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Cellular and Clinical Perspectives on Skeletal Insulin-Like Growth Factor I

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Abstract Insulin-like growth factor (IGF) I, a polypeptide synthesized by skeletal cells, is presumed to act as an autocrine regulator of bone formation. IGF I stimulates bone replication of preosteoblastic cells and enhances the differentiated function of the osteoblast. The synthesis of skeletal IGF I is regulated by systemic hormones, most notably parathyroid hormone and glucocorticoids, as well as by locally produced factors, such as prostaglandins and other skeletal growth factors. Whereas hormones and growth factors regulate IGF I synthesis, the exact level of regulation has not been established and may involve both transcriptional and posttranscriptional mechanisms. The IGF I gene contains six exons, and both exon 1 and 2 contain transcription initiation sites. Extrahepatic tissues, including bone, express exon 1 transcripts, and regulation of the exon 1 promoter activity in osteoblasts is currently under study. It is apparent that the regulation of IGF I gene transcription as well as the regulation of mRNA stability is complex and tissue specific. It is possible that abnormalities in skeletal IGF I synthesis or activity play a role in the pathogenesis of bone disorders. In view of its important anabolic actions in bone, it is tempting to postulate the use of IGF I for the treatment of disorders characterized by decreased bone mass. An alternative could be the stimulation of the local production of IGF I in bone.

Key words: insulin-like growth factor I, transcriptional control, posttranscriptional control, gene expression, insulin-like growth factor binding proteins, osteoblast, bone formation, osteoporosis

Insulin-like growth factor (IGF) I, a 7.5 kilodalton (kd) polypeptide, is one of the most abundant growth factors present in bone [Canalis et al., 1989]. IGF I acts as a regulator of bone cell function, and it stimulates the proliferation of preosteoblastic cells, thereby increasing the number of cells capable of producing bone matrix. Additionally, IGF I increases collagen expression while decreasing collagen degradation, causing an anabolic effect in bone tissue [McCarthy et al., 1989]. The mechanism of these effects is still under study, but IGF I is known to increase type I collagen transcripts and to decrease interstitial collagenase expression in osteoblast cultures [Canalis et al., unpublished observations]. This would indicate that changes in collagen and collagenase synthesis are central to the role of IGF I in bone formation. Recently, IGF I was found to increase the recruitment of cells of the osteoclast lineage, consequently enhancing the resorption of bone [Mochizuki et al., 1992]. As such, IGF I may play a dual role in bone remodeling and in the coupling of bone resorption and formation.

IGF I circulates in serum as part of a large molecular weight complex consisting of IGF I and II, IGF binding proteins (IGFBP), and an acid labile subunit [Daughaday and Rotwein, 1989]. Since there is no free IGF I present in serum, its role as a systemic regulator of bone formation could be questioned. The origin of circulating IGF I is the liver, and growth hormone is the major agent governing the synthesis of hepatic IGF I. In contrast, IGF I synthesis in most extrahepatic tissues is not under growth hormone control. IGF I is synthesized by most skeletal cells including fibroblasts and osteoblasts, and it is believed to act as a paracrine and autocrine regulator of osteoblast function. In osteoblasts, IGF I expression is modulated by hormones and growth factors, most notably parathyroid hormone (PTH), while IGF I activity is modulated by a family of IGFBPs [Canalis et al.,

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1993b]. Thus, the regulation of skeletal IGF I at multiple levels plays a central role in bone homeostasis.

Studies from this and other laboratories have revealed that systemic and local factors regulate IGF I levels in osteoblast cultures. For the most part, changes in IGF I polypeptide levels have paralleled changes in IGF I transcripts suggesting alterations in IGF I synthesis [Canalis et al., 1993a]. There have been no reports on modifications of IGF I polypeptide degradation in bone cell cultures, possibly because of technical difficulties associated with these studies. However, changes in IGF I degradation also may play a critical role in determining the availability of IGF I to its target bone cell. IGF I polypeptide degradation may depend on its association with a specific IGFBP, and changes in IGFBP synthesis, degradation, and IGF I affinity could play a role in the stability of IGF I and in the regulation of IGF I degradation.

