# Unraveling Insulin-Like Growth Factor Binding Protein-3 Actions in Human Disease

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The IGF system plays critical roles in somatic growth in an endocrine fashion (somatomedin hypothesis) as well as proliferation and differentiation of normal and malignant cells in a paracrine/autocrine fashion. IGFBP-3 is known to modulate the actions of IGFs in circulation as well as the immediate extracellular environment. Interestingly, apart from the ability to inhibit or enhance IGF actions, IGFBP-3 also exhibits very clear, distinct biological effects independent of the IGF/IGF-I receptor axis. Over the past decade it has become widely appreciated that IGF/IGF-IR-independent actions of IGFBP-3 (antiproliferative and proapoptotic effects) contribute to improving the pathophysiology of a variety of human diseases, such as cancer, diabetes, and malnutrition. Recent studies have implicated interaction of IGFBP-3 with a variety of proteins or signaling cascades critical to cell cycle control and apoptosis; however, the actual mechanism of IGFBP-3 action is still unclear. This review reinforces the concept in support of the IGF/IGF-IR axis-independent actions of IGFBP-3 and delineates potential underlying mechanisms involved and subsequent biological significance, focusing in particular on functional binding partners and the clinical significance of IGFBP-3 in the assessment of cancer risk. **(*Endocrine Reviews* 30: 417–437, 2009)**



## I. Introduction

### A. The IGF system

The IGF system is well characterized, with profound effects on the growth and differentiation of normal and malignant cells. The established components of the IGF system include GH, IGF-I/-II peptides, type I and II IGF receptors, IGF binding proteins (IGFBPs), and IGFBP proteases.

#### *1. GH*

GH is the major regulator of IGF synthesis in the liver. GH binding to the hepatic GH receptor stimulates IGF-I synthesis and release from the liver, and IGF-I is transported to the main target organs via circulation to act as

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/er.2008-0028 Received June 23, 2008. Accepted May 26, 2009.
First Published Online May 28, 2009

Abbreviations: ALS, Acid-labile subunit; AMF, autocrine motility factor; CEF, chick embryo fibroblast; DNA-PK, DNA-dependent protein kinase; IGFBP, IGF binding protein; IGF-IR, type I IGF receptor; IGF-IIR, type II IGF receptor; IκBα, inhibitory protein κBα; IKK, IκBα kinase; IR, insulin receptor; MMP, matrix metalloproteinase; NF-κB, nuclear factor κB; PGI, phosphoglucose isomerase; RXR, retinoid X receptor; SNP, single nucleotide polymorphism; TGF-βRI, type I TGF-β receptor; TGF-βRII, type II TGF-β receptor; TGF-βRV, type V TGF-β receptor.

an endocrine factor (1). Circulating IGF-I further exerts negative feedback on the somatotropic axis and suppresses the release of GH from the pituitary (1).

#### *2. IGF-I/-II peptides*

These hormones share approximately 50% homology to insulin. They are ubiquitously expressed, highly homologous small peptide hormones of approximately 7 kDa molecular mass with multiple endocrine and paracrine/autocrine activities (2). Most circulating IGF-I is produced by the liver and is responsible for growth and development. However, recent studies of knockout mice with specific gene deletion of liver IGF-I demonstrated that the reduction of circulating IGF-I (>75%) has no discernible effect on postnatal body growth, raising a question about the endocrine function of circulating liver IGF-I (3, 4). Increased GH and extrahepatic tissue IGF-I production may compensate for the lack of the liver IGF-I.

#### *3. Type I and II IGF receptors*

IGFs interact with specific cell surface receptors, designated type I and type II IGF receptors, and can also interact with the insulin receptor (IR). The type I IGF receptor (IGF-IR) is a transmembrane heterotetramer consisting of two $\alpha$-subunits and two $\beta$-subunits. There is approximately 60% sequence homology between IGF-IR and IR (5). IGF-IR, like IR, possesses intrinsic tyrosine kinase activity. IGF-II and insulin also bind to IGF-IR but with 2- to 15-fold and 1000-fold lower affinity, respectively (5). The type II IGF receptor (IGF-IIR), which is identical to the cation-independent mannose-6-phosphate receptor, binds IGF-II with 500-fold increased affinity over IGF-I (6). IGF-IIR does not bind insulin. Most of the biological actions of IGF-II are thought to be mediated via IGF-IR (6). IGF-IIR is known to function primarily as a scavenger receptor, regulating the internalization and degradation of extracellular IGF-II, thus regulating the circulating IGF-II levels. IR does not bind IGF-I and IGF-II with high affinity. However, the IR isoform and the IR-IGF-IR hybrid bind IGFs as well as insulin. IR-A, the IR fetal isoform, binds not only insulin but also IGF-II. Secondly, the insulin-IGF-I hybrid receptors containing an insulin half receptor and IGF-I half receptor have broad binding specificity because they bind IGF-I and also IGF-II and insulin. These IR isoform and hybrid receptors mediate certain actions of IGFs and insulin (7).

#### *4. The IGFBPs*

A total of six high-affinity binding proteins have been identified: IGFBP-1 through IGFBP-6 (8). Hepatic IGF-I circulates almost entirely (>99%) bound to IGFBPs. IGFBP-3, a major IGFBP species in circulation, binds 75 to 90% of circulating IGF-I in a large ternary complex consisting of IGFBP-3, acid-labile subunit (ALS), and IGF. It has been postulated that circulating IGFBP-3 originates in the liver and is regulated by GH based upon the presence of a putative GH-response element in IGFBP-3 gene (9, 10). However, recent findings demonstrated that GH administration had no effect on the expression of hepatic IGFBP-3 mRNA but increased circulating IGFBP-3 in human subjects, due to increased formation of the ternary complex (11). ALS is produced in the liver as a direct effect of GH. The ALS stabilizes the IGF–IGFBP-3 complex, reduces the passage of IGF-I to the extravascular compartment, and extends its half-life (12). It has become clear that IGFBPs 1–6 have intrinsic biological activity (IGF/IGF-IR-independent actions) in addition to their actions to bind IGFs and sequester the active hormone, thereby reducing IGF biological activity (IGF/IGF-IR-dependent actions) (8, 13–17). This IGF/IGF-IR-independent action, in particular IGFBP-3's action, will be discussed in detail in *Section III*.

#### *5. IGFBP proteases*

The IGF/IGFBP complex can be dissociated by proteases that cleave IGFBP-3. To date, several proteases for IGFBP-3 have been described in a variety of cell culture systems. These include serine proteases, cathepsins, and matrix metalloproteinases (MMPs) (18). IGFBP-degrading proteases favor the release of IGF, which then becomes available for biological actions. In addition, certain free IGFBPs can also be acted upon by proteases, resulting in loss of high affinity for IGFs.

## II. Structure of IGFBP-3

IGFBP-3 is a multifunctional protein that is found to play a variety of roles in circulation, in the extracellular environment, and inside the cell. Mature deglycosylated human IGFBP-3 has a molecular mass of 28.7 kDa and is comprised of 264 amino acids. The primary structures of mammalian IGFBPs appear to contain three distinct domains of roughly equivalent sizes, with additional critical subdomains or functional motifs within each major domain that contribute to their diverse actions (Fig. 1). In addition to the structural and sequence homology among IGFBPs, some IGFBPs possess distinctive characteristics such as integrin recognition sequences in IGFBP-1 and IGFBP-2; phosphorylation on serine residues of IGFBP-1 and IGFBP-3; heparin binding motifs in IGFBP-3, -5, and -6; and nuclear localization sequences in IGFBP-3 and IGFBP-5. These distinctive characteristics could be important for the ability of IGFBPs to modulate IGF/IGF-IR-dependent actions as well as IGF/IGF-IR-independent actions (8).

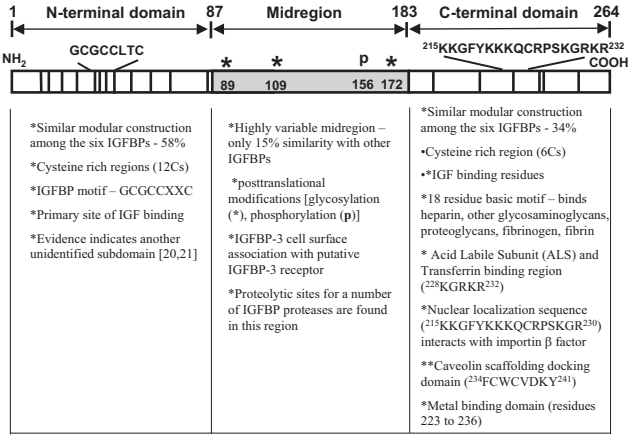


**FIG. 1.** Structure of human IGFBP-3. This figure depicts the three distinct domains of the IGFBP-3 molecule and lists the important functions and motifs of each of these key regions. The *vertical lines* represent cysteine residues.

