellular carcinomas: an immunohistochemical study. Pathol Int 44:131–137
329. **Iizuka N, Oka M, Tamesa T, Hamamoto Y, Yamada-Okabe H** 2004 Imbalance in expression levels of insulin-like growth factor 2 and H19 transcripts linked to progression of hepatocellular carcinoma. Anticancer Res 24:4085–4089
330. **Minuto F, Del Monte P, Barreca A, Fortini P, Cariola G, Catrambone G, Giordano G** 1986 Evidence for an increased somatomedin-C/insulin-like growth factor I content in primary human lung tumors. Cancer Res 46:985–988
331. **Tode B, Serio M, Rotella CM, Galli G, Franceschelli F, Tanini A, Toccafondi R** 1989 Insulin-like growth factor-I: autocrine secretion by human thyroid follicular cells in primary culture. J Clin Endocrinol Metab 69:639–647
332. **Yee D, Paik S, Lebovic GS, Marcus RR, Favoni RE, Cullen KJ, Lippman ME, Rosen N** 1989 Analysis of insulin-like growth factor I gene expression in malignancy: evidence for a paracrine role in human breast cancer. Mol Endocrinol 3:509–517
333. **Paik S** 1992 Expression of IGF-I and IGF-II mRNA in breast tissue. Breast Cancer Res Treat 22:31–38
334. **Gebauer G, Jager W, Lang N** 1998 mRNA expression of components of the insulin-like growth factor system in breast cancer cell lines, tissues, and metastatic breast cancer cells. Anticancer Res 18:1191–1195
335. **Yee D, Morales FR, Hamilton TC, Von Hoff DD** 1991 Expression of insulin-like growth factor I, its binding proteins, and its receptor in ovarian cancer. Cancer Res 51:5107–5112
336. **Elkas J, Gray K, Howard L, Petit N, Pohl J, Armstrong A** 1998 The effects of tamoxifen on endometrial insulin-like growth factor-1 expression. Obstet Gynecol 91:45–50
337. **Liao Y, Abel U, Grobholz R, Hermani A, Trojan L, Angel P, Mayer D** 2005 Up-regulation of insulin-like growth factor axis components in human primary prostate cancer correlates with tumor grade. Hum Pathol 36:1186–1196
338. **Neuvians TP, Gashaw I, Hasenfus A, Hacherhacker A, Winterhager E, Grobholz R** 2005 Differential expression of IGF components and insulin receptor isoforms in human seminoma versus normal testicular tissue. Neoplasia 7:446–456
339. **Burrow S, Andrulis IL, Pollak M, Bell RS** 1998 Expression of insulin-like growth factor receptor, IGF-1, and IGF-2 in primary and metastatic osteosarcoma. J Surg Oncol 69:21–27
340. **Schips L, Zigeuner R, Ratschek M, Rehak P, Ruschoff J, Langner C** 2004 Analysis of insulin-like growth factors and insulin-like growth factor I receptor expression in renal cell carcinoma. Am J Clin Pathol 122:931–937

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