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The Role of Circulating IGF-I

Lessons from Human and Animal Models

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Insulin-like growth factors (IGF-I and IGF-II) play a crucial role in regulating cell proliferation and differentiation. The IGFs have mitogenic and antiapoptotic effects on normal and transformed cells. These peptide growth factors are produced by virtually all tissues and act in an endocrine, autocrine, and paracrine fashion. The endocrine form of IGF-I originates mostly (75%) from the liver and IGF-binding proteins regulate its bioactivity. Compared to other peptide growth factors, the IGFs are in abundant supply in circulation. The role of this large reservoir of IGFs has been debated for many years. In the last few years substantial progress has been made in understanding the function of the endocrine IGF-I using new animal models. This review will revisit the IGF system with particular attention to the role of circulating IGF-I in growth regulation, metabolism, and cancer.

Key Words: IGF-I, -II; circulation.

Introduction

In the 1950s Daughaday and Salomon demonstrated that GH stimulated [^{35}S]sulfate uptake into cartilage *(1)*. This effect was indirect and required a serum protein later termed "sulfation factor" or "somatomedin," which is secreted by the liver. About 20 yr later the somatomedins were purified and characterized. A new term was proposed—"insulin-like growth factor" (IGFs)—to emphasize the structural and biological similarities of these peptides to insulin. In the 1980s the mRNA sequences of IGF-I and IGF-II were determined and Isaksson and colleagues demonstrated that direct injection of GH into the tibial epiphysis caused tibial growth and raised the possibility that GH stimulates local production of IGF-I *(2)*. Later, D'Ercole and colleagues reported that multiple tissues from the fetal mouse synthesized IGF-I *(3,4)* and Isgaard et al. showed that the GH effect on tibial growth was associated with an increase in IGF-I mRNA in growth plate chondrocytes *(5)*.

The cloning of the human and rat IGF-I genes in the late 1980s enabled investigators to convincingly demonstrate autocrine and paracrine functions of locally produced IGF-I. It was shown that IGF-I is expressed in almost all tissues and that GH treatment of hypophysectomized rats increased IGF-I mRNA levels in liver and non-hepatic tissues *(6)*. In addition to GH, other factors also enhanced IGF-I mRNA levels in non-hepatic tissues. Thus, in hypophysectomized rats, GH not only stimulates liver IGF-I gene expression and, by extrapolation, circulating IGF-I levels, but also produces local tissue effects via tissue IGF-I production.

This review will summarize the endocrine (vs autocrine/paracrine) effects of IGF-I on linear growth, metabolism, and tumor development.

The IGF-I Gene and Protein Structures

The IGF-I gene and complementary DNA sequences (cDNA corresponding to the mRNA sequence) were isolated from different species. The mature IGF-I polypeptide is composed of 70 residues and arranged in four highly conserved domains A–D (Fig. 1).

The IGF-I gene sequence contains six exons. Exons 1, 2, 5, and 6 could be alternatively spliced and therefore lead to four potential mRNA precursors (Fig. 1). Exons 3 and 4 encode for a portion of the leader peptide at the N-terminal, the entire sequence of the mature polypeptide, and the first 16 residues of the C-terminal (E domain). The N-terminal leader peptide can be encoded by either one or two 5' sequences leading to class 1/class 2 IGF-I mRNA subtypes. The E domain, at the C-terminal, could be encoded by two different 3' untranslated regions (UTR), leading to Ea or Eb IGF-I mRNA subtypes. Therefore, within the same species one can detect four IGF-I mRNA subtypes corresponding to 1/Ea, 1/Eb, 2/Ea, 2/Eb. Class 1/Ea IGF-I mRNA is expressed in all tissues during development *(7–10)*. Class 1/Ea is the most abundant mRNA in liver at early postnatal age prior to the onset of growth hormone (GH) responsiveness, and then there is a further increase in its transcription when GH is released *(11–13)*. This class of transcripts contributes to

