Inflamm. res. 47 (1998) 90–100
1023-3830/98/030090-11 $ 1.50+0.20/0

Inflammation Research

Review

IGF/IGFBP axis in cartilage and bone in osteoarthritis pathogenesis

J. Martel-Pelletier, J. A. Di Battista, D. Lajeunesse and J.-P. Pelletier

Osteoarthritis Research Unit, Centre de Recherche L.-C. Simard, CHUM, Pavillion Notre-Dame, 1560 est, rue Sherbrooke, 2e DeSève, Montréal, Québec H2L 4M1, Canada, Fax +1 514 896 4680

Received 27 November 1997; accepted by I. Ahnfelt-Rønne 8 December 1997

Abstract. In the context of joint biology, insulin-like growth factor-1 (IGF-1) is the most likely candidate to affect the anabolism of cartilage matrix molecules. Mechanisms for controlling the effects of IGF-1 include alterations in the level of this growth factor, its receptor and/or the IGF-1 affinity or availability to its receptor. Disturbance of any one of the above elements may induce a disregulation of the mechanisms involved in the local control of joint tissue integrity. This review focuses on recent studies of the IGF system, and the potential relevance of these results to in vivo effects in osteoarthritic (OA) tissues. It has been shown that, although the IGF-1's expression and synthesis are increased in OA cartilage, chondrocytes are hyporesponsive to IGF-1 stimulation. This phenomenon appears to be related, at least in part, to an increased level of IGF-binding proteins (IGFBP). The IGFBP have a high affinity for IGF-1, and appear to be important biomodulators for IGF action. Though to date seven IGFBP have been cloned and sequenced, disregulation in IGFBP-3 and -4 appears instrumental to arthritic disorders. Proteolytic activity directed against IGFBP has been found in both cartilage and bone; this activity appears to belong to serine- and/or metallo-proteases families. It has been suggested that a thickening of the subchondral bone participates in OA pathophysiology, and that IGF-1 production by bone and/or subchondral bone cells may contribute to these changes. An abnormal regulation of subchondral bone formation via an increase in the local activation of IGF-1 in bone cells, possibly via abnormal IGFBP synthesis due to aberrant PA/plasmin regulation of the IGF-1/IGFBP system, is believed to be a plausible hypothesis.

Key words: IGF-1 – IGFBP – Osteoarthritis – Cartilage – Bone

Correspondence to: J. Martel-Pelletier

Introduction

Osteoarthritis (OA) is the most common of the various articular disorders affecting humans. Articular cartilage, subchondral bone and synovial membrane are the sites of major abnormalities in this disease process. OA can be described as a degradation and loss of articular cartilage, accompanied by hypertrophic bone changes with osteophyte formation, subchondral bone thickening and, at the clinical stage of the disease, an inflammation of the synovial membrane [1].

Research efforts have led to major advances in understanding the pathophysiology of OA. Enzymes capable of degrading proteoglycan and collagen appear to play a significant role in OA catabolism, and the metalloprotease family (MMP) is a likely candidate [1–3]. Prior to the loss of cartilage mass and the onset of proteoglycan depletion, biosynthetic activity of the chondrocytes may lead to an increase in the proteoglycan concentration of the cartilage [4, 5], resulting in a thickening of the articular cartilage during the earlier stages of OA [6]. However, these new molecules appear abnormal as their structure is significantly altered [5]. Nevertheless, the repair process appears to keep pace with the disease, and this response may be sufficient to maintain the joint for many years. As the disease progresses, however, the degradative process will eventually exceed the anabolic one, leading to a total loss of cartilage and eburnation of bone. This appears to happen when the physiological balance between the synthesis and degradation of the extracellular matrix is disturbed. At the clinical stage of the disease, an inflammatory reaction of the synovial membrane is often seen [1, 7, 8]. This process favors the synthesis of proinflammatory cytokines, which have an impact on cartilage matrix homeostasis by altering chondrocyte metabolism to enhance catabolism while reducing the anabolism.

Insulin-like growth factor (IGF-1) and cartilage

Efforts have been devoted to identifying conditions favorable to the formation of a durable, functional articular surface

following cartilage damage. This has led to the study of factors able to stimulate cartilage repair. The list of growth factors that have a putative effect on the development and proliferation of many cells throughout the body has grown considerably. However, for the majority, studies have been conducted to examine their potential role in vitro, and relevance to cartilage metabolism is often lacking. In contrast, a large body of data from in vitro and in vivo studies has substantiated the importance of IGF-1 in promoting cartilage growth and development. IGF-1 appears to be one of the most important growth factors affecting the anabolism of the principal molecules found in cartilage, namely collagen and proteoglycan. Although IGF-1 has been shown to be moderately mitogenic in human adult articular cartilage, it also strongly stimulates production of chondrocyte extracellular matrix components [9, 10]. IGF-1 induces the expression and synthesis of both collagen type II and proteoglycan core protein, and stabilizes chondrocyte phenotype in pathological conditions where homeostasis is perturbed [11–13].

There are two forms of IGF: IGF-1 and IGF-2. Circulating IGF-1 is synthesized and secreted by the liver in response to growth hormone (GH) stimulation. Several recent findings suggest that the IGF-1 produced locally in tissues may be as physiologically important as the circulating hormone [14, 15]. In cartilage, IGF-1 is believed to act as an enhancer of matrix synthesis and/or growth promoter, depending on the state of the chondrocytes [9, 10, 16, 17], and IGF-2 as a glucose regulator at all stages of differentiation, and as a growth stimulator in a non-differentiated state [18, 19].

IGF-1 belongs to a family of peptide hormones that includes relaxin and insulin, and shares a high degree of structural similarity with proinsulin. IGF-1 is a basic 70 amino acid polypeptide (7,649 daltons) showing mitogenic effects, stimulation of protein and proteoglycan synthesis, DNA and RNA synthesis, and cell proliferation and differentiation. The human IGF-1 gene consists of six exons, numbered 1 through 6 [20], that are located within a region of over 90 kb on the chromosome 12. The gene is transcribed into a large mRNA precursor, which is alternatively spliced to yield two different classes of mRNA: IGF-1A and IGF-1B. It has been suggested that these putative proteins may not be inert propeptides, but may display a range of specific functions. For example, Sara et al. [21] reported that, in the liver, the prohormone IGF-1B has a greater ability to bind to IGFBP and serve for endocrine functions, whereas the prohormone IGF-1A acts as an autocrine agent.

This peptide interacts with specific IGF membrane receptors (IGF-R) as well as with the insulin receptor. The IGF-R is similar to, but distinct from, the insulin receptor. The type I IGF-R is a glycoprotein with a molecular weight of approximately 300,000 daltons, and consists of two extracellular α-subunits (about 130,000 daltons) and two ß-subunits (about 90,000 daltons) joined by disulfide bridges linked in an $\alpha_2ß_2$. The ß-subunits traverse the cell membrane and contain a tyrosine kinase domain in their cytoplasmic protein. The type I IGF-R mediates the action of both IGF-1 and IGF-2, as these hormones bind to and activate this receptor. The affinity (Kd) of the type I IGF-R for IGF-1 is approximately 1 nM, 2- to 10-fold lower for IGF-2 and 100- to 500-fold lower for insulin. Studies suggest type I IGF-R mediates most of the known effects of IGF-1 and IGF-2 [22].

In contrast, the type II IGF-R is a single polypeptide chain (about 220,000 daltons) with a large extracellular domain and a short cytoplasmic tail [23]. Type II IGF-R is identical to the cation-independent mannose 6-phosphate (M6-P) receptor [24], which itself functions as a lysosomal enzyme in targeting proteins. This receptor has a higher affinity for IGF-2 than for IGF-1. The limited number of studies indicating a role for the type II IGF-R in mediating metabolic and mitogenic responses of IGF-2 are difficult to interpret, and the function of type II IGF-R with respect to these activities remains vague. While it is recognized that the type II IGF-R acts as a target for lysosomal enzymes, it has also been suggested that this IGF-R also functions as a reservoir and/or clearance for IGF-2.

IGF-1 and OA cartilage

The parallel between serum and skeletal IGF-1 levels may not be readily explained since hormones and factors regulating the synthesis of IGF-1 in the liver – which determines levels of systemic IGF-1 – and in the skeleton are different. Several investigations of the relationship between serum levels of IGF-1 and the presence and severity of OA have been performed over the years, but have yielded conflicting results [25–30]. In one study, both the size and the growth of osteophytes were directly correlated to serum levels of IGF-1 in subjects with OA of the knee, but evidence of a relationship between IGF-1 and cartilage preservation was not obtained [25]. Other studies showed only a modest association with radiographic changes of knee OA [27, 29]. A recent study, however, showed no association between serum IGF-1 concentrations and OA, and the earlier data may have been skewed by the confounding effects of age [28].

