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REVIEW

The role of the insulin-like growth factor (IGF) axis in osteogenic and odontogenic differentiation

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Abstract The insulin-like growth factor (IGF) axis is a multicomponent molecular network which has important biological functions in the development and maintenance of differentiated tissue function(s). One of the most important functions of the IGF axis is the control of skeletal tissue metabolism by the finely tuned regulation of the process of osteogenesis. To achieve this, the IGF axis controls the activity of several cell types—osteoprogenitor cells, osteoblasts, osteocytes and osteoclasts to achieve the co-ordinated development of appropriate hard tissue structure and associated matrix deposition. In addition, there is an increasing awareness that the IGF axis also plays a role in the process of odontogenesis (tooth formation). In this review, we highlight some of the key findings in both of these areas. A further understanding of the role of the IGF axis in hard tissue biology may contribute to tissue regeneration strategies in cases of skeletal tissue trauma.

Keywords Insulin-like growth factor axis · Osteogenesis · Odontogenesis · Differentiation · Dental pulp

Introduction

The insulin-like growth factor (IGF) axis plays an important role in various aspects of cell physiology [1]. In this respect, various components of the axis have been shown to regulate cell mitogenesis [2], apoptosis [3], migration [4], adhesion [5] and differentiation [6]. This last property of the IGF axis is especially important in the establishment of function in tissues as diverse as mammary gland, CNS and muscle [7–9]. In addition to skeletal muscle function the IGF axis also plays an important role in the differentiation and maintenance of the mineralised skeleton with a role in both osteogenesis and chondrogenesis [10, 11]. Although much of the recent literature has clearly identified an important role for the TGF–β/BMP (transforming growth factor beta–bone morphogenic protein) family during embryonic osteogenic development [12, 13] and as local factors involved in adult bone differentiation and morphogenesis, the IGF axis has also been revealed to play an increasingly important role in postnatal and adult skeletal tissue metabolism. Indeed, in some circumstances, the systemic action of the IGF axis regulates the local action of BMP(s) and TGF-β. The IGF axis also plays an important role in the closely related process of odontogenesis (tooth formation), and this review is concerned with highlighting some of the recent findings in the areas of osteogenesis and odontogenesis. However, the IGF axis is a complex, multicomponent molecular axis, and it is appropriate that at this juncture we provide a brief description of the different proteins which comprise the axis along with some limited comments as to their general function.

IGF axis components

The main components of the IGF axis includes two polypeptide growth factors (IGF-1 and IGF-2), their respective cell membrane receptors [IGF-1 receptor (IGF-1R) and IGF-2 receptor (IGF-2R)] together with six soluble IGF binding proteins (IGFBP1–6). IGF-1 is ubiquitously expressed in body tissues, although the majority of serum IGF-1 is derived from the liver and is under positive regulation by pituitary