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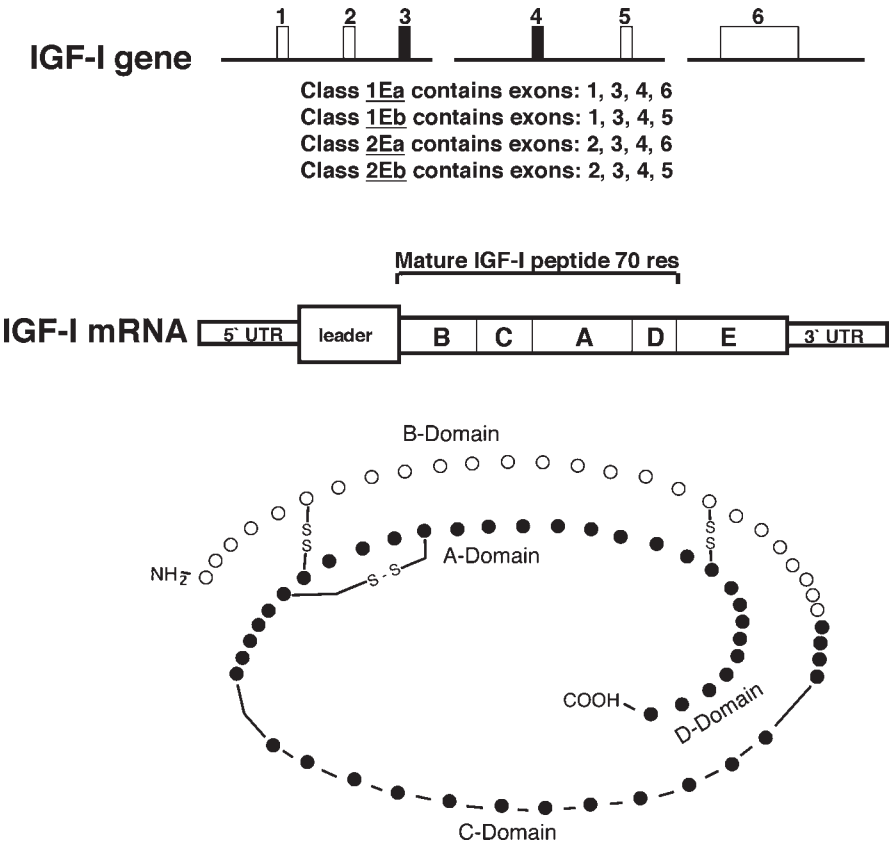

Fig. 1. Schematic illustration of the IGF-I gene structure, transcript, and peptide structure.

the GH-dependent and -independent secretion of IGF-I into circulation. In contrast, class 2/Eb transcripts are undetectable at early embryonic development, and, postnatally, are expressed primarily in the liver *(11,12,14)*.

The evolutionary conserved IGF-I sequence across species and the existence of different IGF-I mRNA precursors within the same species point to the functional significance of IGF-I and its regulation.

Circulating IGF-I and its Regulation

IGF-I Binding Proteins (IGFBPs)

In vivo, the IGFs are bound to a family of six structurally and evolutionary related IGF binding proteins (IGFBP 1–6) *(15)*. The major roles of the IGFBPs are to transport the IGFs in the circulation and across the capillary barriers, to distribute them in the extravascular compartment, and to control their access to the IGF-IR *(16)*.

In circulation, the majority of the IGFs (70–80%) exist in a 150 kDa ternary complex, comprised of one IGF molecule, IGFBP-3, and the acid labile subunit (ALS). A smaller proportion (approx 20%) of the IGFs are associated within a 50 kDa binary complex with other serum IGFBPs, and less than 5% of the IGFs are found in the free form of 7.5 kDa. ALS is a protein that binds to the IGF/IGFBP-3 binary complex, primarily in serum. Association with ALS prolongs the half-life ($t_{1/2}$) of serum IGFs and facilitates their endocrine actions. Compared to other IGF–IGFBP binary complexes, the ternary IGF/IGFBP-3/ALS complex is thought to cross the capillary barrier very poorly *(17,18)*. The ternary complex is not detectable in the lymph nor has ALS been found in extravascular fluids. This suggests that the ALS plays a significant role in regulating the passage of IGFs from circulation into the extravascular compartment.

The liver is the principal source of circulating IGF-I, IGFBP-3, and ALS, which are synthesized by hepatocytes in a growth hormone (GH)-dependent manner *(19)*.

IGFBPs are present in excess in most of the extracellular fluids and because their affinity to the IGFs (IGF-I or IGF-II) is higher than the affinity of the IGF-I receptor to the IGFs, the majority of the IGFs are found in a bound form *(15,16)*. The complexity of the IGFBP–IGF interactions is reflected by the controversial results obtained from in vitro and in vivo studies. IGFBPs were shown to either inhibit or augment IGF-I actions. In many in vitro studies where an intact IGFBP is given in excess together with IGF-I, it was shown that IGF-I binding to its receptor is greatly inhibited *(20)*. In some cases as with IGFBP-1, dephosphorylation of the binding protein causes a reduction of its affinity to IGF-I, and, therefore, even if it is found in excess, IGF-I bioactivity will not be inhibited. In vivo studies, however, show that administration of IGF-I together with IGFBP-3 resulted in

potentiation of IGF-I action most likely through an increase in the half-life of the injected IGF-I *(21)*. In acromegaly there is an increase in IGF-I and IGFBP-3 levels in circulation and yet there is no growth inhibition presumably due to proper equilibrium between IGFBP-3 and IGF-I levels. However, overexpression of the IGFBP-1 or IGFBP-4 in mice resulted in growth retardation consistent with an antagonistic effect on IGF-I bioavailability (when there is disequilibrium between IGBPs and IGF-I levels) *(22)*. Therefore, the regulation of the IGFBPs in the extracellular compartment is an important determinant of IGF-I bioavailability and action.