The observations which focus on serum level of IGF-1 do not exclude a role for local production of IGF-1 in articular tissues. In fact, the level of IGF-1 was found to be enhanced in human OA synovial fluid [31–33]. Although the IGF-2 level was much higher than that of IGF-1, no difference was found between normal and OA [32]. IGF-1 is also produced by chondrocytes, and can act both as a paracrine and autocrine modulator to stimulate matrix synthesis and inhibit matrix degradation [13, 34]. Although human OA chondrocytes express and produce IGF-1 in an increased amount [35–37], these diseased cells are hyporesponsive to IGF-1 [38] (Fig. 1). Further studies have shown that this is related, at least in part, to the presence of the IGFBP (see below) rather than a diminution in number or functionality of the cell receptors on chondrocyte [36, 38–40]. Moreover, the hyporesponsiveness of OA chondrocytes to stimulation by IGF-1 may well account for the decrease in cartilage reparative capacity found at the advanced stages of the disease, and may explain the progressive nature of OA.

Factors controlling IGF-1 production in OA cartilage

Interestingly, proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF-α), which appear to

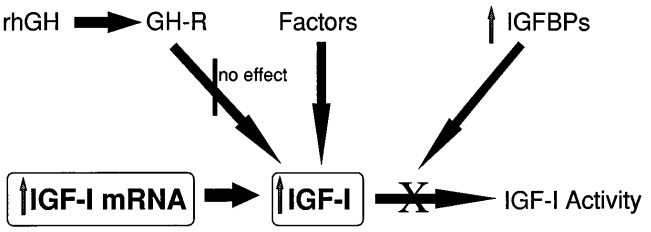

Fig. 1. Human OA chondrocytes express and produce IGF-1 in an increased amount. However, these pathological cells are hyperresponsive to IGF-1, a phenomenon related, at least in part, to the IGFBP. In human adult cartilage, agents such as cytokines and growth factors are suggested to play a role in the increased level of IGF-1. Through cleaving, proteases could also be involved in the local regulation of IGF-1 bioavailability. Interestingly, it appears that in this adult tissue, the increased IGF-1 level did not occur via GH/GH-R mechanism.

play a major role in the structural changes of OA [1, 41], were also implicated in the hyporesponsiveness of pathological chondrocytes to IGF-1 (Fig. 1). As these cytokines activate the release of IGF-1 from chondrocytes, they also trigger the release of IGFBP [42–46]. Hence, it could be postulated that, as IL-1 increases in the synovial fluid and articular tissue of OA patients, it upregulates the production of IGFBP in these tissues. Alternatively, and not exclusively of the latter hypothesis, IL-1 may also participate, via an activation of proteases, in developing the resistance of OA chondrocytes to IGF-1. Indeed, proteases such as the plasminogen activator (PA)/plasmin system, as well as other cell surface proteases – including cathepsin G and gamma-glutamyltranspeptidase, can cleave IGF-1 in other cell systems [47]. Hence, since PA/plasmin and some cathepsins are locally produced by chondrocytes [48, 49], it would be reasonable to hypothesize that these proteases systems are also involved in the local regulation of the IGF-1 system. However, to date, the presence of both cathepsin G and gamma-glutamyltranspeptidase has yet to be demonstrated in chondrocytes.

One of the most important modulating agents of IGF-1 is the GH. Of particular relevance to cartilage, GH has been shown to regulate matrix synthesis and tissue growth in immature cartilage by stimulating IGF-1 expression, and the synthesis of local tissue production via a specific cell membrane receptor, the GH-receptor (GH-R) [50–53]. Although GH probably plays an important role in epiphyseal cartilage metabolism, it has recently been shown that the elevated synthesis of IGF-1 by adult human chondrocytes occurs through a GH/GHR-independent mechanism [35] (Fig. 1). This suggests that other factors are capable of controlling local IGF-1 production in the OA chondrocytes. For example, transforming growth factor ß (TGF-ß) and other pituitary growth factors [54–56] have all been found to stimulate IGF-1 secretion in chondrocytes.

IGF-1 binding proteins (IGFBP)

The action of IGF-1 on cellular metabolism is governed at several levels, including the presence of extracellular, high-affinity IGFBP, which modify the interaction of IGF-1 with its receptor [57]. Six IGFBP (IGFBP-1 to -6) have been identified and are expressed in a number of different tissues. IGFBP-7 has recently been identified in human biological fluids and conditioned medium from human breast cancer cells, but has considerably less affinity for IGF-1 and IGF-2 than the other members of the family [58, 59]. It has recently been reported that there are potentially three other members [60], which have not yet been characterized.

The circulating or local levels of IGFBP are regulated ontogenetically, by various endocrine factors and developmental cues, and by specific cleavage with proteases that compromise the functionality of the IGFBP [61, 62]. The first seven binding proteins have been cloned and sequenced, and each represents an individual gene product. Despite high amino acid homology, they have distinct structural and biochemical properties, and exhibit tissue-specific expression. The latter fact suggests that each plays a specific role in regulating the biological actions of IGF in any given target tissue.

Disulfide bonds may be important in forming high-affinity IGF binding. Unique among IGFBP is the presence of three asparagine residues which provide the potential for N-glycosylation sites on IGFBP-3. Differential N-glycosylation causes IGFBP-3 to run as a doublet on SDS-PAGE with Mr of 39-41 kDa in contrast to the predicted Mr of approximately 29 kDa from cDNA sequence data. Amino acid sequence analysis of the first six IGFBP also reveals the presence of an ArgGlyAsp (RGD) sequence near the C-terminal of IGFBP-1 and -2. The affinity of the IGFBP for IGF may be controlled by their state of phosphorylation, in which the phosphorylated form has a greater affinity for the ligand than the dephosphorylated form [63].

IGFBP-2 to -5 have been shown to be present in human cartilage [36, 38, 39, 64] IGFBP-1 to -4 have also been identified in human synovial fluid [32]. IGFBP-3 and -4 are secreted in the greatest abundance by human chondrocytes in culture, and are produced in dramatically increased amounts in arthritic diseases [36, 38, 65]. The synovial fluids of OA and rheumatoid arthritis (RA) patients have markedly increased levels of IGFBP-3 and -4 vis-à-vis fluid from normal donors [32, 66, 67]. Moreover, the concentration of IGFBP-4 in inflammatory synovial fluid is significantly higher than that measured in serum [67], suggesting that local production by joint tissues is of some consequence physiologically. These latter two binding proteins appear to be of major importance in articular joint tissue.

While the functional role of the binding proteins remains unclear, some of their actions are known. Secreted IGFBP-3 associates preferentially with the cell surface, and it is proposed that IGFBP-3 could exert some of its activity hrough a 'specific' membrane receptor [38, 68, 69]. Of particular importance, recent evidence suggests that IGFBP-3 could directly inhibit DNA synthesis independently of IGF-1 binding in chick embryo fibroblasts [57,70] and human breast cancer cells [68, 69]. In addition, IGFBP-3 has been shown to act independently of IGF-1 in inhibiting cell growth of mouse embryo fibroblasts transfected with IGFBP-3 and having a targeted disruption of the IGF-1 receptor (null) gene [71]. IGFBP-3 can potentiate the action of IGF-1 when the binding protein is preincubated with the cells, but is inhibitory when added concurrently with IGF-1 [72]. In contrast, IGFBP-4 inhibits IGF-1 actions under most, if not all, experimental conditions [57, 73] and also appears to bind strongly to the extracellular matrix. There are IGF-1-dependent IGFBP-4 proteases secreted by a number of cell

types that regulate the bioavailability of IGFBP-4. Interestingly, IGFBP-3 can function as an IGF-1-reversible inhibitor of IGFBP-4 proteolysis [74], suggesting that proteolysis is dependent on the relative proportions of the different binding proteins and the level of IGFs [75].

IGFBP-3 and -4 gene and protein structure

The identification and structural characterization of human IGFBP-3 and -4 (hIGFBP) was made possible by the development of a number of biochemical and protein assay techniques, although molecular biology (cloning and sequencing) has proven its considerable worth in the elucidation of the finer structural/functional details of hIGFBP-3 and -4. Studies characterizing the hIGFBP-3 chromosomal gene and promoter region have demonstrated the presence of a single copy of the gene in the human genome. The gene spans 8.9 kilobases with the protein-coding region divided into four exons, while a fifth exon contains the 3′-untranslated region including the polyadenylation signal. On the chromosomal gene, the CAP site is located 30 base pairs 3′ to the start of a consensus TATA box, and 97 base pairs 3′ to a consensus GC upstream promoter element, an organization common to many eukaryotic promoters [76]. Putative promoter and 5′ flanking sequences in the IGFBP-3 gene direct high-level production of reporter gene protein (e.g. chloramphenicol acetyltransferase, CAT) product in transfection assays indicating that this region can function as a very strong promoter. Specific *cis* elements required for basal and hormone-mediated activity of the IGFBP-3 promoter have not been categorically identified. However, upstream promoter elements that may mediate basal activity surround the GC box, and have affinity for the Sp1 transcription factor. In addition, in the same region, there are sequences which resemble binding sites for the transcription factor AP-2. Indeed, AP-2 is believed to contribute not only to basal promoter activity, but also to phorbol ester-induced (e.g. PMA) increases [76]. The importance of AP-2 in this regard may be independent of the cellular context because, even in terminally differentiated cells like human chondrocytes, PMA induces a massive increase in IGFBP-3 expression and synthesis that is dependent on a concomitant increase in the nuclear accumulation of AP-2 [65]. Increased AP-2 activity may be responsible for the dramatic upregulation of IGFBP-3 observed in OA chondrocytes. The latter chondrocytes are refractory to IGF-1 stimulation, supporting the notion that IGFBP-3 exerts mostly inhibitory effects.