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growth hormone (GH) [14]. Conversely, IGF-1 feedback at the level of the pituitary somatotrophs regulates GH secretion [15]. Despite this classical feedback mechanism, the ubiquity of IGF-1 expression has established the view that this growth factor does not belong within the classic trophic hormone–end organ target family. IGF-2 is also expressed by many tissues, but is not regulated by GH and instead may be expressed in a tissue- and developmental stage-dependent manner [16]. The IGF-1R and IGF-2R are structurally divergent. IGF-IR is a heterotetrameric receptor comprised of two transmembrane α subunits and two cytosolic β subunits covalently bonded through disulphide bridges. As such, the IGF-1R bears a close structural and functional homology with the insulin receptor (IR), and both display receptor tyrosine kinase activity within their β subunits which is critical for signal transduction [17]. The IGF-2R is identical to the cation-independent mannose-6-phosphate receptor and is a single pass transmembrane receptor [18]. The signalling mechanisms associated with IGF-2R remain largely unknown, although IGF-2 and IGF-2R have the unusual distinction of being paternally and maternally imprinted, respectively, in many mammalian genomes [19, 20]. The growth promoting effects of IGF-2 are believed to be largely mediated via the IGF-1R and, indeed, there is promiscuity displayed in the binding specificities of IGF-1 and IGF-2, not only for their cognate receptors but also for the IR and for hybrid receptors which combine either isoform of IR (IR-A or IR-B) with IGF-IR [21, 22] (see Fig. 1). The six soluble IGFBPs, like IGFs, are ubiquitously expressed. They are under multi-factorial, tissue-specific control and, amongst their most important features, is an affinity for IGF-I and -2 which is higher than that displayed by cell surface receptors [23–25]. Intuitively, this leads to the conclusion that sequestration of IGFs by IGFBPs inhibits access of the growth factors to cell surface receptors and attenuates their activity. Such a model provides a rationale for the activity of a group of IGFBP proteases which act to hydrolyse IGFBPs and release IGFs into the immediate vicinity of cell surface receptors [26, 27]. However, IGFBPs also display IGF-independent effects, including the stimulation of mitogenesis, differentiation and cell migration [9, 28, 29], and may also act to enhance the activity of locally expressed IGFs. This latter property may be related to the ability of some IGFBPs to bind to extracellular matrix (ECM) structures [30]. Figure 1 shows a diagrammatic representation of components of the IGF axis outlining most of the features discussed above.

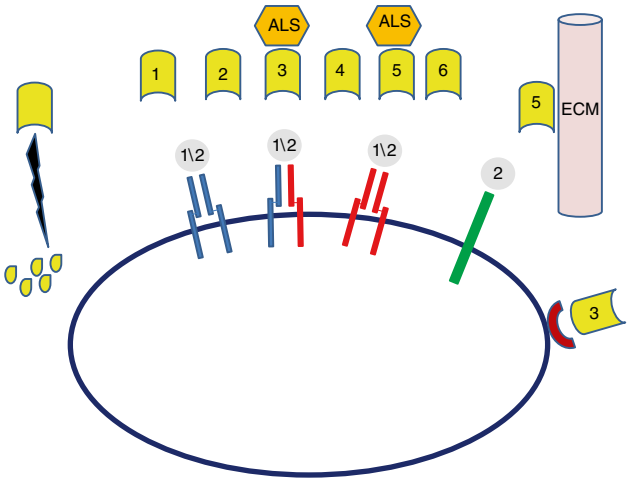

Fig. 1 The IGF axis comprises IGF-1 and -2 polypeptide growth factors (*grey*), six soluble high affinity IGF binding proteins (IGFBP1–6, *yellow*) together with the cell surface IGF-1 receptor (IGF-1R, *blue*) and IGF-2 receptor (IGF-2R, *green*). The insulin receptor (IR, *red*) and hybrid IR/IGF-1R are also able to bind IGFs although with lower affinity than cognate receptors. Similarly, insulin and IGF-2 are able to bind to IGF-1R but with lower affinity than IGF-1 itself. Most IGFBPs can associate with various extra-cellular matrix (ECM) structures (here, IGFBP-5 is shown), and can be hydrolysed by IGFBP proteases present in the interstitial fluid. Both these features are used to regulate the access of pericellular IGFs to cell surface receptors. Recent evidence also suggests that some IGFBPs (here, IGFBP-3 is shown) display IGF-independent effects by association with specific IGFBP receptors. In serum and other biological fluids, IGFBP-3 and -5 can associate with an acid labile subunit (ALS) which may also regulate the activity of these IGFBPs