Hormones

It is well established that circulating IGF-I levels are regulated posttranscriptionally mainly by GH at the postnatal period *(14,23–25)*. However, there is no identified GH-inducible regulatory element within the IGF-I gene and the transactivating factors that mediate GH regulation of IGF-I are poorly defined. The liver is the most responsive organ in regard to GH-dependent increases in IGF-I mRNA. However, non-hepatic tissues like skeletal muscle, adipose tissue, and kidney also respond to GH administration by increasing IGF-I gene transcription *(19)*. Other hormones also regulate circulating IGF-I levels. For example, insulinopenic diabetes is associated with decreased transcription and protein levels of IGF-I, which could be reversed by insulin treatment *(11,26)*. Indeed, it was shown that insulin has a direct effect on IGF-I transcription in isolated hepatocytes *(27)*.

Glucagon increased IGF-I gene expression in primary rat hepatocytes *(28)*. Glucocorticoids decrease IGF-I production in osteoblasts, which may mediate some actions of steroids on bone in both normal and pathological states *(29)*. Chronic estrogen administration reduced GH-induced hepatic IGF-I gene expression and serum concentrations *(30)*. In contrast, in acute experiments estrogen alone had no effect on hepatic IGF-I expression, but acted in a synergistic fashion when administered with GH and resulted in significantly greater accumulation of IGF-I mRNA *(30)*. Thus, the growth retardation effect of estrogen in chronic treatments involves inhibition of GH-dependant hepatic IGF-I expression (mainly the endocrine form of IGF-I).

There is strong evidence that IGF I is a critical determinant of vascular growth responses in vitro and in vivo. Regulation of vascular smooth muscle cell (VSMC) IGF IR expression appears to be crucial for the control of VSMC growth, and, as such, is at a convergence point for the effects of multiple growth factors. It was shown that angiotensin II stimulates transcription of the IGF-I gene in isolated VSMCs and that IGF-I appears to mediate the mitogenic effects of angiotensin II in that system *(31)*.

Thyroid hormone has both direct and indirect effects on IGF-I expression *(32)*. Studies of hypophysectomized rats showed that T4 alone could stimulate IGF-I expression and increase its serum levels *(33)*. In hypothyroidism, seen in humans, IGF-I levels are very low and hyperthyroidism is associated with increased immunoreactive IGF-I, but its bioactivity is lost (which could be explained by an increase in IGF-I inhibitory factors as well) *(34)*. GH mediates the indirect effect of thyroid hormone on IGF-I expression. It was shown that T3 and hydrocortisone could each stimulate GH-releasing-hormone receptor (GHRH-R) mRNA expression in rat primary pituitary culture cells *(35,36)*. The increase in T3 could then stimulate GH transcription, which then could act on hepatocytes to increase IGF-I transcription.

IGF-I expression is also induced in response to immunologically mediated proinflammatory processes and the wound-healing response to inflammation or injury *(37–40)*. IGF-I expression is induced in animal models of inflammatory bowel diseases *(41,42)*. In these models the primary sites of IGF-I expression are mesenchymal cell sites in which IGF-I increases collagen deposition.

IGF-I mRNA is dramatically increased in stem cells stimulated with colony stimulating factor 1 (CSF1), which induces macrophage differentiation *(43,44)*. Advanced glycosylated end products (AGEs) associated with diabetes and aging induce peripheral macrophages to synthesize and secrete pro-IGF-I, a process that is mediated by IL-β *(45)*. Similarly, TNF-α induces IGF-I expression, which could be inhibited by interferon-γ *(46,47)*.

In summary, the endocrine form of IGF-I is regulated mainly by GH secretion. On the other hand, a complex of hormones, factors, and their interactions regulates the autocrine/paracrine form of IGF-I in non-hepatic tissues.

Protein Calorie Intake

Nutritional status (i.e., fasting, dietary protein, and/or energy intake) is a critical element in regulation of circulating IGF-I levels *(48–50)*. Nutritional restriction causes a reduction in the IGF-I mRNA levels in liver and in non-hepatic tissues and a decrease in its stability *(51)*. This reduction leads to a decrease in circulating levels of IGF-I. However, an indirect effect of nutritional restriction on the IGF-I mRNA could be due to other hormonal changes. For example, in rats, dietary restriction of protein intake leads to a decrease in circulating levels of GH, GH binding protein (GHBP), IGF-I, IGFBP-3, and ALS, whereas IGFBP-1 and IGFBP-2 levels are increased in response to reduced protein intake *(52,53)*. In contrast, in human, protein deprivation causes a marked decrease in circulating IGF-I levels and an increase in GH secretion *(54)*. Therefore, the changes in the endocrine form of IGF-I reflect mainly the changes in the hepatic IGF-I mRNAs production and translation.

