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invited review

Insulin-like growth factor (IGF)-binding proteins: interactions with IGFs and intrinsic bioactivities

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Baxter, Robert C. Insulin-like growth factor (IGF)-binding proteins: interactions with IGFs and intrinsic bioactivities. *Am J Physiol Endocrinol Metab* 278: E967–E976, 2000.—The insulin-like growth factor (IGF)-binding proteins (IGFBPs) are a family of six homologous proteins with high binding affinity for IGF-I and IGF-II. Information from NMR and mutagenesis studies is advancing knowledge of the key residues involved in these interactions. IGF binding may be modulated by IGFBP modifications, such as phosphorylation and proteolysis, and by cell or matrix association of the IGFBPs. All six IGFBPs have been shown to inhibit IGF action, but stimulatory effects have also been established for IGFBP-1, -3, and -5. These generally involve a decrease in IGFBP affinity and may require cell association of the IGFBP, but precise mechanisms are unknown. The same three IGFBPs have well established effects that are independent of type I IGF receptor signaling. IGFBP-1 exerts these effects by signaling through $\alpha_5\beta_1$-integrin, whereas IGFBP-3 and -5 may have specific cell-surface receptors with serine kinase activity. The regulation of cell sensitivity to inhibitory IGFBP signaling may play a role in the growth control of malignant cells.

structural determinants; type I insulin-like growth factor receptor; cancer cell growth

IN 1989, AN INTERNATIONAL MEETING of researchers on insulin-like growth factors (IGFs) reached consensus on a system of nomenclature for the IGF-binding proteins (IGFBPs), a family of proteins under investigation in many laboratories for their high-affinity interactions with IGF-I and IGF-II (6). At that time only three such proteins had been described, previously known by a wide variety of names but by agreement designated IGFBP-1 to IGFBP-3. By 1991, IGFBP-4, -5, and -6 were accepted as additions to this family. The six IGFBPs share distinctive structural and functional characteristics, of which the most obvious are *1*) a conservation of gene organization, *2*) three structural domains in the mature proteins, the conserved amino- and carboxy-terminal domains being cysteine rich, and *3*) the ability to bind IGF-I and IGF-II with association constants generally in the range of 1–100 l/nmol (7). IGFBP-6 differs from the other IGFBPs in two notable respects: it has only eight disulfide bonds compared with nine in the other proteins, lacking two conserved cysteine residues in the amino-terminal domain; and it has a markedly higher affinity for IGF-II than for IGF-I, whereas the other IGFBPs bind the two IGFs with relatively similar affinities (7).

In recent years, structural homologies have been noted between the IGFBP family and a wide variety of other proteins, of which the first described was mac25 (51). The homology is typically confined to the amino-terminal region, which is encoded by the first of four conserved exons in the IGFBP gene family. This partial structural similarity and the demonstration of weak IGF binding activity in some of these proteins have led to the hypothesis that they are products of a single gene superfamily (51). By international censensus they have now been designated IGFBP-related proteins (8).

This review presents a cross-sectional survey of current knowledge of IGFBP actions involving IGFs, and IGFBP actions in which a role for IGFs has not been demonstrated. In a limited space it is impossible to provide a comprehensive coverage of the literature in this rapidly expanding area. Instead, important themes will be illustrated by key examples, although this inevitably results in the omission of many papers from the discussion simply for the sake of brevity. The

viewpoint taken is predominantly a biochemical one, in which the structure-function relationships underlying IGFBP actions are explored wherever possible. It is hoped that this approach will point to the unique features of individual IGFBPs while also emphasizing their structural and functional similarities.

NATURE OF THE INTERACTION BETWEEN IGFBPS AND IGFS

IGF Residues Involved in IGFBP Binding

After the seminal molecular modeling studies of Blundell et al. (11) over 20 years ago, the solution structures of IGF-I and IGF-II have been solved by NMR (30, 90). These studies, in conjunction with IGF mutagenesis and a recent NMR study of an IGFBP-5 fragment (59), have provided valuable information about IGF residues that interact with IGFBPs. The earliest data, however, came from studies of IGF-I naturally truncated at the amino terminus, des-(1—3)-IGF-I. This peptide, isolated from bovine colostrum (42) and human fetal brain (86), binds to IGFBP-3 with several times lower affinity than natural IGF-I and shows greatly reduced binding to other IGFBPs (39). Glu^{3} appears to be an important determinant of binding, because variants like [Arg^{3}]IGF-I and [Gln^{3}, Ala^{4}]IGF-I show considerably reduced IGFBP interaction. Glu^{6} occupies a similar key position in the IGF-II B-domain (41), and it has been proposed that the acidic glutamate side chain interacts with Lys^{68} in the amino-terminal domain of IGFBP-5 (59). However, a basic residue is only found in this position in IGFBP-3, -4, and -5, leaving the interacting residue in IGFBP-1, -2, and -6 unclear. Interestingly, studies with a carboxy-terminal IGFBP-2 fragment suggest that it is this region of the binding protein that interacts with the amino-terminal residues of IGF-II (47), in contrast to the NMR study on IGFBP-5.

Other important IGFBP-binding determinants of IGF-I, as revealed by mutagenesis experiments, include Gln^{15} and Phe^{16} in the B domain of IGF-I and the A-domain residues Phe^{49}, Arg^{50}, and Ser^{51}, because substitution of these for the corresponding residues in insulin considerably reduces IGFBP binding (25). In IGF-II, mutation of Phe^{26} in the B domain has a pronounced effect on binding to all six of the IGFBPs, most notably IGFBP-1, and, as with IGF-I, the A-domain residues Phe^{48}, Arg^{49}, and Ser^{50} are also of key importance (4). It should be noted, however, that the significant binding epitopes on IGFs are not identical for binding to all IGFBPs, because alanine-scanning mutagenesis of IGF-I revealed marked differences in the importance of various residues for binding to IGFBP-1 and IGFBP-3 (34).

IGFBP Residues Involved in IGF Binding

Much of the information about IGFBP residues important in IGF binding has come from studies of IGFBP mutants. Mutagenesis of both amino- and carboxy-terminal residues has been shown to disrupt binding, but it is not always clear whether these loss-of-function mutations affect gross IGFBP structure or point to specific interacting residues.

Amino-terminal domain. Abundant evidence points to the importance of amino-terminal IGFBP residues in interacting with the IGFs. Recombinant amino-terminal fragments of IGFBP-3, such as 1—88 or 1—97, bind IGFs poorly as determined by ligand blotting unless the gel is heavily loaded; however, affinity labeling studies clearly indicate that specific binding is retained, with an affinity loss of ~50-fold compared with the full-length protein (38, 96). In IGFBP-1, deletion of the 60 amino-terminal residues results in a loss of IGF binding (14), and point mutation studies in other IGFBPs also indicate the importance of residues in and beyond this region, particularly those between the 9th and 12th conserved cysteine residues of IGFBP-1 to -5 (Fig. 1). In bovine IGFBP-2, Tyr^{60} (between the 9th and 10th cysteines) has been shown to contribute to IGF affinity (48), with residues 59 and 61–63 apparently not involved. In IGFBP-4, a 20-residue sequence from Leu^{72} (spanning the 12th cysteine) was found to be essential for binding (80).