### A. The conserved N-terminal domain

In the mature IGFBP-3 peptide, the N-terminal third of the protein contains 87 amino acid residues after the signal peptide. IGFBP-3 along with all the other members of the IGFBP superfamily (both high affinity and low affinity IGF binders) share a common IGFBP motif —GCGC-CXXC—a conserved N-terminal cysteine rich domain. IGFBP-3 contains a total of 18 cysteines, 12 of which are located in this domain, which leads to the presence of six disulfide bonds within this domain. Important IGF-binding residues are known to be located within this domain (19, 20). Although no other major functional motifs have been identified in the amino-terminal domain, the observation that amino-terminal proteolytic fragments of IGFBP-3 cause IGF-independent inhibition of mitogenesis (21, 22) implies the presence of another active subdomain in this region. This subdomain remains to be identified.

### B. The highly variable midregion

The midregion segment of IGFBP-3 contains 95 amino acids. This region separates the N-terminal domain from the C-terminal domain and shares less than 15% similarity with other IGFBPs. Intriguingly, posttranslational modifications (glycosylation, phosphorylation) of the IGFBPs have been found in the midregion, but not in the N- or C-terminal domains. IGFBPs might be differentially targeted to tissues depending upon both their primary structure and their posttranslational modifications. Thus, glycosylation can affect cell interactions, phosphorylation can affect IGF-binding affinity and susceptibility to proteases, and proteolysis can affect both IGF/IGF-IR-dependent and IGF/IGF-IR-independent actions (23). Three sites of N-linked glycosylation exist in IGFBP-3 (24). Carbohydrate increases the core protein size of IGFBP-3 from 29 kDa to forms estimated to be 40–43 kDa. Of the three potential glycosylation sites at $Asn^{89}$, $Asn^{109}$, and $Asn^{172}$, the first two are always glycosylated, carrying an estimated 4 kDa and 4.5 kDa of carbohydrate, respectively, whereas the third site alternatively contains either undetectable or about 5 kDa of carbohydrate, accounting for the characteristic doublet form of the protein (24). Other sites of posttranslational modification such as potential phosphoacceptor sites also reside in this central domain. Studies conducted by Yamanaka *et al.* (25) suggest that IGFBP-3 binds to human breast cancer cell surface with typical receptor-ligand interaction and the midregion of the IGFBP-3 molecule is responsible for the interaction.

### C. The conserved C-terminal domain

The C-terminal domain is cysteine rich (six cysteines), with three disulfide bonds within this domain. This IGFBP-3 region is also important in IGF binding (26–28). Because residues involved in IGF binding are present in both the N-terminal and C-terminal domains, the findings imply the likely existence of an IGF-binding pocket involving both domains. A functionally important 18-residue basic motif with heparin-binding activity has also been identified at residues 215-232. Apart from heparin binding, certain other glycosaminoglycans (29) as well as cell surface proteoglycans (30) are bound by this 18-residue basic motif. Furthermore, IGFBP-3 as well as IGFBP-3-IGF-I complexes bind fibrinogen, fibrin (31), and plasminogen (32) via this binding domain. The basic region Lys 228-Arg 232 (14) has also been shown to be essential for interactions with the ALS. Additional basic residues that reside within this domain interact with the cell surface and matrix, the nuclear transporter importin-$\beta$ and other proteins (discussed in *Section V*). Singh *et al.* (33) have also identified a short metal-binding domain in the C-terminal region of IGFBP-3 that binds metals and also has intrinsic effects. Although *in vitro* studies do not suggest a potential role for iron in IGF-independent biological actions of IGFBP-3, it reveals a dose-dependent effect of iron on IGFBP-3 binding to integrins$_{\alpha v}$ and $\beta_1$, caveolin-1, and transferrin receptor by unmasking of metal-binding domain epitopes in the IGFBP-3 molecule (33). IGFBP-3 binding to transferrin has been shown to be dependent on a region in the C-terminal domain (34). Furthermore, a caveolin-scaffolding domain consensus sequence also resides in this region.

Recent studies have determined three-dimensional structures of IGFBPs including IGFBP-1, -2, -4, and -5 using nuclear magnetic resonance spectroscopy and x-ray crystallography (35–39). These structural analyses not only confirm the previous findings indicating IGF binding sites in the N- and C-terminal domains of IGFBPs but also further reveal that a rigid disulfide bond ladder-like structure and the first five amino acids in the N-terminal domain are critical for IGF binding and masking IGF residues

responsible for IGF-I receptor binding. The C-terminal domain and the midregion of IGFBPs also contribute to inhibiting the interaction between IGF-I and IGF-IR either by directly blocking the IGF-IR-binding region of IGF-I or by steric hindrance. Thus, these structural data provide an understanding of the roles of each domain of IGFBP in enhancing/stabilizing IGF binding and modulating IGF binding to IGF-IR. Although the structure of IGFBP-3 has not yet been determined, IGFBP-3 should have very similar structures in those critical domains responsible for cooperative binding to IGF and blocking of the IGF-IR-binding region of IGF-I.

## III. IGF/IGF-IR-Dependent and IGF/IGF-IR-Independent Actions of IGFBP-3

IGF binding to and subsequent activation of the IGF-IR usually results in diverse biological effects in a wide range of cell types, including cellular proliferation and differentiation, an increase in metabolic activity, and cell survival via antiapoptotic pathways. The IGFBPs are known to modulate the actions of IGFs in the circulation as well as the immediate extracellular environment (40). Interestingly, apart from the ability of IGFBPs to inhibit or enhance IGF actions, IGFBPs also exhibit very clear, distinct biological effects independent of the IGF/IGF-IR axis (Fig. 2). These IGF/IGF-IR-independent actions contribute to the diversity of biological outcomes due to IGFBPs. IGFBP-3 is a well-documented inhibitor of cell growth and/or promoter of apoptosis. Although its antiproliferative functions are truly achieved through the attenuation of IGF/IGF-IR interaction (41), to date much light has been shed on the ability of these bioactivities to occur via an IGF/IGF-IR axis-independent means (42–46).

The data for IGFBP-3 are the very first to show clear evidence in support of the IGF-independent actions of IGFBPs. The protein inhibitory diffusible factor 45 (now recognized to be IGFBP-3) was originally isolated as a novel inhibitory factor, being able to inhibit the stimulation of chick embryo fibroblasts (CEF) in the absence as well as the presence of IGF-I, yet when bound to IGF-I, serum-induced growth stimulation of CEF was attainable, suggesting two opposing activities for inhibitory diffusible factor 45 (47). This study was quickly followed by another supporting this hypothesis, where fibroblast growth factor-stimulated DNA synthesis in CEF and mouse embryo fibroblasts was inhibited by mouse IGFBP-3—an action independent of IGFs yet attenuated by the presence of IGFs (48). Similarly, the overexpression of human IGFBP-3 in Balb/c mouse fibroblasts resulted in the inhibition of cellular proliferation in the presence/absence of IGFs or insulin (49). Although it is clear that IGFBP-3 achieves biological effects independent of the IGF/IGF-IR axis, the mechanisms by which these effects are achieved are still not entirely understood. Evidence to date suggests the existence of multiple pathways by which IGFBP-3 elicits its proapoptotic and antiproliferative IGF/IGF-IR-independent effects in an array of different cell systems.

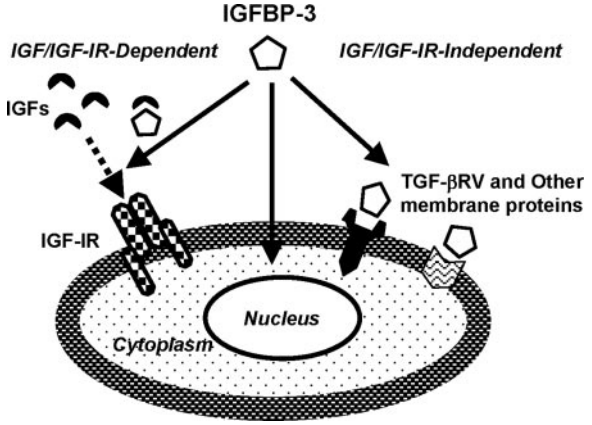


**FIG. 2.** IGF/IGF-IR-dependent and IGF/IGF-IR-independent actions of IGFBP-3.

On the other hand, IGFBP-3 also has been shown to stimulate cell growth or other cell functions in an IGF-independent manner in a variety of cell types. Martin *et al.* (50) demonstrated that IGFBP-3 stimulates growth in MCF-10A human breast epithelial cells via increased epidermal growth factor receptor phosphorylation and activation of p44/42 and p38 MAPK signaling pathways. Similar effect of IGFBP-3 was observed in breast cancer cells. Although expression of IGFBP-3 initially inhibited the growth of T47D human breast cancer cells, long-term culture of these cells resulted in growth stimulation due to an enhanced responsiveness of these cells to the proliferative effects of epidermal growth factor (51). Granata *et al.* (52) reported that IGFBP-3 exerts dual effects on human umbilical vein endothelial cells, potentiating doxorubicin-induced apoptosis but enhancing survival in serum-starved conditions. This study further demonstrated that IGFBP-3 antiapoptotic effects were mediated through activation of sphingosine kinase and increased expression of sphingosine kinase 1. Furthermore, it has been reported that IGFBP-3 has proangiogenic effects on endothelial precursor cells inducing the migration, tube formation, and differentiation of these cells into endothelial cells, thereby promoting proper revascularization and repair after ischemic injury (53, 54). These studies indicate that IGFBP-3 enhances cell growth or other cell functions depending on specific conditions. However, the underlying molecular mechanisms involved in these biological actions of IGFBP-3 are largely unknown.