The fact that bone cells can both synthesize and respond to IGF I suggests its role as an autocrine and paracrine regulator of bone cell function. Osteoblast IGF I receptors have been characterized by Scatchard plot analysis and by chemical cross-linking experiments [Centrella et al., 1990]. IGF I binds to a high affinity receptor, which migrates with an approximate molecular weight of 130 kd, binds IGF I with a dissociation constant of 750 pM, and is presumed to mediate IGF I actions in bone. The typical levels of IGF I in the medium of cultured osteoblastic cells is within the concentration range required for receptor binding and for mediating a measureable physiological effect [Canalis et al., 1989]. Evidence for a role for IGF I as an autocrine modulator of osteoblast function has been demonstrated by the inhibition of a subset of the anabolic actions of PTH on osteoblasts with neutralizing antibodies to IGF I [Canalis et al., 1989]. Neutralizing antibodies to IGF I also have been shown to inhibit the stimulatory effects of growth hormone on rat long bones, where the effect of growth hormone is believed to be mediated by an increase in IGF I synthesis by growth plate chondrocytes [Scheven and Hamilton, 1991]. Autocrine regulation by IGF I also has been demonstrated in a variety of nonskeletal cell types [Moats-Staats et al., 1993; Pietrzkowski et al., 1993]. The autocrine and paracrine nature of IGF I regulation may mediate the effects of numerous local and systemic factors on bone cells as well as on other tissues, and may therefore be part of a general phenomenon.

HORMONE AND GROWTH FACTOR REGULATION OF SKELETAL IGF I

Growth hormone has a minor stimulatory effect on IGF I synthesis in osteoblast cultures, whereas PTH stimulates IGF I synthesis three- to fourfold. The effect of PTH is mediated by an increase in cAMP production, and other agents known to stimulate cAMP in osteoblast cultures also increase IGF I synthesis. The effect of PTH on IGF I synthesis is not dependent on changes in intracellular concentrations of calcium or on modifications of the protein kinase C pathway [McCarthy et al., 1990]. PTH not only increases IGF I synthesis in skeletal cells, but IGF I has been shown to be responsible for selected anabolic actions of PTH in bone [Canalis et al., 1989]. Other hormones, such as 17β estradiol, increase IGF I transcription in cells of the osteoblast lineage, while 1,25 dihydroxyvitamin D_3 decreases IGF I levels in the osteoblastic cell line MC 3T3 but not in primary cultures of osteoblast-enriched cells from rat calvariae [Ernst and Rodan, 1991; Canalis et al., 1992]. Glucocorticoids were recently shown to decrease IGF I transcripts and polypeptide levels in primary osteoblast cultures, and it is reasonable to postulate that a reduction in IGF I synthesis is responsible for the inhibition in bone formation caused by these steroids [Canalis and Avioli, 1992]. However, it appears that glucocorticoids also regulate the production of IGFBPs [Chen et al., 1991; Canalis et al., unpublished observations], and it is likely that they have multiple effects on the skeleton involving IGF I dependent and independent mechanisms. In addition to systemic hormones, locally produced factors regulate the synthesis of skeletal IGF I. Prostaglandin E_2, possibly through an enhancement of cAMP production by bone cells, increases IGF I synthesis in osteoblast cultures [McCarthy et al., 1991]. The effects of transforming growth factor β (TGFβ) on skeletal IGF I synthesis have varied with the culture model studied. In primary cultures of osteoblast-enriched cells TGFβ1 inhibits IGF I mRNA and polypeptide levels. A similar effect is observed with platelet-derived growth factor (PDGF) BB and basic fibroblast growth factor (bFGF) [Canalis et al., 1993a]. Although these growth factors are mitogens for skeletal cells, their inhibitory actions on IGF I synthesis are independent of their effects on cell

replication since DNA synthesis inhibitors do not modify the response. TGFβ, PDGF, and bFGF do not stimulate the differentiated function of the osteoblast, and they may even prevent it and induce dedifferentiation of osteoblasts. This activity seems to parallel their inhibitory actions on IGF I synthesis, and a decrease in IGF I production may impair the differentiated function of bone-forming cells. This hypothesis is supported by the fact that bone morphogenetic protein, a growth factor that stimulates cell replication as well as the differentiation of cells of the osteoblastic lineage, *stimulates* IGF I mRNA and polypeptide levels in osteoblast cultures [Gabbitas and Canalis, unpublished observations]. Whereas hormones and growth factors regulate IGF I synthesis, the exact level of regulation has not been established. It may differ among the various agents, and it is likely to involve transcriptional and posttranscriptional mechanisms.