Perhaps the most compelling evidence to date for an amino-terminal IGF-binding domain comes from the NMR structure of a recombinant IGFBP-5 fragment determined by Kalus et al. (59). This study identified a

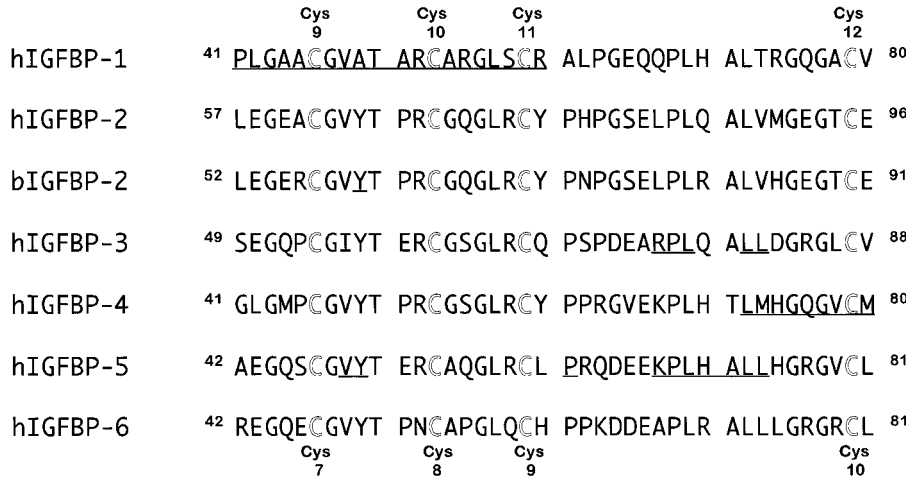

Fig. 1. Comparative sequences of amino-terminal regions of insulin-like growth factor-binding proteins (IGFBPs) shown to be involved in IGF binding. The 9th to 12th conserved cysteine residues of IGFBP-1 to -5 are indicated in outline. For IGFBP-6 only, these correspond to the 7th to 10th cysteine residues. The first and last residue number of each sequence is indicated. Underlined residues have been shown by mutagenesis or deletion to be involved in IGF binding, as described in the text. Note that both the human (h) and bovine (b) IGFBP-2 sequences are shown.

key role for residues beyond the 9th cysteine (Cys^{47}) in IGF binding: Val^{49}, Tyr^{50}, Pro^{62}, and Lys^{68}-Leu^{74}. The importance of residues 68–70 and 73–74, and of the corresponding residues of IGFBP-3, has recently been confirmed by mutagenesis (53). Similarly, Tyr^{50} of IGFBP-5 corresponds to Tyr^{60} of bovine IGFBP-2, and Leu^{73} of IGFBP-5 corresponds to Leu^{72} of IGFBP-4, both residues implicated by mutagenesis studies. Interestingly, Val^{49}, an important IGFBP-5 residue, corresponds to bovine IGFBP-2 Val^{59}, mutagenesis of which was without effect on IGF binding.

Among the IGFBP-related proteins, those designated related protein-1, -2, and -3 have all been shown, like amino-terminal IGFBP-3 fragments, to bind IGFs weakly when determined by affinity labeling (51). Because the structural similarity between these proteins and the IGFBPs occurs mainly in the amino-terminal domain, it might have been predicted that key amino-terminal binding residues identified by NMR and mutagenesis in the IGFBPs would be shared by the IGFBP-related proteins. However, this is not the case. Neither Tyr^{50}, Pro^{62}, nor Leu^{73} (and adjacent residues) of IGFBP-5 is conserved in the IGFBP-related proteins with demonstrable IGF-binding activity (51). Thus the key IGF-binding residues of these proteins remain, like the functional significance of this binding, to be determined.

Carboxy-terminal domain. Mutagenesis studies also indicate that carboxy-terminal residues are important in IGF binding. The earliest were those of Brinkman et al. (15), who showed that deletion of the 20 carboxy-terminal residues of human IGFBP-1, or mutation of Cys^{226} in this region to Tyr, abolished IGF binding. In contrast, bovine IGFBP-2 truncated by 14, 36, or 48 residues appeared to retain normal IGF binding, whereas removal of 63 residues caused a marked loss of activity (40). Removal of most or all of the carboxy-terminal domain of human IGFBP-3 (38, 88), IGFBP-4 (80), or IGFBP-5 (2) similarly disrupts IGF binding, although some residual activity is typically seen. In IGFBP-4, Cys^{205} through Val^{214} have been described as key residues (80), whereas in bovine IGFBP-2, a more amino-terminal sequence (Lys^{222}-Asn^{236}, corresponding to residues 185–199 of human IGFBP-4) was found to be important (40). It should be noted that IGFBP disulfide bond analysis (40) suggests that loss of Cys^{205} and Cys^{207} of IGFBP-4 will disrupt two of the three disulfides in the carboxy-terminal domain, which might introduce a significant conformational change.

Consistent with the existence of specific carboxy-terminal IGF-binding determinants, isolated naturally occurring fragments of rat and human IGFBP-2 have been shown to retain considerable IGF-binding affinity (47, 97). A preparation containing the human fragment IGFBP-2-(169—289) bound IGF-II with an affinity of $\sim 5 \times 10^9$ l/mol, only 10-fold lower than that of full-length IGFBP-2, although its IGF-I binding was much weaker (47). Interestingly, as noted above, this preparation did not bind des-(1—6)-IGF-II, suggesting that the amino terminus of IGF-II is required for interaction with the carboxy-terminal domain of IGFBP-2.

The combined data, supporting interactions of amino- and carboxy-terminal IGFBP residues in IGF binding, suggest that both of the terminal IGFBP domains are likely to be involved in high-affinity IGF-IGFBP complexes. Confirmation of this will await crystallographic or NMR studies on intact IGFBPs.

Natural Modifications of IGFBPs That Influence IGF Binding

Relatively few posttranslational modifications of IGFBPs have been described. As previously reviewed (7), only IGFBP-3 and IGFBP-4 have consensus *N*-glycosylation sites; in the case of IGFBP-4, a single site (Asn^{104}) is variably glycosylated (21), whereas in IGFBP-3, two sites (Asn^{89} and Asn^{109}) are always glycosylated, and a third site (Asn^{172}) is variably glycosylated (37). Glycosylation is reported not to affect the IGF-binding affinity of either IGFBP-3 (37) or IGFBP-4 (20). Similarly, *O*-linked glycosylation of IGFBP-6 does not influence IGF binding (5).

Phosphorylation. At least three of the IGFBPs (IGFBP-1, -3, and -5) are secreted as phosphoproteins (31, 56), but the effect, if any, of phosphorylation on IGF binding has not been reported for IGFBP-5, and in the case of IGFBP-3 there is no effect (49). In contrast, the affinity of phosphorylated human IGFBP-1 for IGF-I is sixfold higher than for the nonphosphorylated protein (56), although this difference is not seen for rat IGFBP-1 (79). Differential measurement of nonphosphorylated and highly phosphorylated IGFBP-1 forms in human serum suggests that the state of phosphorylation is under hormonal control (98), but it is not yet established what physiological consequence this regulation might have. Because IGFBP-1 is suggested to play a role in the acute regulation of IGF bioavailability by binding "free" IGFs (44), regulation of its affinity by phosphorylation may be important in metabolic regulation.

Proteolysis. Whereas the conserved amino- and carboxy-terminal domains of the IGFBPs appear relatively resistant to proteolytic attack, cleavage by endoproteases at central domain sites is common. The resulting fragments typically have greatly reduced IGF-binding affinity; examples are the amino-terminal plasmin-derived fragment IGFBP-3-(1—97) (62) and the carboxy-terminal IGFBP-2 fragment isolated from milk, IGFBP-2-(169—289) (47). Limited proteolysis at the cellular level must therefore be regarded as an important mechanism for regulating the IGF-IGFBP interaction, as we will discuss further. Interestingly, IGFBP-3 in pregnancy serum, which appears fully proteolyzed when analyzed on SDS-PAGE, carries IGFs at normal concentration in the circulation, implying that its IGF-binding activity is unaffected (89). Nevertheless, when stripped of its IGFs and tested for IGF-binding activity in vitro, a considerable reduction in affinity is evident (63). This discrepancy has never been adequately resolved, but it has been observed that the acid-labile subunit (ALS), which normally complexes with IGFBP-3 in serum without affecting its IGF-binding affinity, increases the affinity of pregnancy-

derived IGFBP-3 (9). This suggests that IGFBP-3, which is cleaved by a circulating endoprotease in pregnancy while complexed to IGFs and ALS, retains its binding activity until the complex is disrupted, for example by acidification.