The remainder of this review will focus on the role of IGFBP-3 in human diseases—mainly through its proapop-

totic and antiproliferative IGF/IGF-IR-independent actions. Our aim is to clearly present the evidence to date regarding the IGF/IGF-IR-independent actions and to further characterize the different mechanisms of action of IGFBP-3 in various human diseases.

## IV. Cell Surface Association of IGFBP-3 with Putative Receptors and Downstream Actions

### A. Interaction with IGFBP-3 cognate receptors

The very first evidence for the IGF/IGF-IR-independent actions of IGFBP-3 in human cells was demonstrated using breast cancer cells. Oh *et al.* (42, 55) initially demonstrated cell surface binding between IGFBP-3 and cell surface proteins in Hs578T and MDA-MB-231 human breast cancer cells. An evaluation of IGFBP's binding sites on breast cancer cell membranes by competitive binding studies with IGFBP-1 through -6 and various forms of IGFBP-3 including synthetic IGFBP-3 fragments revealed the existence of high-affinity binding sites for IGFBP-3, typical of receptor-ligand interactions. Further analysis revealed that IGFBP-3 binding was specific and not attributed to nonspecific interaction with glycosaminoglycans.

Recent evidence suggests an interaction between IGFBP-3 and the TGF-$\beta$ signaling pathways as well as an interaction with a TGF-$\beta$ receptor. TGF-$\beta$ is a multifunctional growth factor secreted by many types of cells. TGF-$\beta$ signaling from the cell surface to the nucleus requires a series of interdependent events. It is initiated by the association between TGF-$\beta$ and the type II TGF-$\beta$ receptor (TGF-$\beta$RII), resulting in the recruitment of the type I TGF-$\beta$ receptor (TGF-$\beta$RI) into a heteromeric complex, which allows TGF-$\beta$RII to phosphorylate and activate TGF-$\beta$RI (56). Signaling intermediates Smad2 and 3 are phosphorylated by active TGF-$\beta$RI followed by their association with Smad 4 and the translocation of heteromeric Smad complexes to the nucleus where they can potentially regulate the transcription of target genes either through binding to elements in the DNA or indirectly by binding to other transcription factors (57, 58). Fanayan *et al.* (59) demonstrated that IGFBP-3 inhibitory signals require an active TGF-$\beta$ signaling pathway and implicate Smad 2 and 3 in IGFBP-3 signal transduction. On the other hand, IGFBP-3 has been proposed as a functional ligand for the serine/threonine kinase type V TGF-$\beta$ receptor (TGF-$\beta$RV) and interaction of IGFBP-3 with TGF-$\beta$RV causes cell growth inhibition (44, 60).

The cell signaling pathways activated by IGFBP-3/TGF-$\beta$RV interactions are distinct from those TGF-$\beta$RI/TGF-$\beta$RII signaling cascades outlined above because the key signaling proteins, Smad 2 and 3, are not phosphorylated by IGFBP-3. To date, the cell signaling pathways for IGFBP-3/TGF-$\beta$RV remain to be elucidated. Recent structural and functional analyses of purified TGF-$\beta$RV have revealed that TGF-$\beta$RV is identical to the low-density lipoprotein receptor-related protein-1/activated $\alpha_2$M receptor, which is known as an endocytic receptor and has been shown to mediate the biological functions of the ligands, TGF-$\beta$1 and IGFBP-3 (61).

### B. Induction of apoptosis

One of the ways in which IGFBP-3 can exert its actions on target cells is by inducing apoptosis or programmed cell death. This has been demonstrated by studies showing that IGFBP-3 increases the ratio of proapoptotic (Bax and Bad) to antiapoptotic (Bcl-2 and Bcl-$X_L$) proteins in apoptotic breast cancer cells (62). In paclitaxel-treated Hs578T breast cancer cells, caspase-3 activity and IGFBP-3 levels in conditioned media are increased (63). Although it is well documented that apoptosis is promoted by IGFBP-3, to date the underlying mechanism of action by which it achieves these effects remains elusive.

Apoptosis can be triggered by internal signals (the intrinsic or mitochondrial pathway) or external signals (the extrinsic or death receptor pathway) (64, 65). The initial caspase in the mitochondrial pathway is caspase-9, which subsequently activates the executioner caspase-3, -6, and -7. In contrast, the initial caspase of the death receptor pathway is caspase-8. Caspase-8 (like caspase-9) further activates the cascade of executioner caspases (-3, -6, and -7). Having obtained data to support the existence of a putative receptor for IGFBP-3, through which it can achieve its IGF-independent actions, Oh and colleagues (66) went on to show through a series of caspase activity studies (using specific caspase substrates and/or caspase inhibitors) that the growth inhibitory effect of IGFBP-3 results mainly from its induction of apoptosis via activation of caspase-8 and -7. Further analyses of caspase-9 and measurement of release of cytochrome c into the cytosol confirmed that the mitochondria-mediated pathway is not involved. These findings suggest that IGFBP-3 induces apoptosis through the activation of caspases, via a death receptor-mediated pathway. Hence, IGFBP-3 could interact with the known death receptors such as TNF-$\alpha$ receptor or a unique yet unidentified death receptor (Fig. 2). On the other hand, recent studies also demonstrated that IGFBP-3 induces apoptosis through the mitochondrial pathway (for details, see *Section V*).

In addition, other studies indicate that IGFBP-3 is essential for TNF-$\alpha$-induced apoptosis and show that this IGFBP-3 effect includes the inactivation of Bcl-2 through serine phosphorylation (67). However, the exact mechanism for IGFBP-3 involvement on the TNF-$\alpha$ signaling pathways is largely unknown.

### C. Inhibition of NF-κB

Nuclear factor κB (NF-κB) represents a family of inducible transcription factors found ubiquitously in all cells. Unlike most cells where the basal level of active NF-κB is minimal, many cancers express elevated levels of active NF-κB (68, 69). Much evidence exists in the literature to support the presence of constitutively active NF-κB in a variety of different cancers, including prostate cancer (68, 69). Active NF-κB complexes are dimers of various combinations of the Rel family of polypeptides, the major NF-κB dimer in most cell types being the p65 (Rel A)/p50 (NF-κB1) heterodimer. In most resting cells, NF-κB is retained in the cytoplasm by binding to the inhibitory protein IκBα, which blocks the nuclear localization sequences of NF-κB (70). NF-κB is activated in response to a wide variety of stimuli that promote the dissociation of IκBα through phosphorylation, ubiquitination, and degradation, thus unmasking the nuclear localization sequence of NF-κB and allowing NF-κB to enter the nucleus and bind κB-regulatory elements (71). The phosphorylation of IκBα is a critical step in the pathway leading to NF-κB activation, which is catalyzed by the IκBα kinase (IKK) complex (consisting of IKK-α, IKK-β, and IKK-γ/NEMO) (72). Active NF-κB is commonly known to participate at multiple steps in the pathways associated with cancer, two of which are to block apoptosis and promote cell proliferation (73, 74).

With respect to potential interaction between IGFBPs and NF-κB signaling cascade, Butt *et al.* (75) reported that IGFBP-5, but not IGFBP-3, sensitized MDA-MB-231 breast cancer cells to the inhibitory effects of TNF-α through blockage of TNF-α-induced nuclear translocation of Rel A. On the other hand, H-Zadeh *et al.* (76) reported that IGFBP-3 inhibits TNF-α-induced NF-κB activity in human colonic carcinoma cells. In this report, they further demonstrated that the secreted 16-kDa 1-95:IGFBP-3 fragment is as effective as the intact IGFBP-3 protein at potentiating apoptosis as well as inhibiting NF-κB activity. Williams *et al.* (77) also demonstrated that IGFBP-3 significantly enhances TNF-related apoptosis-inducing ligand-induced cell death in colonic carcinoma-derived cell lines by inhibiting NF-κB activation in response to the induction of apoptosis by TNF-related apoptosis-inducing ligand. Thus, in terms of unraveling the IGF/IGF-IR-independent actions of IGFBP-3, there is now evidence to show that IGFBP-3 interferes with NF-κB signaling cascades. However, the underlying mechanisms of action by which IGFBP-3 achieves inhibition of NF-κB signaling cascades are yet to be elucidated. In addition, because a persistent constitutive nuclear activation of NF-κB has been shown to induce resistance to various chemotherapeutic agents and radiotherapy (78–81), IGFBP-3 may have therapeutic potential for treatment of chemodrug- and radiotherapy-resistant cancer associated with activated NF-κB.