IGF axis and osteogenesis

GH and IGF

The importance of the growth hormone (GH) insulin-like growth factor (IGF) axis for the development and growth of bone tissue is well recognised. Early studies of rat tibiae in organ culture demonstrated an up-regulation of both alkaline phosphatase (AP, an odontogenic marker) and IGF-I secretion following growth hormone treatment [31], and subsequent studies with IGF neutralising antibodies strongly suggested GH had both IGF-dependant and -independent effects on the growth of primary cultures of human osteoblasts and on the human osteogenic sarcoma cell line SaOS-2 [32]. GH and IGFs were subsequently reported to act synergistically in stimulating odontoblast proliferation, although effects on differentiation were not synergistic and were IGF-type-specific [33]. Further studies confirmed the expression and secretion of both IGF-I and IGFBPs from rodent cell lines and primary osteoblast cultures [34–36] and the presence of both IGF-I and IGF-2 receptors (IGF-IR and IGF-2R) on mouse osteoblast cell membranes [37]. In addition, osteogenic agents [e.g. oestradiol and parathyroid hormone (PTH)] up-regulate IGF-1 expression [38], and co-transfection of osteogenic protein-1 with IGF-I results in synergistic effects on the differentiation of rat calvaria cells [39]. Earlier studies suggested that PKA activation was required for increased IGF-I and IGF-IR expression during osteogenesis, and more recently the importance

of transcription factors including oestrogen receptor α and C/EBP delta have been identified as trans acting factors at the IGF-1 promoter during osteogenesis [40]. An especially interesting study reports the IGF-1-induced phosphorylation and intracellular redistribution of forkhead transcription factor 1, preventing its association and inhibition of the osteoblast-specific transcription factor Runx2. This allows access of Runx2 to its trans acting site at the osteocalcin-2 promoter [41]. IGF-I was also demonstrated to be a potent chemotactic factor for osteoblasts and, through this route, may play a role in the recruitment of osteoblasts during bone accretion [42]. The differentiative effect of IGF-1 may also be associated with an up-regulation of claudin-1 in tight junctions through a MAP kinase-dependent mechanism with associated decreases in paracellular permeability [43], and more recent evidence also suggests a role for protein kinase D in the osteogenic differentiation signalling mechanism of IGF-I [44].

IGF receptors

Some elegant genetic studies have documented the importance of IGF-1R in odontogenic differentiation. A landmark study using Cre-mediated-specific knock-out of IGF-IR expression in mouse osteoblasts demonstrated that functioning IGF-1R was required for the biosynthesis and mineralisation of bone matrix [45]. A similar knock-out strategy in primary mouse calvarial osteoblast cultures using adenoviral-expressed Cre recombinase against LoxP-flanked *Igf-1R* alleles showed that IGF-1R expression was required for GH activation of the Stat-5 transcription factor. Re-expression of IGF-1R via an adenoviral vector restored responsiveness to GH, and the authors suggested the direct facilitation of GH activity following the formation of a GH-IGF–1R-Stat5 complex [46]. Although some limited earlier studies indicated the presence of a functional IGF-2R in rat calvarial osteoblasts [47], a clear role for this receptor in the process of osteogenesis remains to be established, and, as in other tissues, many of the anabolic effects of IGF-2 may be mediated via the IGF-1R.