Effect of Cell Association on IGF Binding

Several of the IGFBPs have been reported to bind to the surface of cells, or to extracellular matrix, and various cell-association molecules, or putative receptors, have been identified, as described later. IGF-binding studies generally show a decrease in affinity when the IGFBP is associated with the cell surface or matrix. For IGFBP-3, a 40-fold lower IGF-I affinity was reported when bound to the human fibroblast cell lines GM10 and T98G compared with IGFBP-3 in solution (71); IGFBP-2, which associates with cell-surface proteoglycans in the brain, shows a threefold decrease in IGF affinity when bound to chondroitin sulfate (84).

Some caution is needed in interpreting these findings, because under conditions of restricted volume and ligand concentration, a ligand that is immobilized, for example on a cell membrane, might exhibit different binding kinetics than the corresponding ligand in solution. Nevertheless, the observed affinity changes may be of biological significance, and a decrease resulting from cell association might be an important mechanism by which bound IGFs could be released to act on cells. In the case of IGFBP-5, binding to fibroblast matrix decreases its affinity for IGF-I by sevenfold (57). For both IGFBP-3 and IGFBP-5, an important cell-, matrix-, and glycosaminoglycan-binding determinant is found in a basic carboxy-terminal domain, IGFBP-3-(215—232) and IGFBP-5-(201—218) (12, 38). Interestingly, whereas substitution of key basic residues within this domain abolishes cell binding but has minimal effect on IGF affinity (38), mutagenesis of either of two highly conserved nonbasic residues within this domain in IGFBP-5 (Gly^{203}, Gln^{209}) reduces IGF-I affinity by six- to eightfold (13). This may explain how cell attachment of IGFBP-3 and -5, involving the carboxy-terminal binding domain, decreases IGF affinity.

FUNCTIONAL CONSEQUENCES OF IGF-IGFBP INTERACTIONS

Inhibition of IGF Activity by IGFBPs

The diverse activities of IGF-I and IGF-II in stimulating mitogenesis, increasing substrate uptake and metabolic activity, inhibiting apoptosis, and modulating a variety of specific functions in highly differentiated cell types are for the most part mediated through binding and activation of the type I IGF receptor (IGF1R). Although there are notable exceptions, which will be discussed, the interaction of an IGFBP with IGF-I or IGF-II generally blocks receptor activation. Examples of this inhibitory effect of IGFBPs on IGF action, at all levels from cell DNA synthesis to blood glucose regulation to whole body growth, exist for all six of the IGFBPs (3, 23, 32, 33, 50, 52, 66) and have been extensively reviewed (68, 74, 82).

Use of IGF analogs. Whereas the inhibitory effects of IGFBPs introduced as exogenous protein, or expressed from transfected cDNA, are readily observed, effects of endogenously produced IGFBPs may be harder to demonstrate. A powerful tool in these studies has been the use of IGF analogs with reduced affinity for the IGFBPs but normal affinity for the IGF1R, produced by truncation or mutation of key residues as previously discussed. Valuable reagents in these studies have been [Gln^3,Ala^4,Tyr^{15},Leu^{16}]IGF-I (QAYL) and an amino-terminally extended form of [Arg^3]IGF-I (LR^3). For example, QAYL IGF-I has been used to demonstrate that IGFBP-3 and IGFBP-6, induced by treatment of cultured breast cancer cells with forskolin and retinoic acid, are directly inhibitory to IGF-I-stimulated DNA synthesis. When QAYL IGF-I was used in place of normal IGF-I, the induction of IGFBPs by these agents was no longer associated with inhibition of DNA synthesis (70). At the level of whole animal metabolism, LR^3 IGF-I was demonstrated to be up to three times more potent than IGF-I in stimulating muscle protein synthesis and restoring body growth in diabetic rats (92), indicating that the effect of IGF-I on these processes is limited by interaction with IGFBPs. Des-(1—3)-IGF-I, also used in studies of this kind, is perhaps less definitive, as it retains considerable affinity for the main circulating carrier protein, IGFBP-3 (39).

IGFBP regulation by proteases. As noted above, IGFBP fragments generated by the action of cellular endoproteases typically show a marked loss of IGF binding, and proteolytic degradation of IGFBPs to these low-activity forms appears to be an important mechanism by which cells can regulate IGF activity. Thus IGFBP-degrading proteases secreted by prostate and breast cancer cells have been proposed to act as growth stimulators by increasing local IGF availability (29, 85), and the timed release of IGFBPs and their proteases during differentiation of a variety of cell types suggests that IGFBP degradation could play a key regulatory role. This phenomenon is apparent during osteoblast differentiation (91) but is perhaps best illustrated in ovarian follicular development, in which IGF actions, including the stimulation of estradiol release, are blocked (in the human ovary) by IGFBP-4 during follicle atresia and restored by the action of an IGFBP-4 protease induced during follicular development (55). In the rat, a follicle-stimulating hormone-inducible IGFBP-5 protease may similarly inactivate IGFBP-5 during folliculogenesis (36).

The development of protease-resistant IGFBP mutants has further enhanced the study of IGFBP actions and the role of proteolysis. Conover et al. (28) identified a cleavage site in IGFBP-4 after Met^{135} and constructed a mutant with relative stability to degradation by fibroblast cultures. The mutant protein was able to inhibit IGF-II action in fibroblasts, even after prolonged incubation that caused total inactivation of wild-type IGFBP-4. In a similar approach, mutation of an IGFBP-5 cleavage site identified after Arg^{138} generated a form resistant to degradation by porcine smooth muscle cells even after 24 h of incubation (52). The

protease-resistant protein inhibited IGF-I-stimulated insulin receptor substrate-1 phosphorylation, DNA and protein synthesis, and cell migration, whereas native IGFBP-5 was degraded into inactive fragments. These studies serve to illustrate the predominantly inhibitory role of IGFBPs on IGF actions and the importance of proteases in modulating this activity.

Stimulation of IGF Activity by IGFBPs

Studies in vitro. The paradoxical potentiation of IGF activity by some of the IGFBPs, first described for IGFBP-1 and IGFBP-3, is now also well documented for IGFBP-5, although the mechanisms involved are unclear. Although the expression of IGFBP-2 is reported to cause enhanced growth of tumor cells (72), evidence that it can potentiate IGF activity is sparse. In the case of IGFBP-1, two charge variants were isolated from human amniotic fluid, one inhibitory to IGF-I-stimulated DNA synthesis in porcine smooth muscle cells and the other stimulatory (17). The two isoforms were subsequently shown to be differentially phosphorylated (56); as described above, phosphorylation increases the IGF-binding affinity by several multiples of increase. Potentiation of IGF-I activity appears to be associated with the low-phosphorylation, low-affinity form, but the precise mechanism is unknown. Furthermore, phosphorylation of rat IGFBP-1 has no effect on its IGF-binding affinity, and both the phospho- and nonphosphoforms inhibit IGF activity (79).

Potentiation of IGF activity by IGFBP-3 has been demonstrated in many cell culture systems. The observation that precincubation of cells with IGFBP-3 was necessary for this effect to be seen (33) led to the concept that it involved the cell association and processing of IGFBP-3 to a form of reduced affinity (26), which might in some way enhance the presentation of IGFs to the IGF1R. Agents that alkalinize acidic intracellular compartments were shown to block the potentiating effect (27), supporting the concept that IGFBP-3 processing may be involved. However, preincubation with IGFBP-3 has not always been necessary for this effect to be observed (10), and it remains unclear how low-affinity forms of IGFBP-3 could actually lead to stimulation of IGF1R activity. Indeed, membrane-associated IGFBP-3 remains inhibitory to IGF1R activity in some studies (60), and a recent study using recombinant nonglycosylated IGFBP-3 has been interpreted as showing that IGFBP-3 interacts with the IGF1R and blocks its activation by IGF-I (73).