### D. Other evidence for cell surface association

Mishra and Murphy (82) have used cross-linking strategies to show that IGFBP-3 bound to multiple, unknown proteins on the cell membrane of a different breast cancer cell line T47D. Their studies further suggest that binding of IGFBP-3 to breast cancer membranes is accompanied by phosphorylation at the plasma membrane and that both processes are inhibited by IGF-I. However, once phosphorylated, the ability of IGFBP-3 to bind to IGF-I is enhanced, resulting in increased association of IGF-I with the cell membrane. Mishra and Murphy (83) have also provided evidence that tissue transglutaminase has intrinsic kinase activity and that it is a major component of the IGFBP-3 kinase activity present on breast cancer cell membranes. Further evidence for IGFBP-3 association with cell membrane comes from the identification of an autocrine motility factor (AMF)/phosphoglucose isomerase (PGI)/IGFBP-3 complex from cross-linking experiments of biotinylated IGFBP-3 to breast cancer cell membranes (84). AMF/PGI, apart from its enzymatic function, is an antiapoptotic cytokine that stimulates proliferation and migration of a variety of cells in an autocrine fashion. Current findings suggest that IGF/IGF-IR-independent actions of IGFBP-3 are mediated, at least in part, through the interaction with a variety of cell surface proteins, including the known receptors such as TGF-βRV and other cell surface proteins such as AMF/PGI.

## V. Nuclear Association and Actions of IGFBP-3

Apart from evidence supporting the IGF/IGF-IR axis-independent signaling from several potential cell surface receptors, there exists evidence to suggest that IGFBP-3 can translocate into the nucleus from the extracellular compartment in rapidly dividing human breast cancer cells even if bound to IGFs (85). Nuclear localization of IGFBP-3 is a well-described phenomenon that has been demonstrated in a wide variety of cellular models (45, 86–88). IGFBP-3 possesses a consensus bipartite nuclear localization sequence (89), and nuclear transport is facilitated by importin-β factor (46). Recent evidence shows that the nuclear retinoid X receptor (RXR)-α is a binding partner of IGFBP-3 (45, 90). RXRs belong to the nuclear receptor superfamily, consisting of a large number of ligand-regulated transcription factors that mediate the diverse physiological functions of their ligands, such as ste-

roid hormones, retinoids, thyroid hormone, and vitamin $D_3$, in embryonic development, growth, differentiation, apoptosis, and homeostasis (91, 92). In addition, RXRs form heterodimers with many members of subfamily 1 of nuclear receptors, including the orphan receptor Nur77 (91, 92). Heterodimerization of RXR with its partners dramatically enhances their DNA binding and subsequent transcriptional regulation. Nur77 is a nuclear receptor transcription factor and is an important regulator of apoptosis in different cells (93). In response to synthetic apoptotic stimuli, Nur77 translocates from the nucleus to the mitochondria to induce cytochrome c release and apoptosis in leukemia (94) and lung (95), ovary (96), stomach (97), colon (98), and prostate cancer cells (99). The subcellular localization of Nur77 is important in determining its biological function. Nur77 functions as a transcription factor in the nucleus whereby it mediates cell proliferation events. Nur77 also heterodimerizes with RXR$\alpha$ and participates in its transcriptional activities (100). However, Nur77 functions as a mediator of apoptosis when targeted to the mitochondria. Studies by Lee *et al.* (101) show that in response to IGFBP-3, Nur77 rapidly undergoes translocation from the nucleus to the mitochondria, initiating an apoptotic cascade resulting in caspase activation (Fig. 3). Furthermore, IGFBP-3 and Nur77 possess additive effects in inducing apoptosis, and RXR$\alpha$ is required in this process (101). Because IGFBP-3 is a binding partner of RXR$\alpha$, Lee *et al.* (101) proposed that IGFBP-3 modifies the RXR$\alpha$/Nur77 heterodimeric DNA binding complex, thus shifting the heterodimer from a DNA-binding state to one that targets the mitochondria and induces apoptosis. More recently, Lee *et al.* (102) demonstrated that IGFBP-3 and Nur77 associate in the cytoplasmic compartment in 22RV1 prostate cancer cells, and dose-response experiments further revealed that a small component of IGFBP-3-induced apoptosis is Nur77-independent.

Further work by Cohen and colleagues (103) showed that phosphorylation of IGFBP-3 at S156 by DNA-dependent protein kinase (DNA-PK) is a critical step in the growth-inhibitory and apoptosis-promoting actions of IGFBP-3 in prostate cancer cells. Their results revealed that DNA-PK-mediated phosphorylation enhanced the nuclear accumulation of IGFBP-3 and is also critical for interactions with its nuclear binding partner RXR$\alpha$ (103). It was also demonstrated that nuclear translocation of IGFBP-3 requires IGFBP-3 secretion and reuptake. The authors further found the involvement of distinct endocytic pathways for IGFBP-3 internalization via binding to transferrin and caveolin (104). It should be noted that another study showed that when a mutant form of IGFBP-3 failed to translocate to the nucleus, IGFBP-3 can still induce apoptosis in breast cancer cells, suggesting either that RXR-IGFBP-3 interaction between these proteins may not be required for apoptosis or possibly that the interaction between these proteins in the cytoplasm may be sufficient (105). Recent studies from Bhattacharyya and Rechler's groups (106, 107) further confirmed that IGFBP-3 is capable of inducing apoptosis without being secreted and concentrated in the nucleus or binding RXR$\alpha$ in human prostate cancer cells. These findings indicate that IGFBP-3 is a potent antiproliferative and proapoptotic factor in cancer cells whose biological action is mediated through the interaction with a variety of binding partners on the cell surface and within the cells.

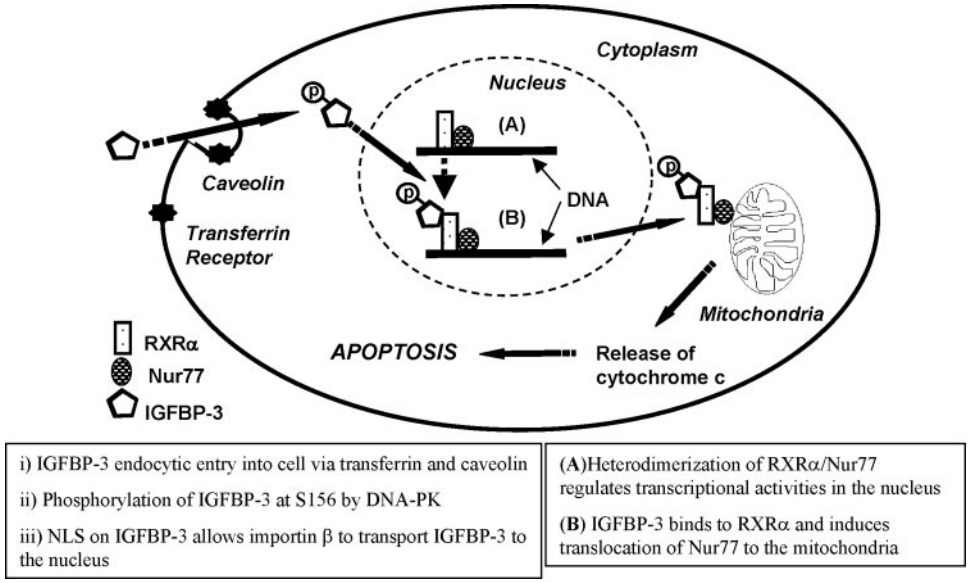


**FIG. 3.** Nuclear localization of IGFBP-3. In response to IGFBP-3, Nur77 rapidly translocates from the nucleus to the mitochondria and induces apoptosis.

## VI. Factors That Influence the Induction or Increased Expression of IGFBP-3

GH, the main regulator of IGF-I, acts by inducing hepatic as well as local tissue production of IGF-I and also stimulates IGFBP-3 directly or indirectly through enhancing IGF-I production (108, 109). IGFBP-3, by binding to IGF-I, prolongs its half-life and also plays a crucial role in modulating human growth. IGFBP-3 concentrations in the circulation are usually low in individuals with GH deficiency (110, 111) and in contrast, IGFBP-3 is increased in cases of acromegaly and GH excess (112). Insulin is another important hormone in IGFBP-3 up-regulation (108). Furthermore, IGFBP-3 can be induced by many cell cycle regulators and growth-inhibitory (and apoptosis-inducing) agents such as TGF-$\beta$1 (113–115), retinoic acid (114, 116, 117), TNF-$\alpha$ (116–118), vitamin D (119), antiestrogens such as tamoxifen (120), antiandrogens (121), and tumor suppressors.

