Insulin-like Growth Factors and Cancer

**Moderator:** Derek LeRoith, MD, PhD; **Discussants:** Renato Baserga, MD, PhD; Lee Helman, MD; and Charles T. Roberts Jr., PhD

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The insulin-like growth factor (IGF) family of peptides, binding proteins, and receptors are important for normal human growth and development and are involved in the specialized functions of most physiologic systems. Most members of the IGF system are expressed by different cancer cells and may play an important role in the propagation of these malignancies. New therapies aimed at modulating various components of the IGF system could affect the progression and metastasis of cancer.


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**Dr. Derek LeRoith (Molecular and Cellular Physiology Section, National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK], National Institutes of Health [NIH], Bethesda, Maryland):** The insulin-like growth factor (IGF) system comprises a collection of ligands, receptors, and binding proteins (Table 1) (1). Insulin, IGF-I, and IGF-II are polypeptides that affect many tissues and result in diverse biological actions. The major role of insulin is controlling metabolic homeostasis. In contrast, IGF-I and IGF-II are vital for normal growth and development during fetal, neonatal, and pubertal stages (2). In addition, IGFs have specialized functions in differentiated tissues, including the reproductive, cardiovascular, and neurologic systems. The biological functions of the IGFs are initiated by their interactions with cell-surface receptors, in particular the IGF-I receptor. When activated, this receptor initiates a cascade of events that begins with the activation of tyrosine kinase and results in divergent effects depending on specific cell types (3).

Circulating IGFs are synthesized primarily in the liver and serve an endocrine function, whereas locally produced IGFs act in an autocrine-paracrine mode. Both forms are bound by a family of binding proteins, six of which have been well characterized (Table 1) (4). Insulin-like growth factor–binding proteins are responsible for protecting IGFs in the circulation, prolonging their half-lives, and delivering them to their specific target tissues. At the local level, IGF-binding proteins may regulate the interaction of IGFs with their receptors by either inhibiting or augmenting the interaction. In addition, IGF-binding proteins may have some actions that are independent of interactions between IGF and IGF receptors.

As could be predicted from the importance of IGFs, their binding proteins, and their receptors in normal cellular growth and development, it has become apparent over the past few years that IGFs are important mitogens in many types of malignancies (5). Although these conclusions were initially derived from in vitro studies, IGFs may enhance in vivo tumor cell formation, growth, and even metastasis. Insulin-like growth factors may reach tumors either from the circulation (endocrine) or as a result of local production by the tumor itself (autocrine) or by adjacent stromal tissue (paracrine). Tumors also express many of the IGF-binding proteins, which modulate IGF action, and IGF receptors, which mediate the effects of IGFs on tumors. We highlight important aspects of IGFs in normal cell growth and their role in certain malignancies.

The syndrome of hypoglycemia with non-islet cell tumors, although not covered in this review, deserves special mention because it was one of the earliest links of IGFs to tumors and was derived from studies done in the mid-1970s at the Diabetes Branch of the NIDDK. The clinical syndrome was described shortly after insulinomas were first described in the late 1920s. The advent of the radioimmunoassay for insulin in the early 1960s showed that insulinomas release insulin but that the nonislet tumors that produce hypoglycemia usually lack insulin, a finding that triggered intense speculation about the mechanism of hypoglycemia. In the mid-1970s, the NIH group devised a novel radioreceptor assay for IGF-II. Using this assay, they showed that in patients with this syndrome, IGF-II-like material is often present in elevated amounts in the circulation and in the tumors (6). Of further interest is that the major clinical culprit may be a higher-molecular-weight precursor form of IGF-II; this IGF-II–like material probably binds to insulin receptors, activates them, and thereby produces hypoglycemia (7, 8).

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The Role of the Insulin-like Growth Factor I Receptor in Cell Growth and Transformation

Dr. Renato Baserga (Jefferson Cancer Center, Jefferson Medical College, Philadelphia, Pennsylvania): Mammalian cell growth in vitro and in vivo is regulated by factors that interact with specific cell-surface receptors. Most normal cells require at least two factors for optimal growth. Insulin-like growth factor I is often one of them and is required for the growth of such cells as fibroblasts, epithelial cells, bone marrow stem cells, and osteoblasts (9). The other required growth factor varies depending on the cell type, but in fibroblasts, platelet-derived growth factor and epidermal growth factor act with IGF-I to stimulate...
cell proliferation. In culture, neither of these factors alone can sustain cell growth. The recent finding that mice in which the IGF-I and IGF-I receptor genes had been inactivated grew to only 30% of the size of normal littermates underscores the role of the IGF-I receptor in murine development (10, 11). Further support for the role of the IGF-I receptor in growth and tumorigenesis has come from studies showing the transforming potential of transfected cells overexpressing the IGF-I receptor (12) and abrogation of this effect by specific mutations of the receptor (13).