Despite uncertainty about the mechanism involved, enhancement of IGF action by IGFBP-3 appears to be a widely reported phenomenon, evident in various cell types, including osteoblasts (35) and breast cancer cells (22), in addition to the fibroblast models in which it was originally described. IGFBP-5 can also potentiate IGF-I activity in fibroblasts. Jones et al. (57) showed that it binds to extracellular matrix components including collagen, laminin, and fibronectin, causing a loss of IGF affinity; when present in matrix, it is able to potentiate IGF-I-stimulated fibroblast growth.

Studies in vivo. Some studies in vivo also suggest that IGFs may be more effective as therapeutic agents when coupled to an IGFBP than when administered alone. An investigation of the action of IGF-I in reversing the inhibitory effect of corticosteroids on wound healing showed that complexed IGF-I-IGFBP-3 had significantly greater activity than the equivalent dose of IGF-I administered alone (46). Similarly, coadministration of IGF-I with IGFBP-3 was more effective than even a larger dose of IGF-I alone in eliciting weight gain and an increase in epiphysial width in hypophysectomized rats (24). Examples of potentiating effects of IGFBPs other than IGFBP-3 are less common, due in part to the paucity of in vivo studies with these proteins. However, IGF-II has been reported to stimulate the healing of human wounds only if recombinant IGFBP-1 was also added (61), and recombinant human IGFBP-5 administered to mice in combination with IGF-I increased serum osteocalcin levels to a greater extent than either peptide given alone (83). Although stimulatory effects of IGFBPs have been associated with forms of low affinity and are postulated to involve the stabilization of bound IGFs in binary complexes, or the presentation of IGFs to their receptor by cell- or matrix-bound IGFBPs, no definitive mechanism has yet been explicitly demonstrated for this phenomenon.

IGF RECEPTOR-INDEPENDENT ACTIONS OF IGFBPS

Although the dichotomous stimulatory and inhibitory effects of IGFBPs on IGF action have provided fertile ground for research activity over the past 10–15 years, it has become evident that a third set of IGFBP actions, those classed as IGF independent, also fill an important place in the repertoire of these multifunctional proteins. These actions are more accurately described as IGF1R independent, because they may be modulated by IGF binding without requiring the presence of the IGF1R. Such actions are likely to involve structural domains of IGFBPs that are distinct from the IGF-binding determinants.

Effects Involving Integrins: IGFBP-1

The first characterized of these domains was the Arg-Gly-Asp integrin binding motif present in the carboxy-terminus of IGFBP-1 and -2 but in no other IGFBP. For IGFBP-1, this was shown to stimulate Chinese hamster ovary cell migration in an IGF-independent manner, the effect being mediated through interaction with the $\alpha_5\beta_1$-integrin (fibronectin receptor) (58). Similarly, $\alpha_5\beta_1$-integrin binding by IGFBP-1 was found to be important in human trophoblast cell migration, because antibodies against either integrin subunit blocked the stimulatory effect of IGFBP-1 (54). The Arg-Gly-Asp motif in IGFBP-1 also appears to be involved in the induction of focal adhesion kinase dephosphorylation, cell detachment, and subsequent apoptosis, because IGFBP-1 and a synthetic Arg-Gly-Asp-containing peptide had similar effects in initiating these events in breast cancer cells (78). However, in contrast to its clear function in IGF-independent ac-

tions of IGFBP-1, the role of the Arg-Gly-Asp motif in IGFBP-2 function has not been documented, and introduction of this motif into the carboxy terminus of IGFBP-3 had no effect on its cell association (38).

IGFBP-3 and IGFBP-5

Apart from IGFBP-1, the other proteins for which functions independent of IGF1R have been demonstrated are IGFBP-3 and IGFBP-5. Mouse IGFBP-3 was shown in 1991 to inhibit fibroblast growth factor (FGF)-stimulated DNA synthesis, an effect that appeared not to depend on the sequestration of endogenous IGFs but was reversed by IGF-I (95). The lack of involvement of the IGF1R in growth inhibition by IGFBP-3 was shown more definitively by the use of a mouse fibroblast cell line with a disrupted IGF1R gene (94), in which transfection with an IGFBP-3 cDNA and expression of the protein caused a marked growth inhibition. Whether endogenous IGFs might be present and signaling through another receptor, e.g., insulin receptor isoform A, which binds IGF-II with high affinity (43), was not determined in these studies. The same cell line was used to show that a 16-kDa plasmin-generated fragment of nonglycosylated IGFBP-3, without measurable IGF-binding activity determined by affinity labeling, was inhibitory to FGF-stimulated DNA synthesis, whereas intact IGFBP-3 had little effect (100). Interestingly, another IGFBP-3 fragment of 22–25 kDa, which also failed to bind IGFs by affinity labeling, retained preferential affinity for IGF-II when studied in a solution-binding assay. The unequivocal conclusion from these studies is that IGFBP-3 or its proteolytic fragments can inhibit the growth of cells lacking IGF1R. Whether this effect might nevertheless involve an IGF-initiated pathway, acting through an insulin or alternative IGF receptor, seems unlikely but is perhaps not absolutely excluded.

Induction of apoptosis by IGFBP-3. In addition to its effect on DNA synthesis, IGFBP-3 induces apoptosis in a manner that appears independent of the IGF1R. Although the IGFBP-3 gene is a target of the tumor suppressor protein p53 (16), the apoptotic effect of IGFBP-3 does not require p53, because it is observed in p53-negative cell lines such as the prostate carcinoma PC-3 cells (81). IGF1R-negative mouse fibroblasts were again used to show that the effect did not depend upon IGF1R signaling. However, in these studies, the use of high serum supplementation in the culture medium means that IGFs were present, which might conceivably exert an antiapoptotic effect through a non-IGF1R receptor, reversible by IGFBP-3. IGF-I was also shown to partly reverse the apoptotic effect of IGFBP-3 in the receptor-negative cells, a result interpreted as indicating that, in this system, IGF-I acts through a direct interaction with the binding protein (81). IGF-independent IGFBP-3 action is also apparent in the IGF-unresponsive breast cancer cell line Hs578T, in which IGFBP-3 does not induce apoptosis alone but markedly enhances the apoptotic effect of the ceramide analog C2 (45).

Other IGFBP-3 ligands. It is now clear that IGFBP-3 has a number of ligands in addition to IGFs. The 18-residue basic cell-association domain, described above, is important in many of these interactions, and because this domain is also found in IGFBP-5, the same ligands are likely to bind to both proteins. However, the functional consequences of many of these interactions are not yet fully understood. Apart from interactions with cell-surface and matrix components, the best-characterized role of the basic domain in IGFBP-3 and IGFBP-5 is in the binding of the 85-kDa leucine-rich glycoprotein ALS (38, 93), as part of circulating IGF-transport complexes. For both IGFBPs, the affinity for ALS is markedly enhanced by IGF binding, presumably through conformational change in the IGFBP, because no IGF-ALS interaction can be demonstrated. The basic domain is also involved in the transport of both IGFBP-3 and IGFBP-5 to the cell nucleus (87), a process for which no role has yet been definitively described, although it is associated with cell proliferation (67). Although IGF-I has been shown to be cotransported to the nucleus with IGFBP-3 (67), it is not established whether any nuclear function of IGFBP-3 might be modulated by IGF binding. Both IGFBP-3 and IGFBP-5 interact with importin (nuclear import receptor) subunits through their basic carboxy-terminal regions (L. J. Schedlich and R. C. Baxter, unpublished observations), and this may be central to the nuclear translocation process.

Other non-IGF ligands for IGFBPs include plasminogen, which binds IGFBP-3 through its basic domain (19) and is suggested to be involved in physiological proteolysis of the binding protein. The corresponding basic domain of IGFBP-5 appears to be involved in plasmin proteolysis of this protein (18) and also serves as a binding site for plasminogen activator inhibitor-1 (75). These studies point to a complex interplay between IGFBPs and the plasmin system, in which there may be reciprocal modulation of IGFBP and plasminogen/plasmin activity. The carboxy-terminal matrix- and ALS-binding region of IGFBP-3 and IGFBP-5 is clearly central to these interactions.