The antiproliferative actions of vitamin D in prostate cancer cells, acting via its hormonally active form calcitriol, are in part due to its ability to stimulate IGFBP-3 expression (122, 123). High concentrations of androgens are also antiproliferative in LNCaP human prostate cancer cells in culture, and this effect is also mediated by induction of IGFBP-3 (124). Calcitriol and androgens both have been demonstrated to up-regulate IGFBP-3 expression directly through interaction with each cognate receptor, vitamin D receptor and androgen receptor, respectively. The receptor complex subsequently binds with the corresponding response elements (vitamin D receptor element or androgen receptor element) within the IGFBP-3 promoter in androgen-responsive LNCaP cells (122–125). The two response elements, separated by approximately 400 bp, appear to interact to regulate the level of IGFBP-3 expression. Calcitriol and androgens in combination not only result in a synergistic increase in IGFBP-3 mRNA and protein levels but also inhibit cell growth better than either hormone alone (126, 127). IGFBP-3 contributes to the antiproliferative actions of both calcitriol and high concentrations of androgens in part through stimulation of p21 and p27 pathways (122, 127). Androgens cause biphasic effects on the growth of the LNCaP human prostate cancer cells in culture, with low concentrations stimulating growth and high concentrations inhibiting growth (126, 128, 129). In a recent study, Peng *et al.* (127) showed that this concentration-dependent effect of androgens was in part the result of differential regulation of IGFBP-3 expression by androgens. Low concentrations of androgens failed to induce increased IGFBP-3 expression allowing LNCaP growth to be stimulated. Higher concentrations of androgens stimulated IGFBP-3 expression and resulted in growth inhibition. Knock-down of IGFBP-3 with small interfering RNA prevented the growth inhibition, confirming a role for IGFBP-3 in the antiproliferative activity of high androgen concentrations in cultured LNCaP cells.

IGFBP-3 is one of the genes transcriptionally activated by the tumor suppressor gene p53 (130, 131). p53 normally resides in low concentrations in a latent form but is activated to induce cell cycle arrest or apoptosis and thus prevents the propagation of damaged cells. Studies conducted by Grimberg *et al.* (132) show that IGFBP-3 is induced by physiological conditions that also induce p53, although p53 is not always required. They demonstrated that genotoxic drugs such as etoposide and adriamycin increased IGFBP-3 protein levels and secretion in tumor cell lines in a p53-independent manner. Evidence also supports induction by IGFBP-3 of p53-independent apoptosis in a prostate cancer cell line (42). Another tumor suppressor gene, PTEN, up-regulates IGFBP-3 at the transcriptional level via an Akt-dependent pathway in human gastric cancer cells (133). Additionally, 2R4'R8'R-$\alpha$-vitamin E succinate exerts its antiproliferative and proapoptotic effects via up-regulation of IGFBP-3 in prostate cancer cells (134). It is clear that IGFBP-3 could mediate a variety of biological effects of many hormones and peptides, particularly antiproliferative and proapoptotic effects of tumor suppressors.

## VII. Factors That Influence the Suppression or Decreased Expression of IGFBP-3

IGFBP-3 can be down-regulated at multiple levels, such as transcriptional, posttranscriptional, and posttranslational levels. DNA methylation and histone modifications are common mechanisms for epigenetic regulation of gene expression (135). These epigenetic modifications play an important role in the pathophysiology of cancer as well as other human disease by silencing specific genes, including tumor suppressor genes such as p53 (136–138). DNA hypermethylation and histone deacetylation of CpG islands within the promoter regions of tumor suppressor genes result in undesirable gene silencing and are found in virtually every type of human cancer (139, 140). Recent studies have demonstrated that DNA methyltransferase as well as histone deacetylase inhibitors can reactivate epigenetically silenced tumor suppressor genes and decrease tumor cell growth *in vitro* and *in vivo* (141, 142).

Aberrant DNA methylation and histone acetylation have been shown to play an important role in the silencing of IGFBP-3 expression in several human cancers, including gastric cancer (143), colorectal cancer (143), breast cancer (143), malignant mesothelioma (143), ovarian cancer (144), renal cancer (145), and hepatocellular carcinomas (146). Chang *et al.* (147, 148) reported that IGFBP-3

is frequently methylated in lung cancer and its methylation status is significantly associated with poor prognosis in stage I non-small-cell lung cancer. Yamashita *et al.* (149) showed that IGFBP-3 expression is significantly up-regulated by 5-Aza-CdR treatment in gastric cancer cell lines. This provides further supporting evidence to suggest that IGFBP-3 expression is regulated by DNA methylation in human cancer. A recent study identified a response element for the methylation-dependent transcription factor in close proximity to the IGFBP-3 promoter that participates to insulin-stimulated transcriptional activity of the IGFBP-3 promoter in liver cells (150). These findings strongly suggest that methylation status of IGFBP-3 also affects its responsiveness to insulin. Furthermore, it has been shown that histone deacetylase inhibitors, sodium butyrate, trichostatin A, and valproic acid also induce increased synthesis and secretion of IGFBP-3 in a variety of human cancer cells including hepatocellular carcinoma, breast, and prostate cancer cells (151–154). The current findings suggest that these epigenetic modifications of the IGFBP-3 gene may play an important role not only in cancer development, progression, and prognosis but also in the pathology of many other diseases.

A variety of transcription factors are involved in repression of the IGFBP-3 transcription. CDX2, a *Drosophila* caudal-related homeobox transcription factor has been reported to promote tumorigenicity in a subset of human colorectal cancer cell lines (155). CDX2 also negatively regulates IGFBP-3 by specifically binding to the IGFBP-3 gene promoter and repressing IGFBP-3 transcription, protein expression, and secretion (155). Interestingly, CDX2 has recently been identified as a transcriptional regulator essential for trophoblast differentiation (156). Taken together with findings that IGFBP-3 is the only IGFBP produced by trophoblasts and that these cells produce a neutral IGFBP-3 protease, CDX2 might play a critical role in the production and biological actions of IGFBP-3 in placenta (157).

An Ewing's sarcoma fusion protein, EWS/FLI1, binds the IGFBP-3 promoter *in vitro* and *in vivo* and can repress the activity of IGFBP-3 (158, 159). Ewing's sarcoma is a rare disease in which cancer cells are found in the bone or in the soft tissue. It is the result of translocation between chromosomes 11 and 22, which fuses the EWS gene of chromosome 22 to the FLI1 gene of chromosome 11. When the EWS/FLI1 fusion gene in Ewing's cells is inactivated by RNA interference, the loss of expression of EWS/FLI1 results in complete growth arrest of the Ewing's sarcoma by induction of apoptosis. Gene profiling of these Ewing's cells identified IGFBP-3 as a crucial downstream target of the fusion gene (158). These findings provide evidence that identifies the repression of IGFBP-3 as a key event in the development of Ewing's sarcoma. Further study confirmed that IGFBP-3 inhibits the growth, migration, invasion, as well as angiogenic and metastatic potential of Ewing's cells (159). These data thus indicate that IGFBP-3 may hold therapeutic promise for the treatment of patients with Ewing's sarcoma.

IGFBP proteases also modulate the actions of IGFBPs, especially IGFBP-3. Prostate-specific antigen in seminal plasma was the first IGFBP protease to be identified (160). Prostate-specific antigen fragmented IGFBP-3s have lower affinity for IGFs and less inhibitory effects on cell growth (161). Other kallikrein-like serine proteases that cleave IGFBP-3 include γ-nerve growth factor (162) and plasmin (163). More recently, studies indicate that human kallikrein 11 expressed in estrogen receptor (+) breast cancer cells may play a crucial role in breast cancer progression by increasing the bioavailability of IGFs via degradation of IGFBP-3 (164). Inflammation-related proteases (cathepsin D and elastase) also play a key role in IGFBP-3 proteolysis. Research shows that these two proteases cleave IGFBP-3 *in vitro* and *in vivo* in a concentration-dependent manner (165). The aspartic protease cathepsin D is also reported to be involved in the proteolytic processing of IGFBP-3 (166, 167). More recent evidence also supports the involvement of the cysteine protease cathepsin L in the intracellular degradation of IGFBP-3 (168).

MMPs are a key family of proteolytic enzymes involved in tissue remodeling by degradation of extracellular matrix and basement membrane components. The activity of MMPs has been implicated in tumor cell invasion, morphogenesis, trophoblast invasion, cartilage and bone repair and turnover, wound healing, and angiogenesis (169–173). In addition to this general role of MMPs, MMP-7, which is exclusively synthesized by cancer cells, has been implicated in the activation of growth factors and cytokines by degrading their precursors or inhibitors, thereby allowing the cancer cells to control the tissue microenvironment (174–176). Recent evidence by Miyamoto *et al.* (177) indicates that MMP-7 produced by various cancer cells also catalyzes the proteolysis of IGFBP-3 *in vitro*, thereby regulating IGF-I bioavailability. Mochizuki *et al.* (178) showed that MMP-7 first activates proADAM28 and further that ADAM28 digests IGFBP-3. ADAM28, a member of a disintegrin and metalloproteinase (ADAM) family, has two isoforms, membrane-type form (ADAM28m) and secreted form (ADAM28 s). Although ADAM28 is expressed and synthesized in a precursor form (proADAM28) by lymphocytes and some cancer cells, its activation mechanism and substrates remain unclear. The authors reported that proADAM28 s of 65 kDa is processed with active MMP-7 to 42- and 40-kDa forms, which corresponds to active ADAM28 s without

propeptide. Processed ADAM28 s digested IGFBP-3 in both free and complex forms with IGF-I or IGF-II, and the digestion was prevented by EDTA, 1,10-phenanthroline, KB-R7785, and tissue inhibitor of metalloproteinase-3 and -4 (178). More recently, Mitsui *et al.* (179) found overexpression of ADAM28 in human breast carcinoma cells. They found that the ADAM28 was expressed in the active form and the levels of expression correlated with the proliferation rate of the cells. They suggested that ADAM28 was involved in stimulating breast cancer cell proliferation by enhancing the bioavailability of IGF-I by cleavage of IGFBP-3.