In the absence of any identifiable c-AMP response elements (CRE) in the promoter region of IGFBP-3, it is conceivable that prostaglandin E_2 (PGE_2)-induced increases in IGFBP-3 expression and synthesis in human chondrocytes may also result from AP-2 induction by PGE_2. Prostaglandin E_2 cell signaling is often the result of induced elevations in cAMP and AP-2 has been shown to mediate increases in promoter activity (e.g. IGFBP-5) induced by cellular elevations in cAMP [77, 78].

However, further studies have failed to identify elements which might mediate the stimulatory effects of insulin or IGF peptides on IGFBP-3 production. For example, there is no serum response element (SRE) in the promoter or 5′ flanking region of the IGFBP-3 gene, which is normally required for gene (*c-fos*) induction by insulin [76]. A rather interesting finding is the recent identification of p53 (tumor suppresser gene and inducer of apoptosis) regulatory elements in the first and second intronic sequences of the IGFBP-3 gene. Additional experiments have shown that IGFBP-3 expression is under the control of p53, and taken together, the data may provide a rationale for the growth inhibitory effects of IGFBP-3 demonstrated in many cell models [79].

Amino acid sequence data from the coding region derived from molecular cloning studies have revealed that the IGFBP-3 is synthesized with a signal peptide, and the mature protein has 264 amino acids and a molecular weight of 28,717. In addition, there are 18 conserved cysteine residues in IGFBP-3 which have been localized to both N-terminus and C-terminus regions of the protein. Disulphide bonding provides the binding site for IGF-1, and indeed, reduction of this type of bonding abrogates IGF-1 binding to its cognate site [63].

As mentioned above, specific membrane receptors have been suggested for IGFBP-3, even though the protein lacks the consensus RGD tripeptide structure. Interestingly, close amino acid sequence analysis of IGFBP-3 indicated the presence of consensus, bipartite nuclear localization signal (NLS) [80]. Since the uptake of proteins by the nucleus is very selective, nuclear proteins must contain in their primary structure a signal that specifies selective accumulation in the nucleus. Such a sequence has been identified in hIGFBP-3 at residues 217-234, prompting speculation that the protein can accumulate in the nucleus, interact with cognate sites/proteins, and inhibit cell growth and proliferation. One such potential interactive species is the nuclear anti-oncogene product retinoblastoma protein (RB) and the RB-binding protein [81,82]. Thus IGFBP-3 may be a tumor suppresser gene product.

Unlike IGFBP-3, the complete genomic sequence and structure of the hIGFBP-4 gene has not been described (as of this writing). Complimentary DNA has been isolated and sequenced, and a large portion of the promoter and 5′ flanking DNA has been reported [83, 84]. However, the rat IGFBP-4 gene structure has been elucidated in considerable detail, and the data indicate that the gene spans 12 kb and consists of four exons separated by three introns [85]. In addition, the rat gene also possesses a typical TATA box and a CAAT box; the latter sequence is lacking in the human promoter [84]. Other differences include the presence of multiple CRE and AP-1 *cis* elements in the rat IGFBP- 4 promoter region, whereas in the human promoter, only one CRE and AP-1 site has been detected. Interestingly, the human promoter also has *cis* elements for early growth factor-1 (Egr-1) and multiple Sp1 sites as well.

IGFBP-4 gene transcription increases in the presence of cAMP elevating agents such as forskolin or PGE_2 [86, 87] (Fig. 2). This response may be mediated by the CRE element, or possibly by the Egr-1, *cis* element, since the expression and synthesis of Egr-1 (transcription factor) is increased dramatically by PGE_2 particularly in human synovial fibroblasts and chondrocytes [87] (and our own unpublished observations). Whether the AP-1 element increases IGFBP-4 promoter activity and gene transcription is unclear because, in certain cell types, phorbol ester (PMA or TPA) does not increase

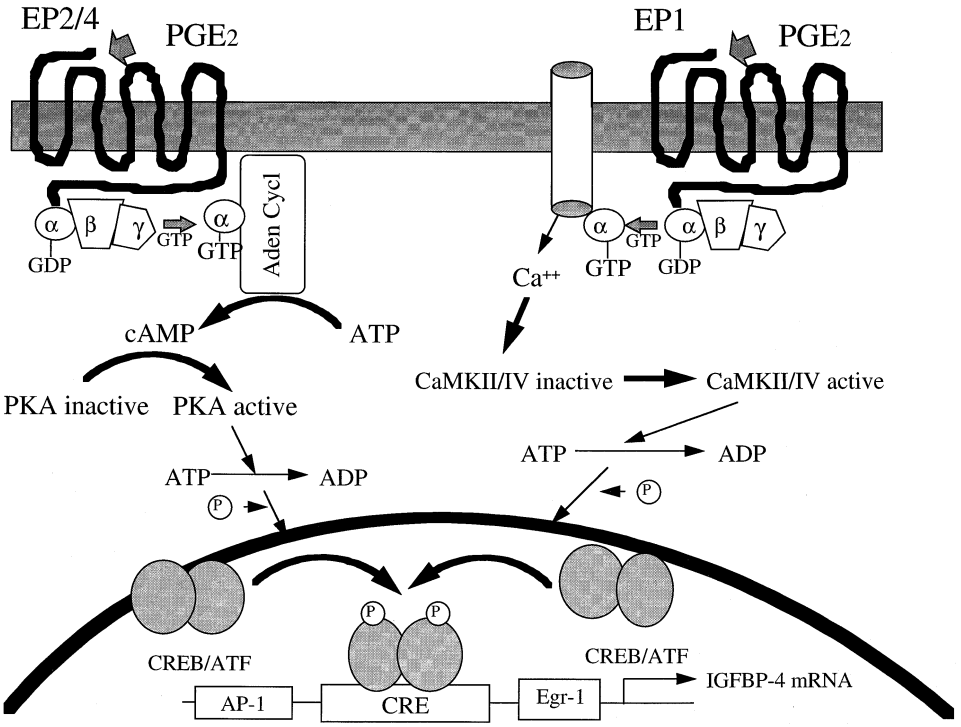

Fig. 2. Signaling pathways mediating PGE_2 induction of IGFBP-4 gene expression in human chondrocytes and synoviocytes. EP1, and associated pathways, mediates PGE_2 signaling in chondrocytes, while EP2/4 predominates in synoviocytes. The CREB/ATF-CRE pathway is the most important, but PGE_2 can upregulate Egr-1 and c-Fos expression and synthesis in both cell types. PKA = protein kinase A; CaMKII/IV = calcium/calmodulin dependent protein kinase; EP2/4 and EP1 = prostaglandin receptor isoform 2/4 and 1; Aden Cycl = adenylyl cyclase; CRE = cyclic AMP response element; AP-1 = activator protein-1 response element; Egr-1 = early growth response protein-1 response element; CREB = cyclic AMP response element binding protein; ATF = activating transcription factor.

IGFBP-4 expression [87]. It should be mentioned that PGE_2 can rapidly induce AP-1 expression and synthesis in many cell types including chondrocytes, and further studies are necessary to resolve what function c-FOS/c-JUN plays in mediating PGE_2 action in terms of IGFBP-4 expression.

Through the manipulation of cellular cAMP levels by different pharmacological means, it would appear that the expression of IGFBP-4 is also negatively regulated by elevations in the levels of cAMP in chondrocytes. As mentioned above, noncanonical cAMP response elements have been located in the promoter region of the IGFBP-4 gene, although how they function to control gene expression is not well-defined [85]. The chondrocyte data, however, are in contrast to other studies that demonstrate a marked increase by cAMP of IGFBP-4 levels in osteoblasts [83, 86]. Tissue-specific trans-acting/transcription factors may be responsible for these differences. PGE_2, though often considered a cAMP mimetic, does not increase cellular levels of cAMP or protein kinase A (PKA) activity in human articular chondrocytes, and this insensitivity may be due to a 'constitutively' activated cAMP-dependent phosphodiesterase IV(PDE IV) [65]. Hormonally-induced, time-dependent increases in PDE IV activity with a resultant decrease in cellular cAMP levels, and subsequent cellular desensitization, is now a widely accepted phenomenon [88, 89]. Whether the latter mechanism is responsible for the 'constitutivity' of PDE IV in chondrocytes awaits further investigation.