Transgenic and Knockout Models Affecting GH-IGF-I Axis

Much of our knowledge of normal and abnormal physiology has been derived from clinical syndromes, including GH overproduction, which results in gigantism/acromegaly,

GH resistance (i.e., Laron dwarfs), GH deficiency, IGF-I deficiency, and the treatment of these disorders.

The critical role of the IGF system in somatic growth has been established in mice by ablation of the genes for IGF-I and IGF-II and their receptors using homologous recombination technology. Mice that are homozygous for a disruption of the IGF-I gene (IGF-I KO) show marked growth retardation *in utero* and postnatally *(55,56)*. At birth, these animals have body weights only approx 65% of that of wild-type littermates. The postnatal survival rate of these mice varies between specific genetic backgrounds *(57)* and, in some strains, almost 95% of mice born die shortly after birth. Survivors fail to undergo a peripubertal growth spurt and as adults have only approx 30% of the body weight of that of wild-type animals. Interestingly, certain organs such as the lungs show relative hypoplasia, whereas liver, kidney, spleen, and heart show relative hypertrophy. These animals are infertile, indicating that IGF-I plays an essential role in normal reproductive function. In contrast, the IGF-II null mice (IGF-II KO) demonstrated growth retardation exclusively *in utero (55)*. While IGF-II KO mice are born smaller than normal, they continue to grow postnatally. Thus, while both IGF-I and IGF-II are important for fetal growth and development, only IGF-I is critical for postnatal growth, at least in rodents. The IGF-I receptor null mice (IGF-IR KO) provided strong evidence that the IGF-I receptor is the main receptor involved in IGF-I function during these stages of development *(58)*. IGF-IR KO mice show extremely retarded growth patterns and die at birth, probably due to poor organ development. Simultaneous disruption of the IGF-I and IGF-IR genes in mice *(58)* results in a phenotype similar to the IGF-IR KO, suggesting that the actions of IGF-I are primarily mediated via the IGF-IR. IGF-II KO/IGF-IR KO double knockout mice *(58)* are even more growth-retarded, suggesting that some effects of IGF-II *in utero* are mediated via a different receptor, namely, the closely related insulin receptor.

The GH/IGF-I axis was further studied by deletion of the GH receptor gene, which resulted in a mouse model for Laron-type dwarfism (GHR KO) *(59)*. These mice show elevated serum GH levels, reduced IGF-I levels, and growth retardation. The reduced IGF-I levels in the circulation are due to a complete loss of the stimulatory effect of GH on IGF-I gene expression, and the elevated circulating GH levels were secondary to the lower circulating IGF-I levels. This model closely correlates with the Laron dwarf clinical syndrome of GH insensitivity. GH deficiency as seen in the *little* mouse (*lit/lit*) resulted in aberrant body composition, reduced serum levels of IGF-I to approx 10% of control levels, and a marked reduction in the IGFBP-3 levels *(60)*. The *lit/lit* mice were characterized by abnormally large adipose tissue stores and reduced body water, protein, and minerals. These changes are attributed to lack of adequate GH in these mice.

To answer the question as to whether circulating vs autocrine/paracrine IGF-I is critical in postnatal growth, we used the conditional gene deletion technique. Using the Cre/*loxP* system, we created a liver-specific IGF-I gene deletion (LID) *(61)*. LID mice showed a complete ablation of hepatic IGF-I mRNA and normal IGF-I mRNA levels in non-hepatic tissues. Circulating IGF-I levels in the LID mice were markedly reduced to only 25% of that in control animals. This was associated with an approximately fourfold increase in circulating GH levels, presumably due to the lack of negative feedback control of circulating IGF-I on pituitary GH secretion. LID mice exhibited normal growth and development as measured by body weight and body length. Further experiments with the LID mice (on a different genetic background) revealed a mild decrease of 6% in linear growth *(62)*. As discussed below, LID mice exhibited a reduction in bone volume, bone mineral density (BMD), and demonstrated muscle insulin insensitivity *(62)*.

Gene deletion of the acid labile subunit (ALS), which stabilizes the IGF-I in circulation, resulted in a phenotype similar to the LID mice *(63)*. Circulating levels of IGF-I in ALSKO mice were reduced to 40% of control levels (presumably due to increased protein degradation). However growth and development were only mildly affected (approx 10% reduction in body weight).