Grzmil *et al.* (180) demonstrated that a direct correlation exists between the inhibition of IGF-IR gene expression and either up-regulation of IGFBP-3 or down-regulation of MMP-2 expression in androgen-independent PC-3 prostate cancer cells. Unlike most other MMPs, MMP-19 is expressed in undifferentiated basal keratinocytes of healthy human skin. MMP-19 has also been shown to be associated with degradation of IGFBP-3, and Sadowski *et al.* (181) concluded that MMP19 could be the major IGFBP-3 degrading MMP in the quiescent epidermis. This activity might have widespread consequences for the behavior of epidermal keratinocytes.

Other evidence indicates that MMP-1 levels in asthmatic airway tissue extracts were 12-fold higher than those found in control samples (182). In addition, IGFBP-2 and IGFBP-3, which have previously been demonstrated to be proteolytic substrates of MMP-1, were found to be cleaved in asthmatic airway tissue extracts. Furthermore, the asthmatic airway extracts contained IGFBP proteolytic activity that was shown by immunodepletion studies to be due to MMP-1. These observations demonstrate that MMP-1 may play a significant role in inducing airway smooth muscle hyperplasia and airway obstruction in asthma by modulating the IGF-IGFBP-IGF-IR axis (182). MMP-2 and -3 have also been reported to be functional in the cleavage of IGFBP-3 (183). These findings strongly indicate that IGFBP-3 is regulated at multiple levels, including transcriptional regulation by epigenetic modification, activation of specific transcription factors, and post-translational regulation by proteolysis, thereby suggesting that IGFBP-3 status may be a critical factor for the pathogenesis of a variety of human disease.

## VIII. Other Important Binding Partners of IGFBP-3

Humanin is a 24-amino acid peptide that is important in specifically inhibiting neuronal cell death induced by familial Alzheimer's disease mutant genes and amyloid-$\beta$. Recent data have identified humanin as an IGFBP-3 binding partner. This interaction is pleiotrophic in nature, may prove to be important in neurological disease processes, and could provide important targets for drug development (184).

In addition, several IGFBP-3-interacting proteins have been discovered using the yeast two-hybrid assay. For example, IGFBP-3 can interact with RNA polymerase II binding subunit 3 (Rpb3). Rpb3 facilitates recruitment of the polymerase complex to specific transcription factors and is necessary for the transactivation of many genes. Its association with IGFBP-3 plays a functional role for IGFBP-3 in the direct modulation of gene transcription (185). Although some interacting proteins have been identified, the ones that are involved in IGFBP-3 signal transduction remain unclear. To understand the molecular mechanism by which IGFBP-3 induces apoptosis in an IGF/IGF-IR-independent manner, it is essential to study the cellular proteins that interact with IGFBP-3. Recently, Wu *et al.* (186) used the yeast two-hybrid assay to identify proteins that bind to IGFBP-3 from a human fetal liver cDNA library and showed that GalNAc-T14 binds IGFBP-3 *in vitro* and *in vivo*. Its role and that of other interacting proteins remains to be defined.

## IX. Clinical Significance of IGFBP-3 in Assessment of Cancer Risk

### A. Evidence from epidemiologic studies

Accumulating evidence of cancer risk from *in vitro* cell growth experiments, *in vivo* tumor models, and epidemiological studies all suggest that IGFBP-3 contributes to cancer risk protection (187). Unlike classic tumor suppressors, IGFBP-3 knockout mice do not develop spontaneous tumors, and there is no identified IGFBP-3-related familial cancer syndrome. Thus, IGFBP-3 more likely serves as one of the multiple, low-penetrance tumor susceptibility and resistance genes that determine cancer incidence and therapeutic responsiveness (188).

According to early epidemiological studies, higher IGF-I and lower IGFBP-3 levels were independently associated with a greater risk of common cancers, including prostate cancer (189, 190), colorectal cancer (191), lung cancer (192), and premenopausal breast cancer (193). Fewer positive associations were found among postmenopausal women (194–196).

Mammographic breast density is an important determinant of breast cancer risk. Studies have also shown that low IGFBP-3 levels are associated with increased premenopausal mammographic breast density (197). However, Becker and Kaaks (198) in a recent study did not find a consistent relationship between IGF-I or IGFBP-3 and either mammographic density or breast cancer risk.

However, more recent studies have not always confirmed these findings of an association between IGFBP-3 and reduced cancer risk (199–202). In the study by Severi *et al.* (201), IGFBP-3 was associated with increased risk of prostate cancer. Baglietto *et al.* (203) found increased breast cancer risk correlated with IGFBP-3 levels in older but not younger women. Allen *et al.* (200), in a large nested case-control study in the European Prospective Investigation into Cancer and Nutrition (EPIC), looked into prediagnostic serum concentrations of IGF-I and IGFBP-3 in 630 incident cases of prostate cancer and 630 matched controls. The IGF-I levels were compatible with a small increase in risk particularly for advanced-stage disease, but no association for IGFBP-3 was observed.

More recent breast cancer studies were similarly variable. Several systematic reviews and meta-analyses of both prospective and case-control studies have been published on the relationship between total IGF-I and IGFBP-3 concentrations in blood and breast cancer (204, 205). The findings indicated an overall increase in breast cancer risk in premenopausal women with increasing endogenous levels of total IGF-I. There was an increase in risk between 42 and 74% for women in the highest IGF-I category *vs.* women with IGF-I levels in the lowest category. No association was observed for postmenopausal women. For circulating IGFBP-3, epidemiological studies have shown heterogeneous relationships with the risk of any cancer, including breast cancer. These studies (204–206) suggested an overall increase in breast cancer risk with increasing IGFBP-3 levels in blood in premenopausal women, but results were less consistent than for IGF-I. No association between IGFBP-3 and breast cancer was observed for postmenopausal women.

Rinaldi *et al.* (207) in a large case-control study nested within the EPIC study included 1081 incident breast cancers and 2098 matched control subjects. No association between IGFBP-3 concentrations in blood and breast cancer risk in young women was observed. These results do not confirm the previous suggestion that total IGF-I and IGFBP-3 concentrations are mainly related to breast cancer in young women (204, 206). Some studies showed an increase in risk with increasing blood levels of IGFBP-3 (194, 195), whereas others reported an inverse association of IGFBP-3 concentrations with breast cancer risk (193, 200) and also with other cancers (189, 191).

Friedrich *et al.* (208) studied the IGF axis in nonmetastatic colorectal cancer. They found no association between IGF-I or IGFBP-3 on mortality, whereas higher C-peptide and lower IGFBP-1 levels were associated with increased mortality. Fuchs *et al.* (209) found that among colorectal cancer patients receiving first-line chemotherapy, increasing levels of IGFBP-3 were associated with an improved objective response and a prolonged time to progression. On the other hand, Wolpin *et al.* (210) found no association between IGF-I or IGFBP-3 levels and mortality in nonmetastatic colorectal cancer.

Two meta-analyses were recently published on the IGF axis and prostate cancer risk. Roddam *et al.* (211) examined the IGF axis and prostate cancer risk in 12 prospective studies including data on 3700 men with prostate cancer and 5200 matched controls. They found that increasing baseline levels of circulating IGFBP-3 were associated with a significantly longer time to tumor progression and an improved objective response to therapy. IGF-I levels were not found to be significantly associated with these outcomes. Rowlands *et al.* (212) examined data from 29 studies, both prospective and retrospective, involving 6541 men with prostate cancer. The data exhibited substantial heterogeneity. An inverse association between IGFBP-3 levels and prostate cancer risk was found in the retrospective but not the prospective studies. The analysis did find that IGF-I was positively associated with prostate cancer risk, but associations between IGFBP-3 and prostate cancer risk were inconsistent.

In studies of benign prostate hyperplasia risk, Neuhouser *et al.* (213) concluded that high IGF-I to IGFBP-3 ratio was associated with an increased risk. High IGFBP-3 levels were associated with a decreased risk of benign prostate hyperplasia in men with severe symptoms.