On the basis of research using fibroblast cell lines derived from IGF-I receptor-deficient mice (R- cells), it has been possible to show that 1) IGF-I receptors are essential for the growth of cells in serum-free media supplemented with factors that support the growth of normal mouse cells (W cells) that are fibroblasts derived from normal mice or 3T3 cells); 2) IGF-I receptors are not necessary for growth in media containing 10% serum but are required for optimal growth; 3) IGF-I receptors are also required for platelet-derived growth factor–stimulated or epidermal growth factor–stimulated growth and transformation; and 4) IGF-I receptors stimulate both ras-dependent and ras-independent signaling pathways (14).

It has been shown that SV40 T antigen increases IGF-I expression, leading to transformation of BALBc 3T3 cells (15). The obligate role of the IGF-I receptor in T-antigen–mediated transformation was confirmed by the inability of T antigen to transform the R- cells described above. Further studies have shown that R- cells are also refractory to transformation by v-src and by bovine papilloma virus, both of which efficiently transform cells expressing IGF-I receptors; thus, some oncogenic viruses require IGF-I receptors to transform mouse embryo fibroblasts.

To ascertain the role of IGF-I receptors in the growth and transformation of other cell types, C6 rat glioblastoma cells were rendered IGF-I receptor–deficient mice by expressing an antisense RNA that prevented efficient expression of the endogenous IGF-I receptor gene. These cells were then evaluated for their ability to form tumors when transplanted into syngeneic rats in comparison with wild-type C6 cells. In all 50 rats injected with wild-type C6 cells, the C6 cells grew well and formed large tumors. In contrast, none of the 50 rats injected with IGF-I receptor–deficient C6 cells did not grow in the 27 animals into which they were injected, and no tumors were formed (16). These results suggest that IGF-I receptors are extremely important in establishing and maintaining the transformed phenotype and that they may represent a suitable target for the inhibition of cell proliferation in vivo.

It is currently accepted that a major signal transduction pathway triggered by the IGF-I receptor and by other receptor tyrosine kinases such as the receptors for insulin, epidermal growth factor, and platelet-derived growth factor involves activation of ras, the protein kinase Raf-1, and the mitogen-activated protein kinase cascade (17). It is interesting that overexpression of an activated ras or Raf-1 could not confer in IGF-I receptor–deficient R- cells the ability to grow in serum-free medium supplemented with purified growth factors (14, 15, 18). Thus, the IGF-I receptor must also use a ras (and

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* IGF = insulin-like growth factor; IGFBP = insulin-like growth factor–binding protein.

Raf-1)-independent pathway to stimulate cell proliferation and transformation.

**Insulin-like Growth Factors and Breast Cancer**

Derek LeRoith (Section on Molecular and Cellular Physiology, Diabetes Branch, NIDDK, NIH): Breast cancer is a common malignancy that affects almost 1 in every 7 women and is the leading cause of death from cancer in women in North America. During normal development, estrogen is primarily involved in promoting the development of breast ducts, whereas progesterone promotes lobuloalveolar development.

Many cancers, especially those developing in the postmenopausal period, express estrogen and progesterone receptors. The presence of these receptors and the likelihood that these cancers will respond to endocrine therapy are strongly correlated. Initial therapy for breast cancer is primarily surgical, but once metastatic disease has developed, endocrine therapy is appropriate. In premenopausal patients, lowering hormone levels by removing the ovaries is useful, whereas in postmenopausal patients, antiestrogens such as tamoxifen have proved useful (19).

In addition to classic hormones, several growth factors, including transforming growth factors, epidermal growth factors, and IGFs, have been shown to be involved in breast cancer. The cellular proto-oncogene products such as c-myc, c-fos, and c-jun are also involved (Table 2) (20).