In both genetic-manipulated mice lines, LID and ALSKO, where circulating IGF-I levels were markedly reduced, there was only a mild effect on linear growth. However, as discussed below, a more precise characterization of these lines revealed a significant reduction in bone volume and BMD. On the other hand, overexpression of the ALS in transgenic mice caused a milled reduction of approx 6% in body weight and a significant reduction in litter size, most likely due to decrease tissue IGF-I bioactivity *(64)*.

IGFBP transgenic and knockout mice models provided us with new insight into the complexity of the IGF-I/IGFBP axis and their regulation of growth and development. IGFBP-1 overexpression in three different transgenic models resulted in reduced brain size associated with specific morphological alterations *(65–69)*. Brain growth retardation in these lines is attributed to the inhibitory effect of IGFBP-1 and related to its binding capacity to IGF-I and inhibition of its action. Few of the IGFBP-1 lines also demonstrated somatic growth retardation, alterations in glucose homeostasis, and decreased reproductive performance. IGFBP-2 transgenic mice showed a decrease of approx 10% in body weight compared to control mice *(70)*. In contrast, overexpression of the IGFBP-3 in mice resulted in selective organomegaly *(71)*. Analysis of serum samples from those mice revealed a high percentage of IGF-I–IGFBP-3 binary complexes as compared to control mice, which might account for the phenotype observed in those mice. Expression of the IGFBP-3 specifically in the mammary tissue resulted in altered involution *(72)*. Mammary tissue remodeling was retarded most likely

due to reduced apoptosis. Tissue-specific expression of the IGFBP-4 in the gall bladder, aorta, stomach, and uterus under the smooth muscle α-actin promoter resulted in hypoplesia and reduced weight of those organs *(73)*. Inactivation of the IGFBP-2, -3, and -6 genes did not appear to differ significantly from control mice *(22)* (IGFBP-2 null mice demonstrated a 30% reduction in splenic size with no overt phenotypic alterations) *(74)*.

Circulating IGF-I in Pathophysiological Conditions

Linear Growth (Acromegaly, Dwarfism)

The mammalian skeleton is a large organ, which remodels itself and serves as a constant source of calcium for the circulation *(75,76)*. The skeleton is composed of cortical bone, trabecular bone, and marrow. Linear bone growth is mediated by a complex series of events that occur within the epiphysial growth plate. This process is regulated by various nutritional, neuronal, and hormonal mechanisms, each of which is necessary for optimal bone growth.

IGF-I and IGF-II act in an endocrine and autocrine/paracrine fashion on bone. The skeletal matrix is highly enriched with IGFs, the IGFBPs, and IGFBP proteases, suggesting an important role for these factors in bone growth and remodeling *(77)*. There are many in vitro and in vivo studies on osteoblast-like cells from humans and rodents demonstrating IGF-I gene regulation by a variety of growth factors that regulate skeletal development *(75)*. Among them are interleukin-6, transforming growth factor β, parathyroid hormone, steroids, and vitamin D, which their effect on bone is mediated in part through increased local IGF-I gene expression *(75)*. Several lines of evidence from both animal and human studies have previously provided indirect support for the hypothesis that circulating IGF-I plays an important role in the acquisition of peak bone mass and strength. Human studies have supported a link between circulating IGF-I and bone density and/or strength. Langlois et al. reported a relatively strong correlation between serum IGF-I levels and bone mineral density (BMD) in women *(78)*. More recently, Garnero et al. noted that low serum levels of IGF-I were associated with a significantly greater risk of hip fractures among a large cohort of older postmenopausal women in France *(79)*.