Putative Receptors for IGFBP-3 and IGFBP-5

At least some IGF1R-independent actions of IGFBP-3 and IGFBP-5 are assumed to be mediated through signaling receptors located on the plasma membrane of target cells. Cell-surface IGFBP-3-binding proteins of 20–50 kDa, initially described as receptors (77), have not been fully characterized and did not have any signaling function ascribed to them. A more recent report, in which putative receptor sites for nonglycosylated IGFBP-3 were partially characterized in breast cancer cells, is notable because the interaction occurred largely through central domain determinants on IGFBP-3 (99), in contrast to the numerous studies implicating a carboxy-terminal domain sequence in cell binding. Once again, no signaling function was attributed to the interaction. It should be considered in this context that the central domain of IGFBP-3 contains three glycosylation sites that, in the natural protein,

carry up to 15 kDa of carbohydrate. The absence of this carbohydrate increases cell association by up to threefold (37), raising the question whether central domain residues of IGFBP-3, which can interact with cells when the glycosylation sites are unfilled, retain their accessibility to cell-binding sites in the natural, fully glycosylated protein.

In contrast to the relatively small cell-surface proteins identified as IGFBP-3 binding sites in earlier studies, the 400-kDa type V transforming growth factor-β (TGF-β) receptor has recently been proposed as an IGFBP-3 receptor in mink lung cells, and IGFBP-3 was stated to be unable to inhibit the growth of cells lacking this protein (64). Subsequently, IGFBP-4 and IGFBP-5 were also shown to be ligands for the type V TGF-β receptor (65). However, if binding elicits inhibitory signaling, these IGFBPs must be relatively weak ligands, because they had only a slight effect on DNA synthesis. A protein of similar size has been proposed as an IGFBP-5 receptor in osteoblasts (1). This protein interacted with the 18-residue basic carboxy-terminal domain of IGFBP-5 and was demonstrated to be a serine kinase, able to autophosphorylate in an IGFBP-5-stimulated manner and also to act as a casein kinase. The possibility that this protein is related to the type V TGF-β receptor, also a serine-threonine kinase with casein kinase specificity (76), certainly requires exploration.

Although the above studies have broken important new ground in the search for IGFBP-signaling pathways, there is clearly a great deal remaining to be discovered. For example, the precise relationship between cell binding of IGFBPs and IGF-independent signal transduction remains largely unexplored. Although some studies show an association between the degree of cell binding of IGFBP-3 and the extent of growth inhibition (99), it is likely that, as with other ligand-receptor systems, signaling will be regulated to a considerable extent by intracellular factors. In this context it is interesting that cell sensitivity to inhibitory signaling by IGFBP-3 has been shown to be regulated by the Ras-mitogen-activated protein (MAP) kinase pathway (69), with IGFBP-3 inhibition of DNA synthesis blocked by the expression of oncogenic Ras and restored by inhibiting MAP kinase activation.

CONCLUDING COMMENTS

Our understanding of the multifunctional nature of the IGFBP family has made considerable progress from the time, little more than a decade ago, when these proteins were considered simply as passive carriers of IGFs. Given that the circulating IGF concentration in healthy adults is ~100 nM and the IGF1R on most cells is typically approaching saturation at IGF concentrations of 5 nM or lower, it is clear that regulation of circulating IGF bioavailability is a key function of the IGFBPs. In this context, the recent discovery that IGFBP-5 as well as IGFBP-3 can form ternary complexes with IGFs and ALS requires that the physiology of "endocrine" IGFs be reevaluated. However, as highlighted in this review, it is in their roles at the cellular level that the IGFBPs have provided most of the surprises in recent years.

Despite the recent advances in IGFBP cell physiology, many fundamental questions remain. In regard to IGFBP modulation of IGF actions, the mechanisms underlying the potentiation of IGF activity by some IGFBPs are still poorly understood. Even less clear are the pathways by which some IGFBPs exert direct cellular actions independent of the IGF1R. Here the unanswered questions start at the plasma membrane, where the identity and function of specific, saturable, signaling receptors remain to be elucidated. The questions then multiply. Are distinct receptors required to initiate cell cycle and apoptotic effects of IGFBPs? Do IGFBPs enter the cell as part of the signaling process, and if so, by what mechanism? Which intracellular signaling systems are used? What are the nuclear functions of IGFBP-3 and IGFBP-5? Do IGFs modulate IGF1R-independent actions of IGFBPs, and if so, how? What processes lead to cellular resistance to IGFBPs, and what are the consequences of this? As the IGF-IGFBP system is increasingly recognized as being of importance in malignancy, in addition to its role in normal cellular growth, there will be increasing urgency to find answers to these questions.

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REFERENCES

1. **Andress DL.** Insulin-like growth factor-binding protein-5 (IGFBP-5) stimulates phosphorylation of the IGFBP-5 receptor. *Am J Physiol Endocrinol Metab* 274: E744–E750, 1998.
2. **Andress DL, Loop SM, Zapf J, and Kiefer MC.** Carboxy-truncated insulin-like growth factor binding protein-5 stimulates mitogenesis in osteoblast-like cells. *Biochem Biophys Res Commun* 195: 25–30, 1993.
3. **Bach LA, Hsieh S, Brown AL, and Rechler MM.** Recombinant human insulin-like growth factor (IGF)-binding protein-6 inhibits IGF-II-induced differentiation of L6A1 myoblasts. *Endocrinology* 135: 2168–2176, 1994.
4. **Bach LA, Hsieh S, Sakano K, Fujiwara H, Perdue JF, and Rechler MM.** Binding of mutants of human insulin-like growth factor II to insulin-like growth factor binding proteins 1–6. *J Biol Chem* 268: 9246–9254, 1993.
5. **Bach LA, Thotakura NR, and Rechler MM.** Human insulin-like growth factor binding protein-6 is O-glycosylated. *Biochem Biophys Res Commun* 186: 301–307, 1992.
6. **Ballard J, Baxter R, Binoux M, Clemmons D, Drop S, Hall K, Hintz R, Rechler M, Rutanen E, and Schwander J.** On the nomenclature of the IGF binding proteins. *Acta Endocrinol* 121: 751–752, 1989.
7. **Baxter RC.** Molecular aspects of insulin-like growth factor binding proteins. *Adv Mol Cell Endocrinol* 1: 123–159, 1997.
8. **Baxter RC, Binoux MA, Clemmons DR, Conover CA, Drop SLS, Holly JMP, Mohan S, Oh Y, and Rosenfeld RG.** Recommendations for nomenclature of the insulin-like growth factor binding protein superfamily. *J Clin Endocrinol Metab* 83: 3213, 1998.
9. **Baxter RC and Skriver L.** Altered ligand specificity of proteolysed insulin-like growth factor binding protein-3. *Biochem Biophys Res Commun* 196: 1267–1273, 1993.
10. **Blum WF, Jenne EW, Reppin F, Kietzmann K, Ranke MB, and Bierich JR.** Insulin-like growth factor I (IGF-I)-binding protein complex is a better mitogen than free IGF-I. *Endocrinology* 125: 766–772, 1989.
11. **Blundell TL, Bedarkar S, Rinderknecht E, and Humbel RE.** Insulin-like growth factor: a model for tertiary structure