Looking at the overall risk of mortality, Saydah *et al.* (214) found no increased risk of the lowest *vs.* the highest quartile for IGF-I. However, the adjusted relative hazard of all-cause mortality for the lowest compared with the highest quartile of IGFBP-3 was 1.57, and the trend for risk was significant. However, there was no increased risk of either cancer or heart disease, so their results suggest that the association of IGFBP-3 with decreased mortality may differ from associations with the incidence of particular diseases. The increased mortality risk of reduced IGFBP-3 may not be due to increased cancer risk.

There are many possible reasons for the conflicting epidemiological data, including age, ethnicity and racial group differences, body adiposity and other metabolic superimposed risk factors, environmental factors, endogenous hormonal concentrations and hormonal therapy, polymorphisms in the critical genes (see below), and especially problems with the unreliability of assaying these proteins in frozen stored specimens (215). Rinaldi *et al.* (216), studying breast cancer risk in young women, showed that the strength of the association of IGFBP-3 with increased risk was dependent on the assay used. Berrigan *et al.* documented that there was variability in assay results that was dependent on the storage history of the samples (217). They showed that quality control, sam-

ple storage history, and other methodological concerns result in variation in both IGF-I and IGFBP-3 assay results, and they stressed the need for care in large studies of biomarkers for cancer risk highlighting this source of heterogeneity in past studies of the IGF axis and cancer risk. It has also been shown that different assays for IGFBP-3 measurements may lead to different conclusions on the relationship of this protein with cancer (216), and it has been speculated that different assays may measure different, more or less intact forms of IGFBP-3 present in blood (216).

### B. Genetic polymorphisms of IGFBP-3

The gene for IGFBP-3 is highly conserved among species and is present as a single copy on chromosome 7p14-p12. Several single nucleotide polymorphisms (SNPs) have been identified in the promoter region of IGFBP-3 by direct sequencing of genomic DNA. For the most commonly studied SNP at the nucleotide −202 locus (relative to the mRNA CAP site), the genotype was highly correlated to circulating level of IGFBP-3. Significantly higher promoter activity of the A allele (at the −202 locus) compared with the C allele was documented (218). The following relationship was observed between genotype and circulating IGFBP-3 concentrations: AA>AC>CC. Furthermore, a positive correlation was also observed between circulating retinol levels and circulating IGFBP-3 levels, and subset analysis by genotype showed that this relationship was only present among individuals carrying an A allele at −202 (218). Studies indicate that more than half of the interindividual variabilities in the level of circulating IGF-I and IGFBP-3 are genetically determined and that the polymorphic variations at the −202 site of the IGFBP-3 gene promoter mediate the level of circulating IGFBP-3 (219–221). Polymorphic variation of IGFBP-3 might thus influence cancer risk. However, conflicting results in this area are also found. Tamimi *et al.* (222) reported that genetic variation in IGF-I correlated with breast mammographic density, but IGFBP-3 variations did not. Cheng *et al.* (223) studied whether common genetic variations in IGF-I, IGFBP-1, and IGFBP-3 influence circulating levels of the proteins in a cross-sectional study of a multiethnic cohort. They found a correlation with five IGFBP-3 SNPs and circulating levels of IGFBP-3 across all five racial/ethnic groups. Thus, not only does the SNP at −202 in the IGFBP-3 promoter alter expression of the gene, but multiple genetic variations contribute to differences in the IGFBP-3 blood levels found.

In a study of polymorphisms in premenopausal breast cancer, Fletcher *et al.* (205) found that five of eight IGF-I studies and four of six IGFBP-3 studies of circulating levels showed that women in the highest category had more than twice the risk of developing breast cancer of those in the lowest, although in some this effect was only apparent at young ages. In postmenopausal women, however, there was no consistent effect. For the A/C polymorphism at the −202 locus in the IGFBP-3 promoter, all three studies were consistent with a modest effect on circulating levels of IGFBP-3, but no evidence of a direct effect on breast cancer risk was seen in the only relevant study. Fletcher *et al.* (205) concluded that variations within the reference range of IGF-I and IGFBP-3 may confer only modest increases in breast cancer risk, and any single polymorphism may only account for a small proportion of that variation. Patel *et al.* (224) studied common genetic variation in the IGF-I, IGFBP-1, and IGFBP-3 genes in relation to circulating IGF-I and IGFBP-3 levels and breast cancer risk within the National Cancer Institute Breast and Prostate Cancer Cohort Consortium (BPC3). They concluded, similar to Fletcher *et al.* (205), that the impact of genetic variation in igf1 and igfbp3 genes on circulating IGF levels does not appear to substantially influence breast cancer risk among primarily Caucasian postmenopausal women.

## X. Concluding Comment

IGFBP-3 may thus serve as an important factor in evaluating cancer risk, in prognosis and in the future, as a treatment. Although epidemiological studies vary in their conclusions about IGFBP-3 and reduced cancer risk, many population variables and assay problems may contribute to this lack of uniformity. However, extensive basic science experimentation indicates a strong benefit of IGFBP-3 for the reduction of carcinogenesis and cancer progression. It is possible that IGFBP-3 can be developed as a very effective therapeutic agent for cancer patients. Current investigation in this area has opened new horizons for our understanding of IGFBP-3's IGF/IGF-IR-independent actions. Much more research needs to be done because IGFBP-3 may also hold promise in areas of treatment where to date increased concentrations of cytotoxic drugs and higher dosages of irradiation fail to improve the response to therapy. IGFBP-3 may be able to play a unique role in instances where current therapy fails due to the development of resistance to apoptosis in cancer cells. Since IGFBP-3 is a highly effective proapoptotic factor in tumor cells by a variety of mechanisms, therapy directly with exogenous IGFBP-3 or indirectly with hormonal or other up-regulators of IGFBP-3 may be an important mode of cancer therapy in the future.

## Acknowledgments

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This work was supported in part by U.S. Army Grants PC040971 and PC061151 (to Y.O.) and National Institutes of Health Grant DK42482 (to D.F.).

Disclosure Summary: The authors have nothing to declare.

## References

1. **Butler AA, Le Roith D** 2001 Control of growth by the somatropic axis: growth hormone and the insulin-like growth factors have related and independent roles. Annu Rev Physiol 63:141–164
2. **Stewart CE, Rotwein P** 1996 Growth, differentiation and survival: multiple physiological functions of insulin-like growth factors. Physiol Rev 76:1005–1026
3. **Yakar S, Liu JL, Stannard B, Butler A, Accili D, Sauer B, LeRoith D** 1999 Normal growth and development in the absence of hepatic insulin-like growth factor I. Proc Natl Acad Sci USA 96:7324–7329
4. **Sjögren K, Liu JL, Blad K, Skrtic S, Vidal O, Wallenius V, LeRoith D, Törnell J, Isaksson OG, Jansson JO, Ohlsson C** 1999 Liver-derived insulin-like growth factor I (IGF-I) is the principal source of IGF-I in blood but is not required for postnatal body growth in mice. Proc Natl Acad Sci USA 96:7088–7092
5. **Siddle K, Ursø B, Niesler CA, Cope DL, Molina L, Surinya KH, Soos MA** 2001 Specificity in ligand binding and intracellular signalling by insulin and insulin-like growth factor receptors. Biochem Soc Trans 29:513–525
6. **Braulke T** 1999 Type-2 IGF receptor: a multi-ligand binding protein. Horm Metab Res 31:242–246
7. **Belfiore A** 2007 The role of insulin receptor isoforms and hybrid insulin/IGF-I receptors in human cancer. Curr Pharm Des 13:671–686
8. **Walker GE, Kim HS, Yang YF, Oh Y** 2004 IGF-independent effects of the IGFBP superfamily. In: Le Roith D, Zumkeller W, Baxter R, eds. Insulin-like growth factors. Austin, TX: Landes Bioscience; 1–22
9. **Phillips LS, Pao CI, Villafuerte BC** 1998 Molecular regulation of insulin-like growth factor-I and its principal binding protein, IGFBP-3. Prog Nucleic Acid Res Mol Biol 60: 195–265
10. **Albiston AL, Saffery R, Herington AC** 1995 Cloning and characterization of the promoter for the rat insulin-like growth factor-binding protein-3 gene. Endocrinology 136: 696–704
11. **Olivecrona H, Hilding A, Ekström C, Barle H, Nyberg B, Möller C, Delhanty PJ, Baxter RC, Angelin B, Ekström TJ, Tally M** 1999 Acute and short-term effects of growth hormone on insulin-like growth factors and their binding proteins: serum levels and hepatic messenger ribonucleic acid responses in humans. J Clin Endocrinol Metab 84:553–560
12. **Domené HM, Bengolea SV, Jasper HG, Boisclair YR** 2005 Acid-labile subunit deficiency: phenotypic similarities and differences between human and mouse. J Endocrinol Invest 28(5 Suppl):43–46
13. **Hwa V, Oh Y, Rosenfeld RG** 1999 The insulin-like growth factor-binding protein (IGFBP) superfamily. Endocr Rev 20:761–787
14. **Zhu W, Shiojima I, Ito Y, Li Z, Ikeda H, Yoshida M, Naito AT, Nishi J, Ueno H, Umezawa A, Minamino T, Nagai T, Kikuchi A, Asashima M, Komuro I** 2008 IGFBP-4 is an inhibitor of canonical Wnt signalling required for cardiogenesis. Nature 454:345–349
15. **Perks CM, Newcomb PV, Norman MR, Holly JM** 1999 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
16. **Fu P, Thompson JA, Bach LA** 2007 Promotion of cancer cell migration: an insulin-like growth factor (IGF)-independent action of IGF-binding protein-6. J Biol Chem 282: 22298–22306
17. **Kiepe D, Van Der Pas A, Ciarmatori S, Ständker L, Schütt B, Hoeflich A, Hügel U, Oh J, Tönshoff B** 2008 Defined carboxy-terminal fragments of insulin-like growth factor (IGF) binding protein-2 exert similar mitogenic activity on cultured rat growth plate chondrocytes as IGF-I. Endocrinology 149:4901–4911
18. **Wetterau LA, Moore MG, Lee KW, Shim ML, Cohen P** 1999 Novel aspects of the insulin-like growth factor binding proteins. Mol Genet Metab 68:161–181
19. **Imai Y, Moralez A, Andag U, Clarke JB, Busby Jr WH, Clemmons DR** 2000 Substitutions for hydrophobic amino acids in the N-terminal domains of IGFBP-3 and -5 markedly reduce IGF-1 binding and alter their biologic actions. J Biol Chem 275:18188–18194
20. **Buckway CK, Wilson EM, Ahlsén M, Bang P, Oh Y, Rosenfeld RG** 2001 Mutation of three critical amino acids of the N-terminal domain of IGFBP-3 essential for high affinity IGF binding. J Clin Endocrinol Metab 86:4943–4950
21. **Lalou C, Lassarre C, Binoux M** 1996 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
22. **Salahifar H, Firth SM, Baxter RC, Martin JL** 2000 Characterization of an amino-terminal fragment of insulin-like growth factor binding protein-3 and its effects in MCF-7 breast cancer cells. Growth Horm IGF Res 10:367–377
23. **Firth SM, Baxter RC** 2002 Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev 23:824–854
24. **Firth SM, Baxter RC** 1999 Characterization of recombinant glycosylation variants of insulin-like growth factor binding protein-3. J Endocrinol 160:379–387
25. **Yamanaka Y, Fowlkes JL, Wilson EM, Rosenfeld RG, Oh Y** 1999 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 domain on the IGFBP-3 molecule. Endocrinology 140:1319–1328
26. **Spencer EM, Chan K** 1995 A 3-dimensional model for the insulin-like growth factor binding proteins (IGFBPs); supporting evidence using the structural determinants of the IGF binding site on IGFBP-3. Prog Growth Factor Res 6:209–214