Breast cancer cells in vivo express low levels of IGF-II (21), whereas the adjacent stromal tissue expresses IGF-I (22). In addition, most breast cancer cells express insulin and IGF receptors (23). Different cancers express different combinations of the IGF-binding proteins: In vitro, estrogen receptor–positive cancer cells synthesize IGF-

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binding proteins 2, 4, and 5, and estrogen receptor-negative cancer cells synthesize IGF-binding proteins 1, 3, 4, and 5 (24). Examination of biopsy specimens of breast cancer cells has confirmed this specific pattern of IGF-binding protein expression (25). Because it has been shown that the proliferation of breast cancer cell lines is enhanced by IGFs and that this effect is modulated by IGF-binding proteins, it can be concluded that breast cancer is also influenced by circulating (endocrine) IGFs or autocrine-paracrine production of IGF and IGF-binding proteins, or both. Of particular interest is the effect of estrogen on the IGF system. In estrogen receptor-positive cells, estrogen enhances cellular proliferation and increases IGF-II expression (21).

It is well established that in addition to the standard treatments such as surgery, radiation, and chemotherapy, hormonal manipulation may also be successful, especially for metastatic disease. Estrogen receptor-positive tumors will thus respond to antiestrogens such as tamoxifen, which is widely used clinically (19). Initially, it was thought to affect cancer cells primarily by blocking the activation of estrogen receptors; it has also been shown, however, to decrease circulating IGF-I levels in women with breast cancer (26, 27) and may thus prove effective in treating both estrogen receptor-positive and estrogen receptor-negative cancers.

Another agent that inhibits the proliferation of breast cancer cells is retinoic acid and its derivatives (28). All-trans retinoids inhibit IGF-induced proliferation of breast cancer cells in culture, and these agents are being used clinically to reduce or prevent metastatic disease (29). The mechanism by which retinoids affect the IGF-induced growth of breast cancer cells seems to involve modulation of local IGF-binding protein production; specifically, retinoic acid affects the expression and release of IGF-binding proteins (30). Like tamoxifen, however, retinoic acid may also reduce circulating IGF-I levels and may thus affect tumor growth in vivo by more than one mechanism.

The above data suggest that IGFs are likely to be involved in breast cancer at the level of tumor growth and perhaps at the level of initial development and later metastases. Ongoing studies involve attempts to interfere with the IGF system to develop additional therapeutic regimens.

The Role of Insulin-like Growth Factors in Rhabdomyosarcomas and Osteosarcomas

Dr. Lee Helman (Molecular Oncology Section, Pediatric Branch, National Cancer Institute, NIH): Insulin-like growth factor II is known to be an important fetal growth factor that plays a role in normal skeletal muscle growth and differentiation. In addition, IGF-II is expressed at high levels in normal human fetal skeletal muscle, and that expression is markedly decreased in adult skeletal muscle. Insulin-like growth factor II expression in rhabdomyosarcoma in children is as high as or higher than levels seen in fetal muscle. Further, human rhabdomyosarcoma cell lines secrete IGF-II into conditioned media, express IGF-1 receptors on their cell surfaces, and can grow in mitogen-free, serum-free media at a rate identical to that seen in serum. Finally, a monoclonal blocking antibody to the IGF-I receptor, αIR-3, inhibited proliferation of these cells (31). These data show that IGF-II functions as an autocrine growth factor in rhabdomyosarcoma cell lines and suggest a potential role for IGF-II in the pathogenesis of rhabdomyosarcoma. In situ hybridization analysis of 26 fixed tumor specimens showed that IGF-II was expressed specifically in tumor cells and not in stromal tissue, further suggesting a direct role for IGF-II in the unregulated growth of these tumor cells (32). In addition, both major histologic subtypes of rhabdomyosarcoma (alveolar and embryonal) overexpressed IGF-II.