accounting for immunoreactivity and receptor binding. *Proc Natl Acad Sci USA* 75: 180–184, 1978.
12. **Booth BA, Boes M, Andress DL, Dake BL, Kiefer MC, Maack C, Linhardt RJ, Bar K, Caldwell EEO, Weiler J, and Bar RS.** IGFBP-3 and IGFBP-5 association with endothelial cells: role of C-terminal heparin binding domain. *Growth Regul* 5: 1–17, 1995.
13. **Bramani S, Song H, Beattie J, Tonner E, Flint DJ, and Allan GJ.** Amino acids within the extracellular matrix (ECM) binding region (201–218) of rat insulin-like growth factor binding protein (IGFBP)-5 are important determinants in binding IGF-I. *J Mol Endocrinol* 23: 117–123, 1999.
14. **Brinkman A, Kortleve DJ, Schuller AGP, Zwarthoff EC, and Drop S.** Site-directed mutagenesis of the N-terminal region of IGF binding protein-1: analysis of IGF binding capability. *FEBS Lett* 291: 264–268, 1991.
15. **Brinkman A, Kortleve DJ, Zwarthoff EC, and Drop SL.** Mutations in the C-terminal part of insulin-like growth factor (IGF)-binding protein-1 result in dimer formation and loss of IGF binding capacity. *Mol Endocrinol* 5: 987–994, 1991.
16. **Buckbinder L, Talbott R, Velasco MS, Takenaka I, Faha B, Seizinger BR, and Kley N.** Induction of the growth inhibitor IGF-binding protein 3 by p53. *Nature* 377: 646–649, 1995.
17. **Busby WJ, Klapper DG, and Clemmons DR.** Purification of a 31,000-dalton insulin-like growth factor binding protein from human amniotic fluid. Isolation of two forms with different biologic actions. *J Biol Chem* 263: 14203–14210, 1988.
18. **Campbell PG and Andress DL.** Plasmin degradation of insulin-like growth factor-binding protein-5 (IGFBP-5): regulation by IGFBP-5-(201—218). *Am J Physiol Endocrinol Metab* 273: E996–E1004, 1997.
19. **Campbell PG, Durham SK, Suwanichkul A, Hayes JD, and Powell DR.** Plasminogen binds the heparin-binding domain of insulin-like growth factor-binding protein-3. *Am J Physiol Endocrinol Metab* 275: E321–E331, 1998.
20. **Carr JM, Grant PA, Francis GL, Owens JA, Wallace JC, and Walton PE.** Isolation and characterization of ovine IGFBP-4: protein purification and cDNA sequence. *J Mol Endocrinol* 13: 219–236, 1994.
21. **Ceda GP, Fielder PJ, Henzel WJ, Louie A, Donovan SM, Hoffman AR, and Rosenfeld RG.** Differential effects of insulin-like growth factor (IGF)-I and IGF-II on the expression of IGF binding proteins (IGFBPs) in a rat neuroblastoma cell line: isolation and characterization of two forms of IGFBP-4. *Endocrinology* 128: 2815–2824, 1991.
22. **Chen J-C, Shao Z-M, Sheikh MS, Hussain A, LeRoith D, Roberts CT, and Fontana JA.** Insulin-like growth factor-binding protein enhancement of insulin-like growth factor-I (IGF-I)-mediated DNA synthesis and IGF-I binding in a human breast carcinoma cell line. *J Cell Physiol* 158: 69–78, 1994.
23. **Cheung PT, Smith EP, Shimasaki S, Ling N, and Chernausek SD.** Characterization of an insulin-like growth factor binding protein (IGFBP-4) produced by the B104 rat neuronal cell line: chemical and biological properties and differential synthesis by sublines. *Endocrinology* 129: 1006–1015, 1991.
24. **Clark RG, Mortensen D, Reifsynder D, Mohler M, Etcheverry T, and Mukku V.** Recombinant human insulin-like growth factor binding protein-3 (rhIGFBP-3): effects on the glycemic and growth promoting activities of rhIGF-I in the rat. *Growth Regul* 3: 50–52, 1993.
25. **Clemmons DR, Cascieri MA, Camacho-Hübner C, McCusker RH, and Bayne ML.** Discrete alterations of the insulin-like growth factor I molecule which alter its affinity for insulin-like growth factor-binding proteins result in changes in bioactivity. *J Biol Chem* 265: 12210–12216, 1990.
26. **Conover CA.** Glycosylation of insulin-like growth factor binding protein-3 (IGFBP-3) is not required for potentiation of IGF-I action: evidence for processing of cell-bound IGFBP-3. *Endocrinology* 129: 3259–3268, 1991.
27. **Conover CA, Clarkson JT, and Bale LK.** Factors regulating insulin-like growth factor-binding protein-3 binding, processing, and potentiation of insulin-like growth factor action. *Endocrinology* 137: 2286–2292, 1996.
28. **Conover CA, Durham SK, Zapf J, Masiarz FR, and Kiefer MC.** Cleavage analysis of insulin-like growth factor (IGF)-dependent IGF-binding protein-4 proteolysis and expression of protease-resistant IGF-binding protein-4 mutants. *J Biol Chem* 270: 4395–4400, 1995.
29. **Conover CA, Perry JE, and Tindall DJ.** Endogenous cathepsin D-mediated hydrolysis of insulin-like growth factor-binding proteins in cultured human prostatic carcinoma cells. *Endocrinology* 80: 987–993, 1995.
30. **Cooke RM, Harvey TS, and Campbell ID.** Solution structure of human insulin-like growth factor 1: a nuclear magnetic resonance and restrained molecular dynamics study. *Biochemistry* 30: 5484–5491, 1991.
31. **Coverley JA and Baxter RC.** Regulation of insulin-like growth factor (IGF) binding protein-3 phosphorylation by IGF-I. *Endocrinology* 136: 5778–5781, 1995.
32. **Cox GN, McDermott MJ, Merkel E, Stroh CA, Ko SC, Squires CH, Gleason TM, and Russell D.** Recombinant human insulin-like growth factor (IGF)-binding protein-1 inhibits somatic growth stimulated by IGF-I and growth hormone in hypophysectomized rats. *Endocrinology* 135: 1913–1920, 1994.
33. **De Mellow JS and Baxter RC.** Growth hormone-dependent insulin-like growth factor (IGF) binding protein both inhibits and potentiates IGF-I-stimulated DNA synthesis in human skin fibroblasts. *Biochem Biophys Res Commun* 156: 199–204, 1988.
34. **Dubaquie Y and Lowman HB.** Total alanine-scanning mutagenesis of insulin-like growth factor I (IGF-I) identifies differential binding epitopes for IGFBP-1 and IGFBP-3. *Biochemistry* 38: 6386–6396, 1999.
35. **Ernst M and Rodan GA.** Increased activity of insulin-like growth factor (IGF) in osteoblastic cells in the presence of growth hormone (GH): positive correlation with the presence of the GH-induced IGF-binding protein BP-3. *Endocrinology* 127: 807–814, 1990.
36. **Fielder PJ, Pham H, Adashi EY, and Rosenfeld RG.** Insulin-like growth factors (IGFs) block FSH-induced proteolysis of IGF-binding protein-5 (BP-5) in cultured rat granulosa cells. *Endocrinology* 133: 415–418, 1993.
37. **Firth SM and Baxter RC.** Characterisation of recombinant glycosylation variants of insulin-like growth factor binding protein-3. *J Endocrinol* 160: 379–387, 1999.
38. **Firth SM, Ganeshprasad U, and Baxter RC.** Structural determinants of ligand and cell-surface binding of insulin-like growth factor-binding protein-3. *J Biol Chem* 273: 2631–2638, 1998.
39. **Forbes B, Szabo L, Baxter RC, Ballard FJ, and Wallace JC.** Classification of the insulin-like growth factor binding proteins into three distinct categories according to their binding specificities. *Biochem Biophys Res Commun* 157: 196–202, 1988.
40. **Forbes BE, Turner D, Hodge SJ, McNeil KA, Forsberg G, and Wallace JC.** Localization of an insulin-like growth factor (IGF) binding site of bovine IGF binding protein-2 using disulfide mapping and deletion mutation analysis of the C-terminal domain. *J Biol Chem* 273: 4647–4652, 1998.
41. **Francis GL, Aplin SE, McNeil KA, Milner SJ, Forbes BE, Ross M, Ballard FJ, and Wallace JC.** Molecular interactions of IGF-I and IGF-II with their binding proteins and receptors. In: *The Insulin-Like Growth Factors and Their Regulatory Proteins*, edited by RC Baxter, PD Gluckman, and RG Rosenfeld. Amsterdam: Elsevier, 1994, p. 57–66.
42. **Francis GL, Upton FM, Ballard FJ, McNeil KA, and Wallace JC.** Insulin-like growth factors 1 and 2 in bovine colostrum. Sequences and biological activities compared with those of a potent truncated form. *Biochem J* 251: 95–103, 1988.
43. **Frasca F, Pandini G, Scalia P, Sciacca L, Mineo R, Costantino A, Goldfine ID, Belfiore A, and Vigneri R.** Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. *Mol Cell Biol* 19: 3278–3288, 1999.
44. **Frystyk J, Grøfte T, Skjærbæk C, and Ørskov H.** The effect of oral glucose on serum free insulin-like growth factor-I and -II in healthy adults. *J Clin Endocrinol Metab* 82: 3124–3127, 1997.