IGFBPs

There is an extensive literature on IGFBP expression and function in osteogenic tissue describing the expression of all six IGFBPs in both developing and mature osteoblasts and in various osteoblast cell lines (see, e.g., [36, 48, 49]). Since then the literature has largely been dominated by studies describing the function of IGFBPs which associate with bone matrix ECM constituents (see Fig. 1) and the identification of an IGFBP proteolytic regulatory system which operates in bone tissue. In the former case, most of the focus has been on IGFBP-5, which, since it was first isolated and purified from cultures of mouse osteoblast-like cells, has been shown to exhibit pleiotropic functions in this tissue [50]. IGFBP-5 has been shown to enhance IGF-1- and -2-stimulated mitogenesis and also to demonstrate an IGF-independent stimulatory activity [51, 52]. Further evidence suggested the presence of a specific IGFBP-5 receptor on osteoblast surfaces which may be associated with the IGF-dependent or -independent effects of IGFBP-5 [53]. This putative receptor has not been further characterised, although the ability of IGFBP-5 to stimulate the expression of osteoblastic differentiation markers has been independently confirmed [54, 55]. Using an in vivo model of bone insufficiency (ovariectomized rats), IGFBP-5 as a daily subcutaneous injection stimulates bone accretion by increasing osteoblast proliferation and secretory activity [56], providing further support for the role of IGFBP-5 as an anabolic agent. More recent studies have examined potential signalling mechanisms for IGFBP-5 action in osteoblasts. siRNA knock-down experiments revealed that the proliferative action of IGFBP-5 on osteoblast-like cells may be mediated via a Ras association family isoform C activation of Erk-1/2 phosphorylation [57]. This finding was subsequently confirmed, together with the observation that IGF-1 action in the presence of IGFBP-5 is enhanced by ligand-occupied alphaVbeta3 integrin [58]. Finally, in the mouse osteoblast precursor cell line MC3T3-E1, IGFBP-5 increases cell growth and the synthesis of the ECM proteins osteopontin and thrombospondin-1, and these findings together with those described above confirm a general consensus as to the osteogenic properties of this binding protein.

The other major area of interest in relation to IGFBP metabolism in bone tissue concerns the proteolysis of IGFBPs. Although proteolytic activity towards IGFBP-3 and -5 has been described [49, 59], most interest has been focussed on an IGFBP-4 protease first described in conditioned medium of human osteoblast-like cells [60, 61] and subsequently identified as pregnancy-associated plasma protein-A (PAPP-A) in MC3T3-E1 cell-conditioned medium [62]. Proteolysis by PAPP-A at residues M135-K136 in IGFBP-4 has been reported, together with the demonstration that an engineered protease-resistant IGFBP-4 species was a more potent inhibitor of IGF actions in osteoblast cultures than wt IGFBP-4 [63]. This observation was given added physiological relevance by the finding that overexpression of IGFBP-4 from an osteocalcin promoter in calvariae of transgenic mice caused a decrease in femur length and bone density in transgenic animals. In addition, transgenic animals showed impaired growth, and the inference was drawn that this was due to impaired IGF action in IGFBP-4 over-expressing bone tissue. Note, however, that no data were provided on possible proteolysis of bone-derived IGFBP-4 [64]. These findings

suggest that IGFBP-4, in contrast to IGFBP-5, has inhibitory effects on bone accretion in animal models.

Very little is known with respect to the molecular mechanisms of IGFBP action in osteogenic differentiation. Recently, an inhibitory action of IGFBP-6 on differentiation of MC3T3-E1 cells has been reported, and further investigation by yeast two-hybrid screening identified LIM-mineralizing protein (LMP-1) as a binding partner for IGFBP-6, and that association of IGFBP-6 with LMP-1 may abrogate its inhibitory action on mineralisation [65]. Limited investigations of the regulation of IGFBP-5 expression in osteoblast cultures and osteosarcoma cell lines identified the Nuclear Factor 1 family of transcription factors associating with a *cis*-acting element in the promoter region of IGFBP-5. Subsequent siRNA, EMSA and promoter mutational analysis identified this family of transcription factors as differential regulators of IGFBP-5 expression [66]. However, further details regarding the mechanisms involved in the action of these important proteins is lacking, and this is an area worthy of further investigation.