Studies with genetically altered animals provided investigators with major clues as to the circulating IGF-I–bone mass relationship. Mice overexpressing the IGF-I or IGF-II transgene under the metallothionein promoter show an increased body weight and disproportionate growth of a few organs *(80,81)*. However, their skeletal growth is normal. In contrast, mice lacking the IGF-I gene are about 30% of normal size and have lower cortical bone mass, but their trabecular bone density and connectivity are much greater than controls *(82)*. Mice lacking the IGF-IR demonstrate severe growth retardation, defects in cell and tissue differentiation, extreme organ hypoplasia, and delayed skeletal calcification, and die shortly after birth *(58)*. However, a selective disruption of the IGF-IR in osteoblasts did not affect body weight and size but demonstrated a striking decrease in cancellous bone volume, connectivity, and trabecular number and an increase in trabecular spacing *(83)*. A spontaneous mutant, the GH-releasing-hormone-receptor-resistant *lit/lit* mouse, which has about 10% of control levels of IGF-I in circulation, has significantly reduced volumetric femoral bone density, reduced cortical bone volume, but has normal trabecular bone volume *(84)*. Inbred strains of mice (C3H/HeJ and C57/B6) that exhibit differences in femoral volumetric BMD also show about 30% differences in serum IGF-I concentrations *(85,86)*. Furthermore, growth-hormone-receptor-deficient mice (GHRKO) show a marked decrease in circulating IGF-I levels, a marked growth retardation, and reduced bone growth and bone mineral density *(87)*. Trabecular bone volume of these mice did not change, but bone turnover was significantly reduced. IGF-I treatment of the GHRKO mice almost completely reduced all the effects of GHRKO on bone growth and remodeling, suggesting a direct effect of IGF-I on both osteoblasts and chondrocytes. A combined deficiency of IGF-I and the GHR results in a super small mouse (approx 20% of normal size) with severe skeletal phenotype *(88)*. Genetic analysis of the growth-promoting effects of IGF-I and GH revealed that IGF-I alone accounts for 35% of growth and GH for 14%. IGF-I and GH, however, had an overlap contribution of 34% (the remaining 17% is the contribution of other growth factors).

Mice overexpressing the IGFBP3 or IGFBP4 ubiquitously show growth retardation and small bones, suggesting a reduced skeletal IGF-I bioavailability *(71,73)*. Generation of the LID and the ALS-deficient mice (ALSKO) provided more evidence for the importance of circulating IGF-I in skeletal development. LID and ALSKO mice, which have 25% and 40% of circulating IGF-I, respectively, demonstrated reduced total femoral volumetric BMD *(62)*. Additionally, femorae from LID and ALSKO mice, which were only slightly (about 6%) shorter compared to control mice, had significantly reduced bone volumes even without differences in skeletal IGF-I mRNA expression. These data suggest that circulating IGF-I is critical for the modeling of bone and in particular for periosteal growth because femoral length of the LID and ALSKO mice was only slightly affected. Crossing of the LID mice with the ALSKO mice resulted in severe growth retardation and a marked decrease in bone length and BMD *(62)*.

The mechanism by which circulating IGF-I levels function in the growing skeleton remains to be determined. Further characterization of the role of circulating IGF-I in bone acquisition may have important clinical implications.

Diabetes

Insulin resistance is central to the pathology of a number of diseases and syndromes including obesity, diabetes, acromegaly, and polycystic ovary syndrome. It is defined by the failure of target cells to respond to normal levels of circulating insulin. At the molecular level, insulin resistance is correlated with impaired insulin signaling and involves not only dysfunction of cell surface components like the insulin receptor (IR), but also cellular components like insulin receptor substrate-1 (IRS-1), IRS-2, phosphatidyl inositol 3' kinase (PI3'-K), and other members of the insulin signaling and glucose transport pathways *(89)*. Transgenic and knockout (KO) models of the IR and other key elements in the insulin-signaling pathways help in the understanding of the molecular mechanism of insulin resistance. Disruption of the mouse IR in all the tissues *(90)* or even specifically in liver, muscle *(91)*, or pancreatic-β cells *(92)*, caused impaired insulin action. Interruption of downstream components of the insulin-signaling pathway, such as IRS-1 *(93)*, IRS-2, or both *(93,94)*, showed that the insulin-induced activation of PI3'-K, glucose transport, p70 S6 kinase, and MAP kinase were markedly impaired in muscle.

In contrast to these studies on insulin and IR, total deletion of the *igf-1* or *igf-2* genes caused mainly growth retardation *(55,56)*. Nevertheless, accumulating data suggest a role for IGF-I in glucose homeostasis, lipolysis, proteolysis, and protein oxidation *(95)*. The role of circulating IGF-I in carbohydrate metabolism was assessed in the LID mice in our previous study *(96)*. The LID mice have low levels of circulating IGF-I and as a result high levels of GH. The antagonistic effects of GH on insulin actions are well documented *(97)*; chronic GH excess as seen in the GH transgenic mice is associated with reduced IR levels and reduced insulin-stimulated IR and IRS1 phosphorylation *(98,99)*. These changes appear to be the consequence of increased serum levels of GH followed by increased levels of insulin. Indeed, LID mice showed hyperinsulinemia, which was associated with muscle insulin resistance *(96)*. In humans, chronic elevations of circulating GH, as seen in acromegaly or GH-producing tumors *(100)*, leads to the development of insulin resistance, impaired glucose tolerance, hyperinsulinemia, and, in some cases, diabetes *(101)*. Although the antiinsulin effects of GH are well known, the exact molecular mechanism for GH-induced insulin resistance still needs to be elucidated.