Like IGFBP-3, IGFBP-4 is synthesized with a signal peptide. Two forms have been detected, a minor of 24 kDa and one of 29 kDa. The latter form most probably represents a posttranslationally modified product of the same gene because deglycosylation of the 29 kDa form results in the formation of the 24 kDa IGFBP-4 protein [63]. Human IGFBP-4 also contains 18-20 cysteine residues that form the site for IGF binding. There are no homologous NLS as in IGFBP-3 and no RGD sequences as in IGFBP-1 and -2, although IGFBP-4 can associate with matrix.

Recent evidence has shown that the level of IGFBP (e.g. IGFBP-4) is also regulated by cell-specific proteases that compromise both the IGF-dependent and independent functional activity of growth factor binding proteins [74]. This suggests that the enzymes play a pivotal role in processes associated with cellular homeostasis, differentiation and proliferation. As such, identification and characterization of IGFBP-3 and -4 proteases is of primary interest particularly given the demonstration of increased IGFBP-3 protease activity in the synovial fluid of OA and RA patients [32, 66]. Although not yet completely characterized, these enzymes appear to belong to serine- and/or metallo-proteases families. Furthermore, human chondrocytes constitutively produce an IGFBP-4-specific protease the activity of which is inhibited by PGE_2 in a dose-dependent manner [87]. It is not clear whether PGE_2 inhibition occurs at the level of expression and synthesis of the enzyme(s) or by PGE_2-induced increase in IGFBP-3 synthesis.

IGF-1 can be cleaved at the amino-terminus by proteases to form des(1-3)IGF-1 which can still bind to and activate the IGF-1 receptor, but is not bound by IGFBP. Hence, local

regulation of IGF-1 bioavailability via IGF-1 synthesis, IGFBP and their associated proteases, represents a complex network of factors that regulate the ability of IGF-1 to interact with its receptor.

IGF system and bone

Bone formation and resorption are the two major processes involved in tissue remodeling. Bone cells secrete a variety of cytokines and growth factors that play an important role in both processes. Among these factors, IGFs have important biological activities in bone [90–94] and in regulating bone formation. IGFs stimulate matrix formation in organ cultures of fetal rat calvariae, even in the presence of DNA synthesis inhibitors, supporting the concept that this stimulating effect is only partially dependent on cell replication [95]. Conversely, IGFs enhance the differentiated function of the osteoblast and increase bone formation. Indeed, IGF-1 is mitogen for fetal rat calvaria, and IGF-1 and IGF-2 have mitogenic activities for rat calvaria osteoblasts in vitro. Moreover, IGF-1 not only increases the replication of osteoblasts in serum-free long-term cultures, but also preferentially maintains the cells expressing high alkaline phosphatase activity and parathyroid hormone (PTH) responsiveness [96], which are phenotypic features of mature osteoblasts.

In bone, both IGF-1 and IGF-2 increase type I collagen transcription, bone matrix apposition rates, and inhibit the degradation of bone collagen, probably by inhibiting the expression of collagenase by the osteoblast [95]. Indeed, IGFs are autocrine down-regulators of collagenase expression [97] and can inhibit the effects of other growth factors, such as the platelet-derived growth factor (PDGF), that accelerate the breakdown of collagen [98]. Hence, IGFs not only enhance bone formation, but most likely inhibit bone degradation, although a clearly-defined action on bone resorption has not yet been identified.

The levels of IGFs found in bone cannot be derived from serum since IGFs are synthesized de novo by osteoblasts, and the levels in cultured bone cells correlate with the levels found in cortical bone [99]. However, GH may be playing a similar role in the liver and in osteoblasts [100]. Skeletal cells express GH-R, and GH increases IGF-1 expression in osteoblast-enriched cultures from fetal rat bone in vitro [100, 101]. GH replacement increases both serum IGF-1 and bone mineral density, suggesting a possible link between these processes. Unilateral infusion of GH into the growth plate of young hypophysectomized rats resulted in growth [102], increasing levels of IGF-1 mRNA in osteoblasts and immunoreactive peptide [103]. Antibodies against IGF-1 abrogated the effects of GH on growth, while GH can increase the number of osteoblasts in serum-free cultures. This GH-dependent increase in IGF-1 mRNA in hypophysectomized rats is also inhibited by dexamethasone treatment [104]. In addition, GH stimulated the IGF-1 regulation by human adult primary osteoblast-like cells in short term culture [105].

Calciotropic hormones such as PTH and 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) mediate their effects on osteoblasts in part by regulating the synthesis of IGFs and/or their binding proteins [106, 107]. IGF-1 secretion by UMR-106, a rat osteosarcoma cell model, and primary human bone cells is stimulated by $1,25(OH)_2D_3$ [105, 108]. PTH activates the release of both IGF-1 and IGF-2 in cultured neonatal mouse calvariae [106], and of IGF-1 in rat calvarial cells. This may be due to increases in steady state mRNA for IGF-1. PTH-dependent stimulation of IGF-1 in organ cultures of fetal rat calvariae may also explain the enhanced synthesis of collagen under these culture conditions, while IGF-1 stimulation does not modify PTH-dependent DNA synthesis [109]. The effect of PTH on IGF-1 release is mimicked by cAMP analogues, and cAMP regulates IGF-1 gene expression in rat bone cells. While both PTH and GH increase IGF-1 mRNA in primary cultures of osteoblasts, only PTH causes accumulation of IGF-1 peptide in the culture media. Different roles for PTH and GH on IGFBP could explain this discrepancy.

Osteoblasts can synthesize at least the first six IGFBP, although to variable extents, depending on the particular cell culture system and conditions. In this tissue, three IGFBP appear to be of major importance. The IGFBP-5 stimulates bone formation, whereas IGFBP-4 inhibits this activity. In addition, IGFBP-3 may provide a local enhancer loop by which systemic hormones (PTH, $1,25(OH)_2D_3$, etc.) regulate the availability of IGFs to bone cells [97]. GH stimulates IGFBP-3 release by adult rat calvaria osteoblasts via a direct action on mRNA synthesis [110]. Moreover, GH regulates the expression of IGFBP-3 more tightly than that of IGF-1 in bone cells [97]. This effect is mimicked by PTH, which also increases the release of IGFBP-2 and -4. The difference between GH and PTH may account for the accumulation of IGF-1 in PTH-treated cells as both IGFBP-2 and -4 prevent the degradation of IGF-1.

IGFs and IGFBP are distributed unevenly within long bones, as observed in an animal model [111]. Indeed, bone mineral density is highest in the proximal region of the diaphysis (i.e. closest to the epiphysis and the subchondral bone), consistent with a local increase in total IGF-1 content, whereas IGF-2 is highest in the distal region. This is accompanied by an inverse distribution of the 29 Kd IGFBP (IGFBP-4) in the diaphysis. It is also noteworthy that skeletal unloading using a rat model induces resistance to IGF-1 at the end of unloading (hence, during reloading period), whereas the type I IGF-R rises in parallel, but apparently does not change IGFBP [112]. This strongly suggests that an intrinsic abnormal response to IGF-1 has arisen and/or intracellular signal transduction is altered under these conditions, or that other regulators of this IGF/IGFBP system are abnormal. However, as total IGF-1 was measured exclusively (not free and bound IGF-1), this limits the interpretation one can give to the true impact of IGF-1 on in vivo bone formation at any given site.

Both type I and type II IGF-R have been identified in bone cells. Rat calvarial osteoblasts possess high affinity type I IGF-R [113], but there is no specialization regarding type I and type II IGF-R in both rat and mouse osteoblast-like cells [114, 115]. However, the type II receptor may be more predominant in chicken osteoblasts [116], and has been suggested to mediate the mitogenic response in these cells. The regulation of both receptors has not been carefully investigated in bone, although glucocorticoids [113] and ß2-microglobulin [117] may affect type I IGF-R.

Recent data show a fundamental role of the PA/plasmin

system in the local regulation of IGF bioavailability in bone [118]. Bone resorption involves a well characterized protease system; this comprises PA and their inhibitors, whose equilibrium controls the formation of plasmin [119, 120]. The PA/plasmin system can produce variable proteolysis at different sites on the IGF-1/IGFBP complex, resulting in peptides of variable sizes, and promoting the dissociation of IGFs bound to IGFBP [118, 121, 122]. This generates free IGF-1, which is then available to exert its biologic influence [118]. Since recent data indicate a role for the NH_2-terminal 16 kDa fragment of IGFBP-3, the involvement of the urokinase (uPA)/plasmin system in the proteolytic cleavage of IGFBP may contribute to local cell proliferation control of osteoblasts. At present, these hypotheses have not been tested.