Regulation of IGF axis in osteogenesis

Although ECM association and IGFBP proteolysis are the most intensively studied aspects of IGFBP biology in bone tissue, it is important to highlight some other salient features with respect to IGFBP expression and activity. In bone tissue, IGFBP (and IGF, see above) expression is influenced by agents which are traditionally viewed as permissive for bone accretion. Amongst these are tri-iodothyronine (T3), PTH, GH, 1, 25-dihydroxycholecalciferol (VitD3) and IGF-1 itself [67–69]. In addition, although there has been much interest with respect to IGFBP-4 physiology in bone tissue, the profile of IGFBP expression and function in differentiating osteogenic tissues is more complex. For example, early studies suggested that IGFBP-3 was the principal IGFBP secreted by osteoblast cultures, and that this binding protein inhibited IGF action in a rat-derived bone cell line and in the human osteosarcoma cell line Saos-2 [70]. The mouse MC3T3-E1 cell line expresses IGF-I, IGF-II and IGFBP-2, -4, -5 and -6, and all these genes are co-ordinately down-regulated after treatment of cultures with bFGF [71]. IGF-I, IGFBP-2, -4 and -5 expression are also decreased during differentiation of the cell line. In some instances, biphasic patterns of expression of IGF axis genes have been reported during osteogenic differentiation. In MC3T3-E1 cells, IGFBP-5 protein showed a biphasic profile with levels peaking in conditioned medium at 10–14 days of culture and decreasing subsequently. The authors argued that the persistence of high levels of IGFBP-5 mRNA during this period suggested a post-translational mechanism for regulation of IGFBP-5 protein [72], and a similar biphasic pattern of IGF-I expression was observed during differentiation of rat osteoblast cultures [73]. Although the physiological significance of such patterns of the IGF axis gene expression is not fully understood, these findings have obvious methodological relevance for design of in vitro studies examining IGF axis effects on osteogenesis. These conclusions are also supported by an extremely detailed profiling of IGF axis expression in differentiating rat tibial osteoblasts. During the incubation periods 0–3 days, 4–6 days and 7–9 days, corresponding to, respectively, onset of ALP expression, peak of ALP expression and initiation of matrix mineral deposition, IGF-II expression was higher than IGF-I at all differentiation stages, although for both peptides the highest concentrations were apparent by day 3. All IGFBPs (except IGFBP-1 and -6) were expressed, although in this instance IGFBP-2 was identified as the principal IGFBP species, and this binding protein potentiated IGF-II differentiation activity [74].

Although IGF-1 is often reported as the most important anabolic growth factor for bone accretion [75], as indicated above, the promiscuity displayed by IGF-1, IGF-2 and insulin, with respect to interaction with cognate cell surface receptors, ensures that all three polypeptides display anabolic activity in bone tissues and that local concentrations of growth factors may determine the balance of activity amongst the three. Amongst other subtleties displayed by the IGF axis with respect to bone physiology are the observations of position-specific expression of IGF-I, IGF-II and IGF-IR in the developing osteophyte, and the suggestion that maintenance of this pattern is important for osteogenic differentiation [76]. As indicated above, the association of IGFBP-5 with ECM components derived from osteogenic tissue may have important physiological implications. This is a commonly observed property of this binding protein [30] and is associated with tissue-specific enhancement or inhibition of IGF activity. Intriguingly, the balance of IGFBP-5 activity may be influenced by the nature of the ECM constituents in the pericellular environment of the tissue, suggesting that the ability of tissues to alter the profile of expressed proteoglycans and glycosaminoglycans which constitute this environment may influence growth factor action. A further level of sophistication is revealed by the observation that some IGFBPs will only associate with the ECM in the presence of IGFs. In human osteoblast cultures, IGFBP-2 association with ECM cultures was dependent on the presence of IGF-I, and the binding protein–growth factor complex was as effective as IGF-2 alone in stimulating thymidine and proline incorporation into cell cultures [77]. Such differential engagement of ECM by IGFBPs allows for an extra level of sophistication in the action of these bindings proteins whether bound or unbound to growth factors. Caution should be exercised when interpreting results of studies examining the role of IGF axis involvement in

cell and tissue differentiation. For example, a recent report discussing the osteoblastic differentiation of the NIH3T3 fibroblast cell line using dexamethasone and VitD reported rather unusually the down-regulation of IGF-I but a 50-fold increase in IGFBP-5 expression [78]. However, treatment with dexamethasone alone also caused similar changes in expression of both genes in the absence of overt osteoblastic differentiation. This indicates that alterations in IGF axis profiles during differentiation should be disassociated from changes resulting from simple hormonal challenge. Questions such as these address the significance of causality of IGF axis activity in pro-differentiative functions, and we have previously highlighted that such issues are of importance in mammary gland differentiation [9].