In the muscle of the LID mice we could not detect stimulation of tyrosine phosphorylation of either IR or IRS-1, after insulin injections *(96)*. Negative regulation of insulin action is attributed to agents that increase serine/threonine (Ser/Thr) phosphorylation of the IR or its downstream effectors such as the IRS proteins *(102)*. Ser/Thr phosphorylated sites on IR are thought to interfere with the IR kinase activity and its ability to tyrosine phosphorylate its downstream substrates. Another aspect of regulation is the dephosphorylation of IR and its substrates by protein tyrosine phosphatases (PTPases). It has been shown that PTP1B regulates both mitogenic and metabolic actions of insulin *(103)*. GH and insulin signaling seem to converge at the postreceptor levels. The extent of cross talk between the GHR and the IR and its physiological significance still needs to be elucidated. Gene inactivation of the GHR in mice (GHRKO mice) resulted in reduced serum insulin and glucose levels *(104)*. GHRKO mice exhibited increased abundance of the IR in liver as well as increase in insulin-stimulated tyrosine phosphorylation, concordant with a state of insulin hypersensitivity *(104)*. It has been shown that GH can activate members of the insulin-signaling pathway, IRS-1 and IRS-2 *(105,106)*. Although the nature of the interaction between the IRS molecules and the GHR/JAK2 complex is not clear, it does appear that JAK2 activation is involved in the insulin-like effect of GH on tyrosine phosphorylation of IRS-1 and IRS-2. Moreover, activation of the GHR due to high levels of circulating GH may also cause activation of Ser/Thr kinases that might negatively regulate the insulin kinase activity *(107)*. Indeed, LID mice demonstrated elevated basal levels of tyrosine phosphorylation of IRS-1, which are most likely the result of high circulating levels of GH and/or insulin. Inactivation of the GH action in the LID mice following GH-releasing-hormone receptor (GHRHr) antagonist injections, demonstrate that in the face of low circulating IGF-I levels we could improve insulin sensitivity (although not to normal levels) *(96)*. Administration of rhIGF-I into the LID mice inhibited insulin and GH secretion and thereby improved insulin sensitivity *(96)*. These studies indicate that the effect of circulating IGF-I on insulin sensitivity is most likely indirect by lowering circulating GH levels. Moreover, it is suggested that normal levels of circulating IGF-I are needed for the fine balance between GH and insulin and for maintaining a state of normoglycemia.

Cancer

Tumor cells exhibit abnormal cellular activity, which is maintained and controlled by a large number of growth factors. Among these growth factors, IGFs play a crucial role in regulating cell proliferation and inhibiting apoptosis *(108, 109)*. The IGFs are expressed ubiquitously and act in an endocrine, paracrine, and autocrine fashion. The IGFs bind to the IGF-I receptor (IGF-IR), which is expressed in most cancer cells and was shown to increase cell proliferation rate *(108,109)*. Activation of the tyrosine kinase IGF-IR, initiates a number of intracellular signaling cascades, which enhance cell-cycle progression and inhibit apoptosis. As discussed below, IGFBPs were also shown to affect cancer cell growth by modulating the interactions between the IGFs and the IGF-IR. However, a direct causative relationship between circulating IGF-I and/or IGFBP-3 levels and tumor initiation and development has not yet been established. The correlation between IGF-I/IGFBP-3 and cancer has led

investigators to question whether circulating levels of IGF-I and IGFBP-3 may indeed serve as a risk marker in tumor development.

Increasing numbers of epidemiological studies in humans have shown a correlation between circulating levels of IGF-I and IGFBP-3 and the relative risk of developing colon, breast, prostate, and lung cancer *(110–114)*. Higher levels of IGF-I and lower levels of IGFBP-3 were demonstrated to be independently associated with an increased risk of colorectal cancer *(112)*. Subjects with adenomas, designated as high risk for developing into cancer, had significantly higher serum IGF-I levels and lower IGFBP-3 levels than did subjects with normal colonoscopy examinations or those with adenomas that were designated as low risk *(115)*. Additionally, acromegalic patients who have elevated serum levels of GH and IGF-I are at increased risk for developing colonic premalignant polyps and cancer *(116)*.

Using the LID mouse model we aimed to determine the role of circulating IGF-I levels in tumor growth and metastasis. In our previous study *(117)*, murine colon 38 adenocarcinoma tissue fragments were transplanted onto the cecum of LID and control mice. The growth of cecum tumors and the extent of hepatic metastases were measured. Interestingly, the incidence of cecum tumor growth and hepatic metastases were significantly higher in control mice, as compared to LID mice that have only 25% of circulating levels of IGF-I *(61)*. Administration of rhIGF-I increased tumor growth and metastases in both control and LID mice. This study suggested that the lower levels of total circulating IGF-I in the LID mice impaired tumor development. The association of serum IGF-I levels with the risk for developing colon cancer in humans is therefore particularly significant, in view of the potential use of rhIGF-I as a therapeutic agent for a number of conditions including diabetes, renal failure, various catabolic syndromes, and age-associated tissue degeneration *(118–121)*.