Bone IGF system in OA

It has been proposed that the thickening of the subchondral bone plate may induce and/or participate in the progression of OA [123,124]. Although the exact role of IGF in OA bone metabolism is unclear, the positive action of IGF-1 on bone cells in the subchondral bone plate is suggested to contribute to bone sclerosis in OA. In contrast, osteoporosis is associated with decreased circulating levels of IGFs and a diminution of IGFBP-3 [125], which may explain why OA and osteoporosis do not present concurrently. Indeed, IGF-1 levels in humans have a positive correlation with bone mineral density, and in fact, bone mineral density is increased in OA patients as compared to osteoporotic or normal individuals [10]. Likewise, the aging process in humans is accompanied by a decrease in bone mass and reduced levels of IGF-1 in both serum and cortical bone. Therefore, this could imply a causative role of IGF-1 in the development of the osteopenia of aging, and conversely, the appearance of an increased bone mineral density in OA patients [32, 38]. Recent findings indicated an increase in IGF-1 production by human OA primary osteoblast-like cell cultures compared to normal cells, a situation that reflects the levels of IGF-1 measured in subchondral bone explants from normal and OA individuals [126].

The increase in IGFBP-3 in OA could also result in decreased cell growth and/or apoptosis as observed in other cell systems [79]. Furthermore, the NH_2-terminal 16 kDa fragment of IGFBP-3 could directly inhibit cell proliferation without IGF-1 interaction and/or binding in chick embryo fibroblasts via limited proteolysis with plasmin [127], a similar mechanism could also be implicated in OA chondrocytes and/or osteoblasts, where the uPA/plasmin system is elevated [48, 126, 128, 129].

Plasmin is one of the factors capable of activating the latent form of TGF-ß [130, 131], and TGF-ß is also a very important local regulator of bone formation. In particular, TGF-β1 can reduce IGFBP-4 mRNA expression and enhance IGFBP-4 proteolysis in human primary bone cell cultures [132], thereby increasing free IGF-1 levels. Since TGF-β1 is increased in OA bone extracts [133] and in conditioned-media from ex vivo explant cultures [126], this could account for a local enhanced remodeling by this growth factor. Finally, uPA – and more specifically its amino-terminal fragment – stimulates mitogenic activity and proliferation in human osteoblast-like cells and in rat primary calvaria osteoblast-like cells [134]. Paradoxically, this suggests that, at least in bone cells, uPA can stimulate or inhibit cell proliferation either directly or via proteolytic activation of local growth factor. Hence, this would also contribute to couple bone resorption and formation (remodeling) by the PA/plasmin system [135]. This last hypothesis would (Fig. 3) explain why uPA activity is increased in human OA primary cell cultures, as compare to normal cells, mimicking the situation observed with subchondral bone explants from normal and OA individuals [126].

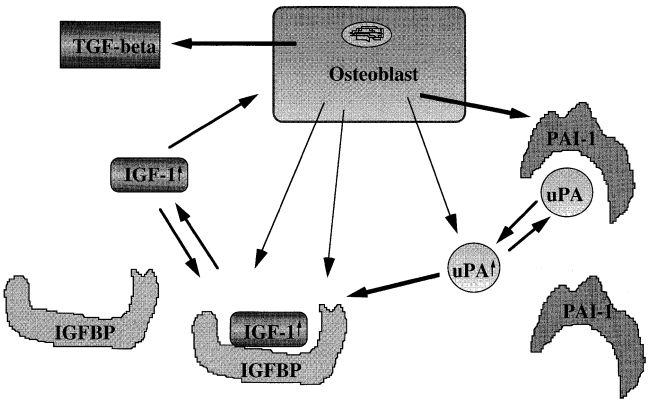

Fig. 3. Schematic representation of subchondral bone pathways involved in human osteoarthritis. Osteoarthritic osteoblasts produce more uPA, IGF-1 and TGF-ß than normal cells, while producing normal levels of PAI-1. The imbalance between PAI-1 and uPA may favor the hydrolysis of IGFBP, resulting in the freeing of IGF-1 locally, which can then act via an autocrine/paracrine pathway to enhance osteoblast cell growth/differentiation. This in turn may ultimately lead to abnormal subchondral bone sclerosis as observed in human osteoarthritis.

Conclusion

Further investigations to clarify which factors modulate the synthesis and/or activity of IGF-1 during OA process have yet to be conducted. Moreover, knowledge of the modifying influences of such factors on the action of the IGF system (IGF, IGFBP) are necessary to evaluate the impact of this system on OA articular joint tissue.

While much information has been published on the IGFBP control of IGF-1 action, new findings on the IGFBP itself open the door to future prospects for these proteins. From the discovery that some IGFBP have IGF- and IGF-R-independent functional activity could be of particular importance. In addition, the possible existence of more IGFBP than the first well known six could lead to a more thorough comprehension of the IGF system process and activity.

Although our knowledge of human OA has evolved in recent years, we still have not been able to completely understand the triggering process(es) that induce OA, or which tissue is the primary target for this disease. As we gain new insights into which mechanisms are altered in OA tissue, we may attain a clearer picture of the key events involved in the onset and/or progression of OA. Finally, the bone sclerosis in OA, although initially considered

secondary, may now be emerging as one of the most important events leading and/or contributing to cartilage degradation in OA.

Our increased knowledge of the IGF-1 system provides the basis upon which we may assert that this system is intimately involved in the pathophysiology of OA. Identification of the exact mechanisms that regulate the action of IGF-1 on OA chondrocytes is the only route towards the possibility of using this growth factor as a means for therapeutic intervention in OA.

References

[1] Pelletier JP, Martel-Pelletier J, Howell DS. Etiopathogenesis of osteoarthritis. In: Koopman WJ, editor. Arthritis and Allied Conditions. A Textbook of Rheumatology. 13th ed. Baltimore: Williams & Wilkins, 1997:1969–84.

[2] Pelletier JP, Roughley PJ, Di Battista JA, McCollum R, Martel-Pelletier J. Are cytokines involved in osteoarthritic pathophysiology? Semin Arthritis Rheum 1991;20:12–5.

[3] Dean DD. Proteinase-mediated cartilage degradation in osteoarthritis [review]. Semin Arthritis Rheum 1991;20:2–11.

[4] Mitchell N, Shepard N. Pericellular proteoglycan concentrations in early degenerative arthritis. Arthritis Rheum 1981;24:958–64.

[5] Mankin HJ, Lippiello L. The glycosaminoglycans of normal and arthritic cartilage. J Clin Invest 1971;50:1712–9.

[6] Vignon E, Arlot M, Hartmann D, Moyen B, Ville G. Hypertrophic repair of articular cartilage in experimental osteoarthritis. Ann Rheum Dis 1983;42:82–8.

[7] Pelletier JP, Di Battista JA, Roughley PJ, McCollum R, Martel-Pelletier J. Cytokines and inflammation in cartilage degradation. In: Moskowitz RW, editor. Osteoarthritis, Edition of Rheumatic Disease Clinics of North America. Philadelphia: W.B. Saunders, 1993:545–68.

[8] Haraoui B, Pelletier JP, Cloutier JM, Faure MP, Martel-Pelletier J. Synovial membrane histology and immunopathology in rheumatoid arthritis and osteoarthritis. In vivo effects of anti-rheumatic drugs. Arthritis Rheum 1991;34:153–63.

[9] Guenther HL, Guenther HE, Froesch ER, Fleisch H. Effect of insulin-like growth factor on collagen and glycosaminoglycan synthesis by rabbit articular chondroytes in culture. Experientia 1982;38:979–81.

[10] McQuillan DJ, Handley CJ, Campbell MA, Bolis S, Milway VE, Herington AC. Stimulation of proteoglycan biosynthesis by serum and insulin-like growth factor-I in cultured bovine articular cartilage. Biochem J 1986;240:423–30.

[11] Franchimont P, Bassler C. Effects of hormones and local growth factors on articular chondrocyte metabolism. J Rheumatol 1991;18(Suppl 27):68–70.

[12] Sandell LJ, Dudek EJ. Insulin-like growth factor I stimulates type II collagen gene expression in cultured chondrocytes. Trans Orthop Res Soc 1988;35:300.

[13] Tesch GH, Handley CJ, Cornell HJ, Herington AC. Effects of free and bound insulin-like growth factors on proteoglycan metabolism in articular cartilage explants. J Orthop Res 1992;10:14–22.

[14] D'Ercole AJ, Hill DJ, Strain AJ, Underwood LE. Tissue and plasma somatomedin-C/insulin-like growth factor I concentations in the human fetus during the first half of gestation. Pediatr Res 1986;20:253–5.

[15] Han VK, D'Ercole AJ, Lund PK. Cellular localization of somatomedin (insulin-like growth factor) messenger RNA in the human fetus. Science 1987;236:193–7.

[16] Schoenle E, Zapf J, Humbel RE, Froesch ER. Insulin-like growth factor I stimulates growth in hypophysectomized rats. Nature 1982;296:252–3.

[17] Trippel SB, Corvol MT, Dumontier MF, Rappaport R, Hung HH, Mankin HJ. Effect of somatomedin-C/insulin-like growth factor I and growth hormone on cultured growth plate and articular chondrocytes. Pediatr Res 1989;25:76–82.