To understand the mechanism of IGF-II overexpression, detailed studies of the IGF-II gene were done. The P3 and P4 promoter regions of the IGF-II gene, regions of the gene involved in transcriptional regulation, are not mutated in rhabdomyosarcoma cells, and studies on the promoter function of these regions showed no alterations compared with normal tissue (unpublished observations). It was recently found that the human IGF-II gene is normally imprinted by a genome-specific modification of one allele, thus leading to parental allele-specific gene expression (33, 34). This can be identified because of the presence of a transcribed Apal restriction-site polymorphism present in the population, such that an informative heterozygote inherits one allele of IGF-II containing the Apal site and another allele without the Apal site. When such a polymorphism is present, one can then identify whether one or both alleles are transcribed into RNA by determining whether the Apal site is present. In normal tissue, this imprinting or differential expression of parental alleles leads to expression of the IGF-II gene from the paternal allele alone, whereas the maternal allele is transcriptionally silent. Loss of imprinting, with expression of IGF-II from both alleles, could occur in these tumors and thereby lead to overexpression of IGF-II. Of further interest, embryonal rhabdomyosarcoma tumors have been shown to have loss of heterozygosity with paternal disomy at 11p15, the chromosomal location of IGF-II (35). Paternal disomy at this allele could also lead to a twofold gene dosage effect because the maternal allele is normally silent. Of 13 alveolar or undifferentiated primary specimens of rhabdomyosarcoma tumors that were analyzed, 5 were informative heterozygotes at the IGF-II allele; 4 of these 5 tumors showed loss of imprinting of the IGF-II gene. This condition contrasted with normal fetal muscle, in which imprinting had already occurred and expression occurred from only one allele (36). These data suggest that one mechanism of IGF-II overexpression in these tumors is caused by relaxation of normal genomic imprinting of the IGF-II locus.

To determine whether the IGF-II signaling pathway was necessary for tumor growth in vivo, we evaluated the ability of the blocking monoclonal antibody directed against the IGF-I receptor, αIR-3, to alter the growth of a human rhabdomyosarcoma xenograft tumor in immunodeficient nude mice. Mice were injected with tumor cells preincubated with αIR-3 and then given twice-weekly subcutaneous injections of the antibody over 50 days. The result was a dose-dependent suppression of tumor formation. In a representative experiment, the tumor-free period was 12 days in groups of mice treated with αIR-3 at 5 μg/dose, 25 days in mice treated with 50 μg/dose, and 35 days in mice treated with 300 μg/dose. The untreated mice all developed tumors within 9 days of tumor implanta-
tation. Subsequently, when twice-weekly 50-μg doses of antibody were started after the tumors had reached a size of approximately 70 mm³, aIR-3 treatment significantly inhibited tumor growth. In a representative experiment, control animals had an average increase in tumor size of 1381 mm³ during a 35-day period, whereas animals treated with 50 μg of aIR-3 twice weekly had an average increase of tumor size of 264 mm³ during the same period (P < 0.01). Further, this observed growth inhibition was not associated with cytotoxic effects but rather with growth arrest as shown by a marked decrease in p34cdc2 expression (a serine-threonine protein kinase whose activity is critical for progression through the cell cycle) in the treated tumors and by histologic examination showing absence of necrosis (37).

Osteosarcoma is the most common bone tumor in children, usually occurring during the adolescent growth spurt at sites of rapid bone growth. Because IGF-I was initially described as the factor produced in the liver that directly mediates the effect of growth hormone on skeletal growth (38), there has been interest in a potential role for IGF-I in the pathogenesis of osteosarcoma. Support for a role for IGF-I in osteosarcoma growth comes from data showing that IGF-I is a potent mitogen for human osteogenic sarcoma cells (39). Further, several reports have shown that a rat chondrosarcoma (a closely related tumor) and a murine osteosarcoma are growth-inhibited in animals that have had hypophysectomy (40, 41), presumably through the inhibition of the growth hormone–IGF-I axis. Unlike rhabdomyosarcoma cell lines, human osteosarcoma cell lines do not survive in serum-free, mitogen-free media but will survive in such media with the addition of 25 ng/mL of recombinant IGF-I. In addition, proliferation of human osteosarcoma cell lines was greatly inhibited in vitro when signaling through the IGF-I receptor was blocked with either the blocking monoclonal antireceptor antibody aIR-3 or with antisense oligonucleotides that inhibit expression of the IGF-I receptor gene (42). These data suggest that human osteosarcoma cells require an intact IGF-I receptor–signaling pathway for both survival and proliferation in vitro.

It therefore appears that the growth hormone–IGF-I axis may play a role in the unregulated proliferation of osteosarcoma tumor cells and that blocking of this axis using somatostatin analogs that reduce the circulating levels of growth hormone and IGF-I may have therapeutic potential.

Insulin-like Growth Factors and Wilms Tumor

Dr. Charles T. Roberts, Jr. (Section on Molecular and Cellular Physiology, Diabetes Branch, NIDDK, NIH): Wilms tumor is a pediatric malignancy that is derived from the metanephrogenic blastema of the developing kidney and that expresses high levels of IGF-II, IGF-I receptors, and, potentially, IGF-binding protein 2. It is the most common abdominal childhood tumor, with an incidence of approximately 1 in 8000 live births. Its etiology suggested the involvement of tumor suppressor genes, one of which, the WT1 tumor suppressor gene, has been extensively studied. Described in this section are the function of the WT1 gene product, the role of IGFs in kidney development, and the relation between WT1 action and the IGF system in normal kidney development and in the pathogenesis of Wilms tumor.