TRANSCRIPTIONAL AND POSTTRANSCRIPTIONAL REGULATION OF IGF I

Mammalian IGF I polypeptides are highly conserved, and the human and rat IGF I genes share similar structural elements [Daughaday and Rotwein, 1989]. Changes in IGF I mRNA levels in osteoblasts are closely followed by changes in IGF I protein levels [Canalis et al., 1993b], suggesting that the regulation of IGF I transcripts is a key control point in the regulation of IGF I expression. However, IGF I gene regulation has the potential to be complex. The rat IGF I gene spans 70 kb of DNA and contains six exons and codes for at least two precursor proteins [Hall et al., 1992]. The mature IGF I protein, 70 amino acids long, is encoded by sequences found in exons 3 and 4 [Daughaday and Rotwein, 1989]. The IGF I mRNA species range in size from 0.8–7.5 kb, resulting from the use of tandem promoters, each with multiple transcription initiation sites, alternative splicing of exons, and variable polyadenylation signals [Hall et al., 1992]. It is possible that the IGF I gene is regulated at the levels of transcription, mRNA processing, and RNA stability, and the mechanisms regulating IGF I expression may be tissue-specific. For example, transcripts containing exon 1 are found primarily in bone and other extrahepatic tissues, while exon 2–containing transcripts are found primarily in liver [Hall et al., 1992; Lowe et al., 1987; Canalis and LeRoith, unpublished observations].

The IGF I promoter found in exon 1 has four transcription initiation sites and does not contain classical promoter elements, such as a TATA or a CCAAT box [Adamo et al., 1991; Hall et al., 1992]. However, this promoter contains a sequence which resembles the "initiator" element [Hall et al., 1992] of the mouse terminal deoxynucleotidyl transferase gene, which can direct transcription initiation in the absence of a TATA element [Smale and Baltimore, 1989]. A second IGF I promoter is found in exon 2. This promoter has two transcription initiation sites but is more like a typical eukaryotic promoter in that it contains putative CCAAT and TATA elements [Hall et al., 1992; Adamo et al., 1991, 1993]. Several groups have used chimeric constructs containing IGF I promoter fragments linked to the reporter gene luciferase [Hall et al., 1992; Adamo et al., 1993] to examine the transcriptional regulation of the rat IGF I gene. They found that exon 1 promoter fragments are more active than exon 2 promoter fragments in Chinese hamster ovary (CHO) cells and in the IGF I producing SK-N-MC neuroblastoma cell line, possibly reflecting the tissue-specific nature of IGF I promoter usage [Adamo et al., 1993; Hall et al., 1992]. The regulation of IGF I transcription in osteoblasts is currently being studied using chimeric rat IGF I promoter/luciferase gene constructs [Adamo et al., 1993]. These experiments will lead to the identification of regulatory elements which play a role in modulating IGF I transcription in response to hormones and growth factors.

Induction or repression of IGF I gene transcription probably is not the only mechanism by which the gene is regulated. IGF I mRNA species range in size from 0.8–7.5 kb, and the biologic significance of these multiple transcripts is still unclear. In rat liver, all IGF I mRNA species are found associated with polysomes [Foyt et al., 1991; Thissen and Underwood, 1992]. In osteoblasts, the largest (7.5 kb) transcript is the most abundant IGF I mRNA species, and this RNA contains about 6 kb of 3′ untranslated sequence [Lund et al., 1989; Hoyt et al., 1992]. The 3′ untranslated region of the 7.5 kb mRNA contains multiple AU-rich regions and potential stem loop structures [Hoyt et al., 1992], which may be associated with the modulation of mRNA stability [Carter and Malter, 1991]. For example, growth hormone was found

to induce the IGF I mRNA species to a similar extent in the liver of normal and hypophysectomized rats, but the 7.5 kb transcripts decayed three times faster than lower molecular weight mRNA species after hypophysectomy [Hepler et al., 1990]. Other reports reveal differential stability of the IGF I mRNA species on polysomes in liver [Thissen and Underwood, 1992], but this may not occur in other tissues. Therefore, like the regulation of the IGF I gene transcription, the regulation of IGF I mRNA stability may be a tissue-specific phenomenon dependent on the complement and quantity of specific nucleases and RNA stabilizing factors found in different tissues.

Several cytoplasmic proteins, which regulate RNA stability through AU-rich elements in the 3′ untranslated regions of transcripts, have been isolated from lymphocytes. These include adenosine-uridine binding factor (AUBF), a 36 kd protein, which binds to AUUUA motifs found in labile cytokine and lymphokine mRNAs [Gillis and Malter, 1991] and which may be responsible for the phorbol ester or calcium ionophore stabilization of mRNA [Malter and Hong, 1991]. AU-A, AU-B, and AU-C are also AU-specific binding factors isolated from lymphocytes. These differ from each other in molecular weight, subcellular location, and inducibility with lymphocyte activation [Bohjanen et al., 1992]. Most recently, the heterogeneous nuclear ribonucleoproteins A1 and C have been shown to be associated with the AUUUA sequences in the 3′ untranslated region of GM-CSF mRNA [Hamilton et al., 1993]. Since the 3′ untranslated region of the largest IGF I mRNA is AU-rich [Hoyt et al., 1992], it is possible that selected AUBFs play a role in regulating the stability of the transcript. Translation and increased transcript degradation are frequently associated [Carter and Malter, 1991]; therefore, it will be important to determine which, if any, of the IGF I mRNA species are preferentially translated in osteoblasts. Preferential translation of the 7.5 kb species may be related to its decreased stability.