Breast cancer cells are responsive to IGFs, and it has been proposed that breast cancer prognosis depends on host-derived IGFs in a manner analogous to the dependence on steroids. Measurements of circulating IGF-I levels in breast cancer patients showed significantly higher levels of circulating IGF-I compared to normal patients, whereas there was no correlation with several prognostic factors such as estrogen receptor and nodal status *(122)*. Other retrospective studies of breast cancer in humans showed significantly higher levels of IGF-I in women with breast cancer compared to the control group. However, the role of IGF-I production by the breast cancer was not ruled out *(123)* and, in addition, IGF-I levels, measured in 69 advanced breast cancer patients, could not be used to identify patients with better or worse short-term survival *(124)*. A prospective study that included 397 women with breast cancer and 620 age-matched control women showed that among premenopausual women those in the top tertile of IGF-I levels had a relative risk of breast cancer of 4.58 (95% confidence limits 1.75–12.0) compared to those in the bottom tertile.

In rodent models, it was shown that energy-restricted diets, which significantly reduce the circulating IGF-I levels, there is a decrease in cancer incidence *(52,125,126)*. Experiments with the *lit/lit* mice, which have only 10% of circulating IGF-I levels, demonstrated a significant attenuation of neoplastic proliferation of human MCF-7 cell xenografts *(127)*. In contrast, transgenic mice that overexpress growth hormone and, consequently, have high levels of circulating IGF-I, exhibit morphological evidence of mammary gland epithelial cell hyperplasia and a high frequency of breast cancer *(128, 129)*. On the other hand, transgenic mice overexpressing the growth hormone antagonist show ductal hypoplasia *(130)*.

IGFBPs can be potentiators or inhibitors of IGF-I action. In a pilot study of IGFBP expression in 80 breast tumor specimens, McGuire et al. found that IGFBPs-2 to -6 were all expressed in the tumors except IGFBP-1 *(131)*. IGFBP-4 expression was correlated with poor prognostic markers. In a wider study of 238 node-negative patients, it was shown that the IGFBPs level correlate with estrogen receptor (ER) expression *(132,133)*. ER-positive tumors expressed high levels of IGFBP-4 and -5 while ER-negative tumors expressed high levels of IGFBP-3 *(134)*. It was shown that in human breast cancer cells, IGFBP-3 expression is inhibited by estrogen, whereas antiestrogens can stimulate IGFBP-3 expression *(134)*. Moreover, IGFBP-3 can be proteolyzed by proteases such as cathepsin D, prostate-specific antigen (PSA), and plasmine, all of which can be detected in breast cancer cells *(135–139)*. In general, it is believed that these proteases cleave the IGFBP-3 and thereby release IGF-I from IGFBP-3 and increasing IGF-I bioavailability. Recent evidence indicates that few of the IGFBPs may also have IGF-I-independent effect on tumor growth. It was demonstrated that IGFBP-3 has a specific and high-affinity cell association and act as a growth inhibitor for ER-negative human breast cancer cells *(140)*. Furthermore, it was shown that the antiproliferative effects of TGF-β and retinoic acid on human breast cancer cells were mediated, in part, through the IGFBP-3 axis *(140)*. Further understanding of the IGF-I-independent effects of IGFBPs on neoplastic cells is important for developing new strategies for the endocrine therapy of breast cancer.

In summary, serum IGF-I concentrations may be useful in determining the risk for breast, prostate, lung and colon cancers, and in development of risk-reduction strategies. However, more studies are needed to provide precise estimation of the endocrine IGF-I effect on tumor initiation and progression.

Summary

IGF-I is an important regulator of somatic growth, carbohydrate metabolism, and tumorigenesis. It functions in an

endocrine and autocrine/paracrine fashion. IGF-I appears to circulate in high molar levels as compared to other hormones and its action in the target tissue is regulated by a complex interaction with the IGF-IR, IGFBPs, and IGFBP-proteases.

Understanding the endocrine vs autocrine/paracrine roles of IGF-I was partially achieved by using the Cre/*loxP* system. Generation of the liver IGF-I deficient mice (LID) enabled us to demonstrate the roles of IGF-I originated from the liver in bone growth and acquisition, diabetes, and cancer. In summary, normal circulating IGF-I levels (1) are important to maintain cortical bone mineral density and skeletal integrity, (2) are necessary for the hormonal balance between GH and insulin and thereby necessary to maintain normoglycemia, and (3) could serve as a risk factor for developing colon and breast tumors.

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