The putative product of the WT1 gene (43) contains an amino terminus rich in glutamine, proline, serine, and threonine residues (characteristic of transcriptional regulatory domains) and a carboxy terminus that includes four zinc finger domains—motifs that bind DNA. As shown in Figure 1, three of the four zinc finger domains of the WT1 protein are thought to bind to a specific DNA sequence that would constitute the target site for the regulatory (generally inhibitory) activity of this protein (44).

Studies of mice in which the WT1 gene has been inactivated by gene-targeting showed that the WT1 protein is necessary for normal kidney development (45). Histologically, Wilms tumors are composed primarily of blastemal cells, with varying proportions of stromal and epithelial elements; as a group, these tumors appear to correspond to various stages of arrested development of the metanephric kidney. These findings suggest that Wilms tumors may arise from the loss of appropriate WT1 function at different stages of metanephric development.

The role of IGFs in kidney development is supported by the presence of messenger RNA (mRNA) that encodes IGF-II, the IGF-I receptor (activated by IGF-II to elicit its biological responses), and several IGF-binding proteins, including binding protein 2 (46, 47). These binding proteins modulate the action of the IGF ligands by influencing their half-lives and, potentially, their interaction with the IGF-I receptor. Insulin-like growth factor–binding protein 2 is pertinent to this discussion because it preferentially binds IGF-II. Evidence for IGF action in the progression of Wilms tumor has come from studies showing that an inhibitory antibody directed against the human IGF-I receptor inhibits the growth of Wilms tumor–derived cell lines in culture and in athymic mice (48).

The relative expression of the WT1 and IGF-II genes during human fetal kidney development was recently in-
vestigated (49). Interestingly, as the metanephrogenic blastema, influenced by the elongating ureteric bud (or ampulla), differentiates into the early condensate, the renal vesicle, and finally into glomerular structures, IGF-II gene expression decreases as WT1 gene expression increases (Figure 2). Such a pattern is consistent with the repression of IGF-II gene expression by the transcriptional repressor encoded by the WT1 gene itself. This process would reduce the levels of the mitogenic IGF-II peptide and allow specific differentiation processes to occur. Although WT1 gene expression subsequently decreases, the WT1 protein remains intact and is also present in the adult kidney (50). This proposed effect of WT1 on IGF-II gene expression is supported by studies that show repression of the promoter of the IGF-II gene by WT1 in vitro (51), as well as by the finding that Wilms tumors (which presumably lack functional WT1) express high levels of IGF-II mRNA.

The other components of the IGF system that appear to function in kidney development are also implicated in the pathogenesis of Wilms tumors. Specifically, IGF-I receptor protein and mRNA levels are elevated in Wilms tumors, and the WT1 protein can also repress the promoter of the IGF-I receptor gene in vitro (52). Finally, the serum levels of IGF-binding protein 2 are significantly elevated in children with Wilms tumor (53).

On the basis of these data, a unifying hypothesis of the involvement of the IGF system in the progression of Wilms tumor can be formulated as follows: During normal kidney development, activation of WT1 gene expression at the time of ureteric bud induction of the metanephrogenic blastema represses synthesis of IGF-II and the IGF-I receptor through which IGF-II action is initiated. The inhibition of this autocrine mitogenic loop is presumably necessary for the appropriate differentiation processes of glomerular development to occur. The lack of WT1 function at this stage of kidney development, resulting from any of several mechanisms affecting the structure or expression of the WT1 gene, would leave the IGF-II autocrine loop intact and possibly contribute to the inappropriate proliferation that leads to Wilms tumor. The potential regulation of IGF-binding protein 2 production by WT1 could also contribute to this process. Although the direct effect of the WT1 gene product on the expression of the IGF-II and IGF-I receptor gene has been shown by in vitro studies, the patterns of expression of WT1, IGF-II, and the IGF-I receptor seen in vivo during normal kidney development and in Wilms tumor are consistent with the hypothesis presented above.

Thus, Wilms tumor may represent a situation in which inactivation of a tumor suppressor gene up-regulates several components of a complex growth factor system (that is, ligands, receptors, and binding proteins) to produce severe pathologic consequences.