[18] Bhaumick B. Insulin-like growth factor (IGF) binding proteins and insulin-like growth factor secretion by cultured chondrocyte cells: Identification, characterization and ontogeny during cell differentiation. Regul Pept 1993;48:113–22.

[19] Bhaumick B, Bala RM. Differential effects of insulin-like growth factors I and II on growth, differentiation and glucoregulation in differentiating chondrocyte cells in culture. Acta Endocrinol 1991;125:201–11.

[20] Holthuizen E, LeRoith D, Lund PK, Roberts CT Jr, Rotwein P, Spencer EM, et al. Modern concepts in insulin-like growth factors. New York: Elsevier, 1991:733–6.

[21] Sara VR, Hall K. Insulin-like growth factors and their binding proteins. Physiol Rev 1990;70:591–614.

[22] Czech MP. Signal transmission by the insulin-like growth factors. Cell 1989;59:235–8.

[23] Kiess W, Blickenstaff GD, Sklar MM, Thomas CL, Nissley SP, Sahagian GG. Biochemical evidence that the type II insulin-like growth factor receptor is identical to the cation-dependent mannose 6-phosphate receptor. J Biol Chem 1988;263:9339–44.

[24] Oshima A, Nolan CM, Kyle JW, Grubb JH, Sly WS. The human cation-independent mannose 6-phosphate receptor. Cloning and sequence of the full-length cDNA and expression of functional receptor in COS cells. J Biol Chem 1988;263:2553–62.

[25] Schouten JSAG, Van den Ouweland FA, Valkenburg HA, Lamberts SW. Insulin-like growth factor-1: A prognostic factor of knee osteoarthritis. Br J Rheumatol 1993;32:274–80.

[26] McAlindon TE, Teale D, Dieppe PA. Insulin-like growth factor 1: Effect of age acounts for apparent correlation with sclerosis and osteophytosis in osteoarthritis of the knee [abstract]. Br J Rheumatol 1992;31(Suppl 2):213.

[27] Denko CW, Boja B, Moskowitz RW. Growth promoting peptides in osteoarthritis: insulin, insulin-like growth factor-I, growth hormone. J Rheumatol 1990;17:1217–21.

[28] Hochberg MC, Lethbridge-Cejku M, Scott WW Jr, Reichle R, Plato CC, Tobin JD. Serum levels of insulin-like growth factor I in subjects with osteoarthritis of the knee. Data from the Baltimore Longitudinal Study of Aging. Arthritis Rheum 1994;37:1177–80.

[29] Lloyd ME, Hart DJ, Nandra D, McAlindon TE, Wheeler M, Doyle DV, et al. Relation between insulin-like growth factor-I concentrations, osteoarthritis, bone density, and fractures in the general population: The Chingford study. Ann Rheum Dis 1996;55:870–4.

[30] Denko CW, Boja B. Growth factors in asymptomatic osteoarthritis – insulin, insulin-like growth factor-1, growth hormone. Immunopharmacology 1993;2:71–6.

[31] Schneiderman R, Rosenberg N, Hiss J, Lee P, Liu F, Hintz RL, et al. Concentration and size distribution of insulin-like growth factor-I in human normal and osteoarthritic synovial fluid and cartilage. Arch Biochem Biophys 1995;1:173–88.

[32] Tavera C, Abribat T, Reboul P, Doré S, Brazeau P, Pelletier JP, et al. IGF and IGF-binding protein system in the synovial fluid of osteoarthritc and rheumatoid arthritic patients. Osteoarthritis Cartilage 1996;4:263–74.

[33] Fernihough JK, Billingham ME, Cwyfan-Hughes S, Holly JM. Local disruption of the insulin-like growth factor system in the arthritic joint. Arthritis Rheum 1996;39:1556–65.

[34] Isaksson OGP, Ohlsson C, Nilsson A, Isgaard J, Lindahl A. Regulation of cartilage growth by growth hormone and insulin-like growth factor I. Pediatr Nephrol 1991;5:451–3.

[35] Doré S, Abribat T, Rousseau N, Brazeau P, Tardif G, Di Battista JA, et al. Increased insulin-like growth factor 1 production by human osteoarthritic chondrocytes is not dependent on growth hormone action. Arthritis Rheum 1995;38:413–9.

[36] Olney RC, Tsuchiya K, Wilson DM, Mohtai M, Maloney WJ, Schurman DJ, et al. Chondroyctes from osteoarthritic cartilage have increased expression of insulin-like growth factor I (IGF-1) and IGF-binding protein-3 (IGFBP-3) and -5, but not IGF-2 or IGFBP-4. J Clin Endocrinol Metab 1996;81:1096–103.