45. **Gill ZP, Perks CM, Newcomb PV, and Holly JM.** Insulin-like growth factor-binding protein (IGFBP-3) predisposes breast cancer cells to programmed cell death in a non-IGF-dependent manner. *J Biol Chem* 272: 25602–25607, 1997.
46. **Hamon GA, Hunt TK, and Spencer EM.** In vivo effects of systemic insulin-like growth factor-I alone and complexed with insulin-like growth factor binding protein-3 on corticosteroid suppressed wounds. *Growth Regul* 3: 53–56, 1993.
47. **Ho PJ and Baxter RC.** Characterization of truncated insulin-like growth factor-binding protein-2 in human milk. *Endocrinology* 138: 3811–3818, 1997.
48. **Hobba GD, Lothgren A, Holmberg E, Forbes BE, Francis GL, and Wallace JC.** Alanine screening mutagenesis establishes tyrosine 60 of bovine insulin-like growth factor binding protein-2 as a determinant of insulin-like growth factor binding. *J Biol Chem* 273: 19691–19698, 1998.
49. **Hoeck WG and Mukku VR.** Identification of the major sites of phosphorylation in IGF binding protein-3. *J Cell Biochem* 56: 262–273, 1994.
50. **Hoflich A, Lahm H, Blum W, Kolb H, and Wolf E.** Insulin-like growth factor-binding protein-2 inhibits proliferation of human embryonic kidney fibroblasts and of IGF-responsive colon carcinoma cell lines. *FEBS Lett* 434: 329–334, 1998.
51. **Hwa V, Oh Y, and Rosenfeld RG.** The insulin-like growth factor-binding protein (IGFBP) superfamily. *Endocrine Rev* 20: 761–787, 1999.
52. **Imai Y, Busby WH, Smith CE, Clarke JB, Garmong AJ, Horwitz GD, Rees C, and Clemmons DR.** Protease-resistant form of insulin-like growth factor-binding protein 5 is an inhibitor of insulin-like growth factor-I actions on porcine smooth muscle cells in culture. *J Clin Invest* 100: 2596–2605, 1997.
53. **Imai Y, Morales A, Clarke JB, Busby W, Fielder P, and Clemmons DR.** Synthesis and characterization of IGFBP-5 and IGFBP-3 mutants that have no affinity for IGF-I (Abstract). *GH and IGF Res* 9: 372, 1999.
54. **Irving JA and Lala PK.** Functional role of cell surface integrins on human trophoblast cell migration: regulation by TGF-beta, IGF-II, and IGFBP-1. *Exp Cell Res* 217: 419–427, 1995.
55. **Iwashita M, Kudo Y, Yoshimura Y, Adachi T, Katayama E, and Takeda Y.** Physiological role of insulin-like-growth-factor-binding protein-4 in human folliculogenesis. *Horm Res* 46, *Suppl* 1: 31–36, 1996.
56. **Jones JI, D'Ercole AJ, Camacho HC, and Clemmons DR.** Phosphorylation of insulin-like growth factor (IGF)-binding protein 1 in cell culture and in vivo: effects on affinity for IGF-I. *Proc Natl Acad Sci USA* 88: 7481–7485, 1991.
57. **Jones JI, Gockerman A, Busby WH, Camacho-Hübner C, and Clemmons DR.** Extracellular matrix contains insulin-like growth factor binding protein-5: potentiation of the effects of IGF-I. *J Cell Biol* 121: 679–687, 1993.
58. **Jones JI, Gockerman A, Busby WJ, Wright G, and Clemmons DR.** Insulin-like growth factor binding protein 1 stimulates cell migration and binds to the alpha 5 beta 1 integrin by means of its Arg-Gly-Asp sequence. *Proc Natl Acad Sci USA* 90: 10553–10557, 1993.
59. **Kalus W, Zweckstetter M, Renner C, Sanchez Y, Georgescu J, Grol M, Demuth D, Schumacher R, Dony C, Lang K, and Holak TA.** Structure of the IGF-binding domain of the insulin-like growth factor-binding protein-5 (IGFBP-5): implications for IGF and IGF-I receptor interactions. *EMBO J* 17: 6558–6572, 1998.
60. **Karas M, Danilenko M, Fishman D, LeRoith D, Levy J, and Sharoni Y.** Membrane-associated insulin-like growth factor-binding protein-3 inhibits insulin-like growth factor-I-induced insulin-like growth factor-I receptor signaling in ishikawa endometrial cancer cells. *J Biol Chem* 272: 16514–16520, 1997.
61. **Kratz G, Lake M, and Gidlund M.** Insulin-like growth factor-1 and -2 and their role in the re-epithelialisation of wounds: interactions with insulin like growth factor binding protein type 1. *Scand J Plast Reconstr Hand Surg* 28: 107–112, 1994.
62. **Lalou C, Lassarre C, and 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* 137: 3206–3212, 1996.
63. **Lassarre C and Binoux M.** Insulin-like growth factor binding protein-3 is functionally altered in pregnancy plasma. *Endocrinology* 134: 1254–1262, 1994.
64. **Leal S, Liu Q, Huang S, and Huang J.** The type V transforming growth factor beta receptor is the putative insulin-like growth factor-binding protein 3 receptor. *J Biol Chem* 272: 20572–20576, 1997.
65. **Leal SM, Huang SS, and Huang JS.** Interactions of high affinity insulin-like growth factor-binding proteins with the type V transforming growth factor-β receptor in mink lung epithelial cells. *J Biol Chem* 274: 6711–6717, 1999.
66. **Lewitt MS, Denyer GS, Cooney GJ, and Baxter RC.** Insulin-like growth factor-binding protein-1 modulates blood glucose levels. *Endocrinology* 129: 2254–2256, 1991.
67. **Li W, Fawcett J, Widmer HR, Fielder PJ, Rabkin R, and Keller G-A.** Nuclear transport of insulin-like growth factor-I and insulin-like growth factor binding protein-3 in opossum kidney cells. *Endocrinology* 138: 1763–1766, 1997.
68. **Martin JL and Baxter RC.** IGF binding proteins as modulators of IGF action. In: *The IGF System, Molecular Biology, Physiology and Clinical Applications*, edited by R Rosenfeld and C Roberts. Totowa, NJ: Humana, 1999, p. 227–255.
69. **Martin JL and Baxter RC.** Oncogenic *ras* causes resistance to the growth inhibitor insulin-like growth factor binding protein-3 (IGFBP-3) in breast cancer cells. *J Biol Chem* 274: 16407–16411, 1999.
70. **Martin JL, Coverley JA, Pattison ST, and Baxter RC.** Insulin-like growth factor-binding protein-3 production by MCF-7 breast cancer cells: stimulation by retinoic acid and cyclic adenosine monophosphate and differential effects of estradiol. *Endocrinology* 136: 1219–1226, 1995.
71. **McCusker RH and Clemmons DR.** Use of lanthanum to accurately quantify insulin-like growth factor binding to proteins on cell surfaces. *J Cell Biochem* 66: 256–267, 1997.
72. **Menouny M, Binoux M, and Babajko S.** IGFBP-2 expression in a human cell line is associated with increased IGFBP-3 proteolysis, decreased IGFBP-1 expression and increased tumorigenicity. *Int J Cancer* 77: 874–879, 1998.
73. **Mohseni-Zadeh S and Binoux M.** Insulin-like growth factor (IGF) binding protein-3 interacts with the type 1 IGF receptor, reducing the affinity of the receptor for its ligand: an alternative mechanism in the regulation of IGF action. *Endocrinology* 138: 5645–5648, 1997.
74. **Murphy LJ.** Insulin-like growth factor binding proteins: functional diversity or redundancy? *J Mol Endocrinol* 21: 97–107, 1998.
75. **Nam TJ, Busby W, and Clemmons DR.** Insulin-like growth factor binding protein-5 binds to plasminogen activator inhibitor-I. *Endocrinology* 138: 2972–2978, 1997.
76. **O'Grady P, Liu Q, Huang SS, and Huang JS.** Transforming growth factor beta (TGF-beta) type V receptor has a TGF-beta-stimulated serine/threonine-specific autophosphorylation activity. *J Biol Chem* 267: 21033–21037, 1992.
77. **Oh Y, Muller H, Pham H, and Rosenfeld R.** Demonstration of receptors for insulin-like growth factor binding protein-3 on Hs578T human breast cancer cells. *J Biol Chem* 268: 26045–26058, 1993.
78. **Perks CM, Newcomb PV, Norman MR, and Holly JM.** Effect of insulin-like growth factor binding protein-1 on integrin signaling and the induction of apoptosis in human breast cancer cells. *J Mol Endocrinol* 22: 141–150, 1999.
79. **Peterkofsky B, Gosiewska A, Wilson S, and Kim Y-R.** Phosphorylation of rat insulin-like growth factor binding protein-1 does not affect its biological properties. *Arch Biochem Biophys* 357: 101–110, 1998.
80. **Qin X, Strong DD, Baylink DJ, and Mohan S.** Structure-function analysis of the human insulin-like growth factor binding protein-4. *J Biol Chem* 273: 23509–23516, 1998.
81. **Rajah R, Valentinis B, and Cohen P.** Insulin-like growth factor (IGF)-binding protein-3 induces apoptosis and mediates the effects of transforming growth factor-β1 on programmed cell