PHYSIOLOGICAL AND CLINICAL RELEVANCE OF IGF I IN BONE

There is little doubt about the importance of IGF I for normal skeletal growth, and it is likely that the systemic form of IGF I plays a pivotal role in achieving normal longitudinal growth. The contribution of systemic IGF I to the maintenance of bone mass is less clear, and there is no compelling evidence demonstrating abnormal levels of serum IGF I in patients with osteoporosis. This is not surprising since serum contains IGFBPs in excess, and there is no free circulating IGF I. There is an age-related decline in circulating levels of growth hormone and IGF I that correlates with changes in physical fitness and may be instrumental in changes in musculoskeletal mass [Kelly et al., 1990]. However, the correlation between serum levels of IGF I and bone mineral density is weak, casting some doubt about the physiological role of circulating IGF I in bone formation and mineralization. It is possible that the activity or the local concentrations of IGF I and IGFBPs in skeletal tissue are abnormal in patients with metabolic bone disorders [Canalis, 1992]. However, this hypothesis is difficult to test, since it may not be possible to extract and quantitate precisely the concentrations of IGF I and its binding proteins in skeletal tissue.

From a therapeutic point of view, it is tempting to postulate the use of systemic IGF I for disorders of decreased bone formation. Although bound by IGFBPs in serum, it should be possible to achieve effective concentrations of IGF I in the skeleton after its systemic administration. Studies in humans and rats [Guler et al., 1987; Jacob et al., 1989] have demonstrated that IGF I infusions cause hypoglycemia and reveal that IGF I has comparable actions to insulin. The hypoglycemic effect of IGF I, as well as its nonspecific actions in skeletal and nonskeletal systems, tempers the enthusiasm for its clinical use. On the other hand, IGF I has been shown to have anabolic actions in healthy and catabolic individuals and to stimulate growth in selected animal and patient populations [Clemmons, 1992; Clemmons et al., 1992; Turkalj et al., 1992; Mauras et al., 1992; Scheiwiller et al., 1986]. Recently, the combination of IGF I with growth hormone was shown to be a possible alternative to the use of IGF I alone. Since the two agents are anabolic and have opposite effects on carbohydrate metabolism, their combination resulted in a substantial increase in their independent anabolic effects with an attenuation of the hypoglycemic effect of IGF I [Kupfer et al., 1993]. Although it is plausible to postulate the systemic use of IGF I for the treatment of bone disorders, its administration to laboratory animals has resulted in controversial effects on bone formation and resorption [Spencer et al., 1991; Ibbotson et al., 1992]. An alternative to

the use of systemic IGF I and growth hormone is the manipulation of the locally produced IGF I in an effort to modify its synthesis, activity, or its binding proteins to achieve an increase in bone formation. Intermittent administration of PTH has been shown to have an anabolic action in the rat skeleton, an effect that depends on the presence of normal growth hormone reserve and likely involves changes in the synthesis of IGF I by bone cells [Hock and Fonseca, 1990]. PTH, as well as other agents that use bone as a primary target tissue and enhance skeletal IGF I synthesis, could be used in the development of novel therapeutic modalities for the treatment of osteoporosis.

CONCLUSIONS

In summary, IGF I is one of the most prevalent growth factors present in bone, where it is synthesized and acts as an autocrine and paracrine regulator of bone formation. The synthesis of skeletal IGF I is regulated by hormones and growth factors, but the exact mechanisms of its regulation are unknown. They likely involve changes at the transcriptional and posttranscriptional levels. Little is known about modulation of IGF I degradation in bone cells, but IGFBPs probably play a role at this level of regulation by modifying IGF I half-life. It is possible that changes in skeletal IGF I and IGFBPs are relevant to the development of various bone disorders, although data substantiating this possibility are lacking. Nevertheless, increases in IGF I synthesis or activity in bone cells could result in an increase in bone formation and offer a therapeutic alternative for the treatment of osteoporosis.

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