IGF axis activity in dental tissues

There is a limited literature describing the role of the IGF axis in the development and function of dental tissues. Early studies reported the stimulation of proliferation and extracellular matrix secretion by dental pulp-derived fibroblasts following IGF-I treatment [79], leading these authors to suggest that IGF-I, together with other polypeptide growth factors (PDGF, EGF, bFGF), may play a role in differentiation of these cells. These findings have been independently confirmed, although some variation in growth factor activity related to cell passage number has been reported [80]. An elegant study used in situ hybridisation to demonstrate developmental stage-dependent expression of IGF-I in the continually erupting rat incisor model [81] and, in related studies, GH and IGF-I acting independently were reported to increase expression of bone morphogenetic protein (BMP)-2 and -4 by up to fivefold, suggesting that the osteogenic activities of both hormones may be mediated by these BMPs [82]. Onishi et al. have shown that IGF-I and -2 increased ALP activity and proliferation in canine dental pulp-derived cells. Insulin was also effective but only at higher doses than IGFs [83], consistent with cross-reactivity but lower affinity at the IGF-1R (see above). Amongst other highly expressed genes of the IGF axis in dental pulp are IGF-2 [84] and IGF-IR [85]. In an important study, high levels of IGF-2 secretion were reported during the osteogenic differentiation of human dental pulp-derived fibroblasts [86], and the same study indicated an increase in IGF-IR and IGFBP-3 expression during osteogenic differentiation of these cells. Whether any of these changes in gene expression are causally associated with the process of dental pulp stem cell differentiation remains to be established. However, our preliminary data suggest an increase in IGF-1 and IGF-1R expression after 3 weeks osteogenic differentiation of dental pulp-derived stromal cells (unpublished observations).

A subsequent detailed immunohistochemical examination of IGF axis components in other dental structures reported the presence of IGF-I and -2 together with all six IGFBPs in the extracellular matrix (ECM) of the periodontal ligament, and the presence of IGF-IR on the surface of periodontal ligament-derived fibroblasts. These authors similarly concluded that the location of specific IGF axis members within dental structures may reflect distinct roles for each of these genes during processes of tooth development [87]. In a further extremely detailed in situ hybridisation/immunohistochemical analysis of IGF axis expression during the life cycle of differentiating ameloblasts, very strong reactivity for IGF-I, IGF-2, IGF-IR and IGF-2R was evident at the outer enamel epithelial layer towards the apical loop in the continuingly erupting rat incisor model. In pulp-facing ameloblasts, reactivity towards these IGF axis genes was somewhat reduced. This indicates once again position-specific expression of IGF axis components, and suggests the importance of this axis in the development of dental tissues [88, 89]. IGFBP-5 is also up-regulated during osteogenic differentiation of dental pulp stem cells [90, 91]. We and others have suggested a role for this protein in the differentiation of other cell types [30], and the role of this IGF binding protein in the differentiation of dental pulp cells warrants further investigation. Finally, an interesting recent study using stem cells isolated from apical papillae reported that IGF-I stimulated cell proliferation, ALP expression and mineralisation activity in these cells. Interestingly ,expression of odontogenic markers (dentin sialoprotein and dentin sialophosphoprotein) was down-regulated. This argues for a bias in IGF-I action towards bone tissue formation and away from chondrogenic structures in this particular tissue niche [92].

Concluding remarks

It is clear from the foregoing that the IGF axis plays an important role in the development and maintenance of mineralised tissue and in the function of different tissues within the oral cavity. It is expected that future research will further elucidate the mechanisms by which this important growth factor axis operates in these tissues, and may assist with those strategies which aim to use stem cell technology for the replacement and repair of structures which have been lost or damaged by trauma or disease.

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