death through a p53- and IGF-independent mechanism. *J Biol Chem* 272: 12181–12188, 1997.
82. **Rechler MM and Clemmons DR.** Regulatory actions of insulin-like growth factor-binding proteins. *Trends Endocrinol Metab* 9: 176–183, 1998.
83. **Richman C, Baylink DJ, Lang K, Dony C, and Mohan S.** Recombinant human insulin-like growth factor-binding protein-5 stimulates bone formation parameters in vitro and in vivo. *Endocrinology* 140: 4699–4705, 1999.
84. **Russo VC, Bach LA, Fosang AJ, Baker NL, and Werther GA.** Insulin-like growth factor binding protein-2 binds to cell surface proteoglycans in the rat brain olfactory bulb. *Endocrinology* 138: 4858–4867, 1997.
85. **Salahifar H, Baxter RC, and Martin JL.** Insulin-like growth factor (IGF) binding protein-3 protease activity secreted by MCF-7 breast cancer cells: inhibition by IGFs does not require IGF-IGFBP interaction. *Endocrinology* 138: 1683–1690, 1997.
86. **Sara V, Carlsson-Skwirut C, Andersson C, Hall E, Sjogren B, Holmgren A, and Jornvall H.** Characterization of somatomedins from human fetal brain: identification of a variant form of insulin-like growth factor I. *Proc Natl Acad Sci USA* 83: 4904–4907, 1986.
87. **Schedlich LJ, Young TF, Firth SM, and Baxter RC.** Insulin-like growth factor-binding protein (IGFBP)-3 and IGFBP-5 share a common nuclear transport pathway in T47D human breast carcinoma cells. *J Biol Chem* 273: 18347–18352, 1998.
88. **Sommer A, Maack CA, Spratt SK, Mascarenhas D, Tressel TJ, Rhodes ET, Lee R, Roumas M, Tatsuno GP, Flynn JA, Gerber N, Taylor J, Cudny H, Nanney L, Hunt TK, and Spencer EM.** Molecular genetics and actions of recombinant insulin-like growth factor binding protein-3. In: *Modern Concepts of Insulin-Like Growth Factors*, edited by EM Spencer. New York: Elsevier, 1991, p. 715–728.
89. **Suikkari A-M, and Baxter RC.** Insulin-like growth factor binding protein-3 is functionally normal in pregnancy serum. *J Clin Endocrinol Metab* 74: 177–183, 1992.
90. **Terasawa H, Kohda D, Hatanaka H, Nagata K, Higashihashi N, Fujiwara H, Sakano K, and Inagaki F.** Solution structure of human insulin-like growth factor II; recognition sites for receptors and binding proteins. *EMBO J* 13: 5590–5597, 1994.
91. **Thrailkill KM, Quarles LD, Nagase H, Suzuki K, Serra DM, and Fowlkes JL.** Characterization of insulin-like growth factor-binding protein 5-degrading proteases produced throughout murine osteoblast differentiation. *Endocrinology* 136: 3527–3533, 1995.
92. **Tomas FM, Knowles SE, Owens PC, Chandler CS, Francis GL, and Ballard FJ.** Insulin-like growth factor-I and more potent variants restore growth of diabetic rats without inducing all characteristic insulin effects. *Biochem J* 291: 781–786, 1993.
93. **Twigg SM, Kiefer MC, Zapf J, and Baxter RC.** Insulin-like growth factor-binding protein 5 complexes with the acid-labile subunit: role of the carboxyl-terminal domain. *J Biol Chem* 273: 28791–28798, 1998.
94. **Valentinis B, Bhala A, DeAngelis T, Baserga R, and Cohen P.** The human insulin-like growth factor (IGF) binding protein-3 inhibits the growth of fibroblasts with a targeted disruption of the IGF-I receptor gene. *Mol Endocrinol* 9: 361–367, 1995.
95. **Villaudy J, Delbé J, Blat C, Desauty G, Golde A, and Harel L.** An IGF binding protein is an inhibitor of FGF stimulation. *J Cell Physiol* 149: 492–496, 1991.
96. **Vorwerk P, Yamanaka Y, Spagnoli A, Oh Y, and Rosenfeld RG.** Insulin and IGF binding by IGFBP-3 fragments derived from proteolysis, baculovirus expression and normal human urine. *J Clin Endocrinol Metab* 83: 1392–1395, 1998.
97. **Wang J-F, Hampton B, Mehlman T, Burgess WH, and Rechler MM.** Isolation of a biologically active fragment from the carboxy terminus of the fetal rat binding protein for insulin-like growth factors. *Biochem Biophys Res Commun* 157: 718–726, 1988.
98. **Westwood M, Gibson JM, Williams AC, Clayton PE, Hamberg O, Flyvbjerg A, and White A.** Hormonal regulation of circulating insulin-like growth factor-binding protein-1 phosphorylation status. *J Clin Endocrinol Metab* 80: 3520–3527, 1995.
99. **Yamanaka Y, Fowlkes J, Wilson E, Rosenfeld R, and Oh Y.** Characterization of insulin-like growth factor binding protein-3 (IGFBP-3) binding to human breast cancer cells: kinetics of IGFBP-3 binding and identification of receptor binding domain on the IGFBP-3 molecule. *Endocrinology* 140: 1319–1328, 1999.
100. **Zadeh SM and Binoux M.** The 16-kDa proteolytic fragment of insulin-like growth factor (IGF) binding protein-3 inhibits the mitogenic action of fibroblast growth factor on mouse fibroblasts with a targeted disruption of the type I IGF receptor gene. *Endocrinology* 138: 3069–3072, 1997.