[37] Middleton JF, Tyler JA. Upregulation of insulin-like growth factor-1 gene expression in the lesions of osteoarthritic human articular cartilage. Ann Rheum Dis 1992;51:440–7.
[38] Doré S, Pelletier JP, Di Battista JA, Tardif G, Brazeau P, Martel-Pelletier J. Human osteoarthritic chondrocytes possess an increased number of insulin-like growth factor 1 binding sites but are unresponsive to its stimulation. Possible role of IGF-1-binding proteins. Arthritis Rheum 1994;37:253–63.
[39] Tardif G, Reboul P, Pelletier JP, Geng C, Cloutier JM, Martel-Pelletier J. Normal expression of type 1 insulin-like growth factor receptor by human osteoarthritic chondrocytes with increased expression and synthesis of insulin-like growth factor binding proteins. Arthritis Rheum 1996;39:968–78.
[40] Verschure PJ, Van Marle J, Joosten LA, van den Berg WB. Histochemical analysis of insulin-like growth factor-1 binding sites in mouse normal and experimentally induced arthritic articular cartilage. Histochem J 1996;28:13–23.
[41] Pelletier JP, Martel-Pelletier J. Evidence for the involvement of interleukin 1 in human osteoarthritic cartilage degradation: Protective effect of NSAID. J Rheumatol 1989;16:19–27.
[42] Matsumoto T, Tsukazaki T, Enomoto H, Iwasaki K, Yamashita S. Effects of interleukin-1 beta on insulin-like growth factor-I autocrine/paracrine axis in cultured rat articular chondrocytes. Ann Rheum Dis 1994;53:128–33.
[43] Linkhart TA, MacCharles DC. Interleukin-1 stimulates release of insulin-like growth factor-I from neonatal mouse calvaria by a prostaglandin synthesis-dependent mechanism. Endocrinology 1992;131:2297–2305.
[44] Olney RC, Wilson DM, Mohtai M, Fielder PJ, Smith RL. Interleukin-1 and tumor necrosis factor-alpha increase insulin-like growth factor-binding protein-3 (IGFBP-3) production and IGFBP-3 protease activity in human articular chondrocytes. J Endocrinol 1995;146:279–86.
[45] Yateman ME, Claffey DC, Cwyfan Hughes SC, Frost VJ, Wass JA, Holly JMP. Cytokines modulate the sensitivity of human fibroblasts to stimulation with insulin-like growth factor-I (IGF-1) by altering endogenous IGF-binding protein production. J Endocrinol 1993;137:151–9.
[46] Scharla SH, Strong DD, Mohan S, Chevalley T, Linkhart TA. Effect of tumor necrosis factor-alpha on the expression of insulin-like growth factor 1 and insulin-like growth factor binding protein 4 in mouse osteoblasts. Eur J Endocrinol 1994;131:293–301.
[47] Laouar A, Wietzerbin J, Bauvois B. Protease-catalyzed conversion of insulin-like growth factor-1 and interleukin-6 into high-molecular-mass species through the sequential action of hematopoietic surface-associated cathepsin G and gamma-glutamyl transpeptidase-related activities. Eur J Biochem 1994;223:617–24.
[48] Martel-Pelletier J, Faure MP, McCollum R, Mineau F, Cloutier JM, Pelletier JP. Plasmin, plasminogen activators and inhibitor in human osteoarthritic cartilage. J Rheumatol 1991;18:1863–71.
[49] Martel-Pelletier J, Cloutier JM, Pelletier JP. Cathepsin B and cysteine protease inhibitors in human OA: Effect of intra-articular steroid injections. J Orthop Res 1990;8:336–44.
[50] Isaksson OGP, Nilsson A, Isgaard J, Lindahl A. Cartilage as a target tissue for growth hormone and insulin-like growth factor I. Acta Paediatr Suppl 1990;367:137–41.
[51] Isgaard J. Expression and regulation of IGF-1 in cartilage and skeletal muscle. 1992;2:16–22.
[52] Barnard R, Haynes KM, Werther GA, Waters MJ. The ontogeny of growth hormone receptors in the rabbit tibia. Endocrinology 1988;122:2562–9.
[53] Isaksson OGP, Lindahl A, Nilsson A, Isgaard J. Mechanism of the stimulatory effect of growth hormone on longitudinal bone growth. Endocr Rev 1987;8:426–38.
[54] Elford PR, Lamberts SW. Contrasting modulation by transforming growth factor-beta-1 of insulin-like growth factor-I production in osteoblasts and chondrocytes. Endocrinology 1990;127:1635–9.
[55] Jones KL, Villela JF, Lewis UJ. The growth of cultured rabbit articular chondrocytes is stimulated by pituitary growth factors by not by purified human growth hormone or bovine prolactin. Endocrinology 1986;118:2588–93.
[56] Kasper S, Friesen HG. Human pituitary tissue secretes a potent growth factor for chondrocyte proliferation. J Clin Endocrinol Metab 1986;62:70–6.
[57] Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: Biological actions. Endocr Rev 1995;16:3–34.
[58] Oh Y, Nagalla SR, Yamanaka Y, Kim HS, Wilson E, Rosenfeld RG. Synthesis and characterization of insulin-like growth factor-binding protein (IGFBP)-7. Recombinant human mac25 protein specifically binds IGF-1 and -II. J Biol Chem 1996;271:30322–5.
[59] Wilson EM, Oh Y, Rosenfeld RG. Generation and characterization of an IGFBP-7 antibody: Identification of 31kD IGFBP-7 in human biological fluids and Hs578T human breast cancer conditioned media. J Clin Endocrinol Metab 1997;82:1301–3.
[60] Rechler MM. Growth inhibition by insulin-like growth factor (IGF) binding protein-3 – what's IGF got to do with it? [editorial]. Endocrinology 1997;138:2645–7.
[61] Lamson G, Giudice LC, Cohen P, Liu F, Gargosky S, Muller HL, et al. Proteolysis of IGFBP-3 may be a common regulatory mechanism of IGF action in vivo. Growth Regul 1993;3:91–5.
[62] Cohen P, Graves HC, Peehl DM, Kamarei M, Giudice LC, Rosenfeld RG. Prostate-specific antigen (PSA) is an insulin-like growth factor binding protein-3 protease found in seminal plasma. J Clin Endocrinol Metab 1992;75:1046–53.
[63] Rosenfeld RG, Pham H, Cohen P, Fielder P, Gargosky SE, Muller H, et al. Insulin-like growth factor binding proteins and their regulation. Acta Paediatr Suppl 1994;399:154–8.
[64] Olney RC, Smith RL, Kee Y, Wilson DM. Production and hormonal regulation of insulin-like growth factor binding proteins in bovine chondrocytes. Endocrinology 1993;133:563–70.
[65] Di Battista JA, Doré S, Morin N, Abribat T. Prostaglandin E_2 up-regulates insulin-like growth factor binding-3 expression and synthesis in human articular chondrocytes by a cAMP-independent pathway: Role of calcium and protein kinase A and C. J Cell Biochem 1996;62:1–14.
[66] Matsumoto T, Gargosky SE, Iwasaki K, Rosenfeld RG. Identification and characterization of insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs), and IGFBP proteases in human synovial fluid. J Clin Endocrinol Metab 1996;81:150–5.
[67] Kanety H, Shimon I, Ehrenfeld M, Israeli A, Pariente C, Karasik A. Insulin-like growth factor I and its binding proteins 3 and 4 are increased in Human inflammatory synovial fluid. J Rheumatol 1996;23:815–8.
[68] Oh Y, Muller HL, Lamson G, Rosenfeld RG. Insulin-like growth factor (IGF)-independent action of IGF-binding protein-3 in Hs578T human breast cancer cells. Cell surface binding and growth inhibition. J Biol Chem 1993;268:14964–71.
[69] Oh Y, Muller HL, Pham H, Rosenfeld RG. Demonstration of receptors for insulin-like growth factor binding protein-3 on Hs578T human breast cancer cells. J Biol Chem 1993; 268:26045–8.
[70] Liu L, Delbe J, Blat C, Zapf J, Harel L. Insulin-like growth factor binding protein (IGFBP-3), an inhibitor of serum growth factors other than IGF-1 and -II. J Cell Physiol 1992;153:15–21.
[71] Valentinis B, Bhala A, DeAngelis T, Baserga R, Cohen P. The human insulin-like growth factor (IGF) binding protein-3 inhibits the growth of fibroblasts with a targeted disruption of the IGF-1 receptor gene. Mol Endocrinol 1995;9:361–7.
[72] De Mellow JS, Baxter RC. Growth hormone-dependent insulin-like growth factor (IGF) binding protein both inhibits and potentiates IGF-1-stimulated DNA synthesis in human skin fibroblasts. Biochem Biophys Res Commun 1988;156:199–204.
[73] Rechler MM. Molecular endocrinology: Basic concepts and clinical correlations. New York: Raven Press Ltd., 1995:155–80.
[74] Fowlkes JL, Serra DM, Rosenberg CK, Thrailkill KM. Insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) functions as an IGF-reversible inhibitor of IGFBP-4 proteolysis. J Biol Chem 1995;270:27481–8.

[75] Donnelly MJ, Holly JM. The role of IGFBP-3 in the regulation of IGFBP-4 proteolysis. J Endocrinol 1996;149:R1–7.
[76] Cubbage ML, Suwanichkul A, Powell DR. Insulin-like growth factor binding protein-3. Organization of the human chromosomal gene and demonstration of promoter activity. J Biol Chem 1990;265:12642–9.
[77] Duan C, Clemmons DR. Transcription factor AP-2 regulates human insulin-like growth factor binding protein-5 gene expression. J Biol Chem 1995;270:24844–51.
[78] McCarthy TL, Casinghino S, Mittanck DW, Ji CH, Centrella M, Rotwein P. Promoter-dependent and -independent activation of insulin-like growth factor binding protein-5 gene expression by prostaglandin E2 in primary rat osteoblasts. J Biol Chem 1996;271:6666–71.
[79] Buckbinder L, Talbott R, Velasco-Miguel S, Takenaka I, Faha B, Seizinger BR, et al. Induction of the growth inhibitor IGF-binding protein 3 by p53. Nature 1995;377:646–9.
[80] Radulescu RT. Nuclear localization signal in insulin-like growth factor-binding protein type 3. Trends Biochem Sci 1994;19:278.
[81] Radulescu RT, Wendtner CM. Proposed interaction between insulin and retinoblastoma protein. J Mol Recognit 1992;5:132–7.
[82] Radulescu RT, Wendtner CM. Hormone and growth factor subunits: A novel perception of cell growth regulation. J Endocrinol 1993;139:1–7.
[83] LaTour D, Mohan S, Linkhart TA, Baylink DJ, Strong DD. Inhibitory insulin-like growth factor-binding protein: cloning, complete sequence, and physiological regulation. Mol Endocrinol 1990;4:1806–14.
[84] Dai B, Widen SG, Mifflin R, Singh P. Cloning of the functional promoter for human insulin-like growth factor binding protein-4 gene: endogenous regulation. Endocrinology 1997;138:332–3.
[85] Gao L, Ling N, Shimasaki S. Structure of the rat insulin-like growth factor binding protein-4 gene. Biochem Biophys Res Commun 1993;190:1053–9.
[86] Yang YW, Pioli P, Fiorelli G, Brandi ML, Rechler MM. Cyclic adenosine monophosphate stimulates insulin-like growth factor binding protein-4 and its messenger ribonucleic acid in a clonal endothelial cell line. Endocrinology 1993;133:343–51.
[87] Di Battista JA, Doré S, Morin N, He Y, Pelletier JP, Martel-Pelletier J. Prostaglandin E_2 stimulates insulin-like growth factor binding protein-4 expression and synthesis in cultured human articular chondrocytes: Possible mediation by Ca^{++}/-calmodulin regulated processes. J Cell Biochem 1997;65:408–19.
[88] Conti M, Jin SL, Monaco L, Repaske DR, Swinnen JV. Hormonal regulation of cyclic nucleotide phosphodiesterases. Endocr Rev 1991;12:218–34.
[89] Sette C, Iona S, Conti M. The short-term activation of a rolipram-sensitive, cAMP-specific phosphodiesterase by thyroid-stimulating hormone in thyroid FRTL-5 cells is mediated by a cAMP-dependent phosphorylation. J Biol Chem 1994;269:9245–52.
[90] Schmid C, Ernst M. Insulin-like growth factors. In: Gowen M, editor. Cytokines and bone metabolism. Boca Raton: CRC Press, 1992:229–65.
[91] Canalis E, McCarthy TL, Centrella M. The role of growth factors in skeletal remodeling. Endocrinol Metab Clin North Am 1989;18:903–18.
[92] Mohan S, Baylink DJ. Bone growth factors. Clin Orthop 1991;30–48.
[93] Mohan S, Baylink DJ. Modern concepts of insulin-like growth factors. New York: Elsevier, 1991:169–84.
[94] Mohan S, Jennings JC, Linkhart TA, Baylink DJ. Primary structure of human skeletal growth factor: Homology with human insulin-like growth factor-II. Biochim Biophys Acta 1988; 966:44–55.
[95] Hock JM, Centrella M, Canalis E. Insulin-like growth factor I has independent effects on bone matrix formation and cell replication. Endocrinology 1988;122:254–60.
[96] Ernst M, Froesch ER. Osteoblastlike cells in a serum-free methylcellulose medium form colonies: Effects of insulin and insulinlike growth factor I. Calcif Tissue Int 1987;40:27–34.
[97] Canalis E. Insulin-like growth factors and osteoporosis. Bone 1997;21:215–6.
[98] Canalis E, McCarthy TL, Centrella M. Effects of platelet-derived growth factor on bone formation in vitro. J Cell Physiol 1989;140:530–7.
[99] Rosen CJ, Dimai HP, Vereault D, Donahue LR, Beamer WG, Farley J, et al. Circulating and skeletal insulin-like growth factor-I (IGF-1) concentrations in two inbred strains of mice with different bone mineral densities. Bone 1997;21:217–23.
[100] Canalis E. Growth hormone, skeletal growth factors and osteoporosis. Endocrine Practice 1995;1:39–43.
[101] McCarthy TL, Centrella M, Canalis E. Parathyroid hormone enhances the transcript and polypeptide levels of insulin-like growth factor I in osteoblast-enriched cultures from fetal rat bone. Endocrinology 1989;124:1247–53.
[102] Isaksson OG, Jansson JO, Gause IA. Growth hormone stimulates longitudinal bone growth directly. Science 1982;216:1237–9.
[103] Hansson HA, Nilsson A, Isgaard J, Billig H, Isaksson OGP, Skottner A, et al. Immunohistochemical localization of insulin-like growth factor I in the adult rat. Histochemistry 1988;89:403–10.
[104] Luo JM, Murphy LJ. Dexamethasone inhibits growth hormone induction of insulin-like growth factor-I (IGF-1) messenger ribonucleic acid (mRNA) in hypophysectomized rats and reduces IGF-1 mRNA abundance in the intact rat. Endocrinology 1989;125:165–71.
[105] Chenu C, Valentin-Opran A, Chavassieux P, Saez S, Meunier PJ, Delmas PD. Insulin like growth factor I hormonal regulation by growth hormone and by $1,25(OH)_2D_3$ and activity on human osteoblast-like cells in short-term cultures. Bone 1990;11:81–6.
[106] Linkhart TA, Mohan S. Parathyroid hormone stimulates release of insulin-like growth factor-I (IGF-1) and IGF-2 from neonatal mouse calvaria in organ culture. Endocrinology 1989;125:1484–91.
[107] Rosen CJ, Donahue LR, Hunter SJ. Insulin-like growth factors and bone: the osteoporosis connection. Proc Soc Exp Biol Med 1994;206:83–102.
[108] Pfeilschifter J, Lempart UG, Naumann A, Minne HW, Ziegler R. Transforming growth factor beta increases insulin-like growth factor I (IGFI) secretion in osteoblast-like cells. Acta Endocrinol 1989;120(Suppl 1):144–5.
[109] Canalis E, Centrella M, Burch W, McCarthy TL. Insulin-like growth factor I mediates selective anabolic effects of parathyroid hormone in bone cultures. J Clin Invest 1989;83:60–5.
[110] Schmid C, Ernst M, Zapf J, Froesch ER. Release of insulin-like growth factor carrier proteins by osteoblasts: Stimulation by estradiol and growth hormone. Biochem Biophys Res Commun 1989;160:788–94.
[111] Benedict MR, Ayers DC, Calore JD, Richman RA. Differential distribution of insulin-like growth factors and their binding proteins within bone: Relationship to bone mineral density. J Bone Miner Res 1994;9:1803–11.
[112] Bikle DD, Harris J, Halloran BP, Morey-Holton ER. Skeletal unloading induces resistance to insulin-like growth factor I. J Bone Miner Res 1994;9:1789–96.
[113] Bennett A, Chen T, Feldman D, Hintz RL, Rosenfeld RG. Characterization of insulin-like growth factor I receptors on cultured rat bone cells: Regulation of receptor concentration by glucocorticoids. Endocrinology 1984;115:1577–83.
[114] Centrella M, McCarthy TL, Canalis E. Receptors for insulin-like growth factors-I and -II in osteoblast-enriched cultures from fetal rat bone. Endocrinology 1990;126:39–44.
[115] Slootweg MC, Hoogerbrugge CM, de Poorter TL, Duursma SA, van Buul-Offers SC. The presence of classical insulin-like growth factor (IGF) type-I and – II receptors on mouse osteoblasts: Autocrine/paracrine growth effect of IGFs? J Endocrinol 1990; 125:271–7.
[116] Mohan S, Linkhart T, Rosenfeld R, Baylink D. Characterization of the receptor for insulin-like growth factor II in bone cells. J Cell Physiol 1989;140:169–76.
[117] Centrella M, McCarthy TL, Canalis E. Beta 2-microglobulin enhances insulin-like growth factor I receptor levels and synthesis in bone cell cultures. J Biol Chem 1989;264:18268-71.

[118] Campbell PG, Novak JF, Yanosick TB, McMaster JH. Involvement of the plasmin system in dissociation of the insulin-like growth factor-binding protein complex. Endocrinology 1992;130:1401–12.
[119] Saksela O, Rifkin DB. Cell-associated plasminogen activation: Regulation and physiological functions. Annu Rev Cell Biol 1988;4:93–126.
[120] Vassalli JD, Sappino AP, Belin D. The plasminogen activator/plasmin system. J Clin Invest 1991;88:1067–72.
[121] Koutsilieris M, Frenette G, Lazure C, Lehoux JG, Govindan MV, Polychronakos C. Urokinase-type plasminogen activator: A paracrine factor regulating the bioavailability of IGFs in PA-III cell-induced osteoblastic metastases. Anticancer Res 1993; 13:481–6.
[122] Martin TJ, Allan EH, Fukumoto S. The plasminogen activator and inhibitor system in bone remodeling. Growth Regul 1993;3:209–14.
[123] Radin EL, Paul IL, Tolkoff MJ. Subchondral changes in patients with early degenerative joint disease. Arthritis Rheum 1970; 13:400–5.
[124] Radin EL, Rose RM. Role of subchondral bone in the initiation and progression of cartilage damage. Clin Orthop 1986;213:34–40.
[125] Wuster C, Blum WF, Schlemilch S, Ranke MB, Ziegler R. Decreased serum levels of insulin-like growth factors and IGF binding protein 3 in osteoporosis. J Intern Med 1993;234:249–55.
[126] Hilal G, Martel-Pelletier J, Pelletier JP, Ranger, Lajeunesse D. Osteoblast-like cells from human subchondral ostoearthritic bone demonstrate an altered phenotype in vitro: Possible role in subchondral bone sclerosis. Arthritis Rheum 1997, in press.
[127] Lalou C, Lassarre C, Binoux M. A proteolytic fragment of insulin-like growth factor (IGF) binding protein-3 that fails to bind IGFs inhibits the mitogenic effects of IGF- I and insulin. Endocrinology 1996;137:3206–12.
[128] Saxne T, Lecander I, Geborek P. Plasminogen activators and plasminogen activator inhibitors in synovial fluid. Difference between inflammatory joint disorders and osteoarthritis. J Rheumatol 1993;20:91–6.
[129] Murphy G, Cockett MI, Stephens PE, Smith BJ, Docherty AJ. Stromelysin is an activator of procollagenase. A study with natural and recombinant enzymes. Biochem J 1987;248:265–8.
[130] Lyons RM, Gentry LE, Purchio AF, Moses HL. Mechanism of activation of latent recombinant transforming growth factor beta 1 by plasmin. J Cell Biol 1990;110:1361–7.
[131] Sato Y, Rifkin DB. Inhibition of endothelial cell movement by pericytes and smooth muscle cells: Activation of a latent transforming growth factor-beta 1-like molecule by plasmin during co-culture. J Cell Biol 1989;109:309–15.
[132] Durham SK, Riggs BL, Conover CA. The insulin-like growth factor-binding protein-4 (IGFBP-4)-IGFBP-4 protease system in normal human osteoblast-like cells: Regulation by transforming growth factor-beta. J Clin Endocrinol Metab 1994;79:1752–8.
[133] Dequeker J, Mohan R, Finkelman RD, Aerssens J, Baylink DJ. Generalized osteoarthritis associated with increased insulin-like growth factor types I and II and transforming growth factor beta in cortical bone from the iliac crest. Possible mechanism of increased bone density and protection against osteoporosis. Arthritis Rheum 1993;36:1702–8.
[134] Rabbani SA, Desjardins J, Bell AW, Banville D, Mazar A, Henkin J, et al. An amino-terminal fragment of urokinase isolated from a prostate cancer cell line (PC-3) is mitogenic for osteoblast-like cells. Biochem Biophys Res Commun 1990; 173:1058–64.
[135] Allan EH, Martin TJ. The plasminogen activator inhibitor system in bone cell function. Clin Orthop 1995;313:54–63.