27. **Ho PJ, Baxter RC** 1997 Characterization of truncated insulin like growth factor binding protein-2 in human milk. Endocrinology 138:3811–3818
28. **Devi GR, Yang DH, Rosenfeld RG, Oh Y** 2000 Differential effects of insulin-like growth factor (IGF)-binding protein-3 and its proteolytic fragments on ligand binding, cell surface association, and IGF-I receptor signaling. Endocrinology 141:4171–4179
29. **Fowlkes JL, Serra DM** 1996 Characterization of glycosaminoglycan-binding domains present in insulin-like growth factor binding protein-3. J Biol Chem 271:14676–14679
30. **Smith EP, Lu L, Chernausek SD, Klein DJ** 1994 Insulin-like growth factor binding protein-3 (IGFBP-3) concentration in rat Sertoli cell-conditioned medium is regulated by a pathway involving association of IGFBP-3 with cell surface proteoglycans. Endocrinology 135:359–364
31. **Campbell PG, Durham SK, Hayes JD, Suwanichkul A, Powell DR** 1999 Insulin-like growth factor binding protein-3 binds fibrinogen and fibrin. J Biol Chem 274:30215-30221
32. **Campbell PG, Durham SK, Suwanichkul A, Hayes JD, Powell DR** 1998 Plasminogen binds the heparin-binding domain of insulin-like growth factor binding protein-3. Am J Physiol 275:E321–E331
33. **Singh B, Charkowicz D, Mascarenhas D** 2004 Insulin-like growth factor-independent effects mediated by a C-terminal metal-binding domain of insulin-like growth factor binding protein-3. J Biol Chem 279:477–487
34. **Weinzimer SA, Gibson TB, Collett-Solberg PF, Khare A, Liu B, Cohen PJ** 2001 Transferrin is an insulin-like growth factor-binding protein-3 binding protein. J Clin Endocrinol Metab 86:1806–1813
35. **Kalus W, Zweckstetter M, Renner C, Sanchez Y, Georgescu J, Grol M, Demuth D, Schumacher R, Dony C, Lang K, Holak TA** 1998 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
36. **Fernández-Tornero C, Lozano RM, Rivas G, Jiménez MA, Ständker L, Díaz-Gonzalez D, Forssmann WG, Cuevas P, Romero A, Giménez-Gallego G** 2005 Synthesis of the blood circulating C-terminal fragment of insulin-like growth factor (IGF)-binding protein-4 in its native conformation. Crystallization, heparin and IGF binding, and osteogenic activity. J Biol Chem 280:18899–18907
37. **Sala A, Capaldi S, Campagnoli M, Faggion B, Labò S, Perduca M, Romano A, Carrizo ME, Valli M, Visai L, Minchiotti L, Galliano M, Monaco HL** 2005 Structure and properties of the C-terminal domain of insulin-like growth factor-binding protein-1 isolated from human amniotic fluid. J Biol Chem 280:29812–29819
38. **Sitar T, Popowicz GM, Siwanowicz I, Huber R, Holak TA** 2006 Structural basis for the inhibition of insulin-like growth factors by insulin-like growth factor-binding proteins. Proc Natl Acad Sci USA 103:13028–13033
39. **Kuang Z, Yao S, McNeil KA, Thompson JA, Bach LA, Forbes BE, Wallace JC, Norton RS** 2007 Cooperativity of the N-and C-terminal domains of insulin-like growth factor (IGF) binding protein 2 in IGF binding. Biochemistry 46:13720–13732
40. **Rajaram S, Baylink DJ, Mohan S** 1997 Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. Endocr Rev 18:801–831
41. **Martin JL, Coverley JA, Pattison ST, Baxter RC** 1995 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
42. **Oh Y, Müller HL, Lamson G, Rosenfeld RG** 1993 Insulin-like growth factor (IGF)-independent action of IGFBP-3 in Hs578T human breast cancer cells. Cell surface binding and growth inhibition. J Biol Chem 268:14964–14971
43. **Rajah R, Valentinis B, Cohen P** 1997 Insulin-like growth factor (IGF)-binding protein-3 induces apoptosis and mediates the effects of transforming growth factor-$\beta$1 on programmed cell death through a p53- and IGF-independent mechanism. J Biol Chem 272:12181–12188
44. **Leal SM, Liu Q, Huang SS, Huang JS** 1997 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
45. **Liu B, Lee HY, Weinzimer SA, Powell DR, Clifford JL, Kurie JM, Cohen P** 2000 Direct functional interactions between insulin-like growth factor binding protein-3 and retinoid X receptor $\alpha$ regulate transcriptional signaling and apoptosis. J Biol Chem 275:33607–33613
46. **Schedlich LJ, Le Page SL, Firth SM, Briggs LJ, Jans DA, Baxter RC** 2000 Nuclear import of insulin-like growth factor binding protein-3 and -5 is mediated by the importin $\gamma$ subunit. J Biol Chem 275:23462–23470
47. **Blat C, Delbe J, Villaudy J, Chatelain G, Golde A, Harel L** 1989 Inhibitory diffusible factor 45 bifunctional activity. As a cell growth inhibitor and as an insulin-like growth factor I-binding protein. J Biol Chem 264:12449–12454
48. **Liu L, Delbé J, Blat C, Zapf J, Harel L** 1992 Insulin-like growth factor binding protein-3 (IGFBP-3), an inhibitor of serum growth factors other than IGF-I and -II. J Cell Physiol 153:15–21
49. **Cohen P, Lamson G, Okajima T, Rosenfeld RG** 1993 Transfection of the human insulin-like growth factor binding protein-3 gene into Balb/c fibroblasts inhibits cellular growth. Mol Endocrinol 7:380–386
50. **Martin JL, Weenink SM, Baxter RC** 2003 Insulin-like growth factor-binding protein-3 potentiates epidermal growth factor action in MCF-10A mammary epithelial cells. Involvement of p44/42 and p38 mitogen-activated protein kinases. J Biol Chem 278:2969–2976
51. **Butt AJ, Martin JL, Dickson KA, McDougall F, Firth SM, Baxter RC** 2004 Insulin-like growth factor binding protein-3 expression is associated with growth stimulation of T47D human breast cancer cells: the role of altered epidermal growth factor signaling. J Clin Endocrinol Metab 89:1950–1956
52. **Granata R, Trovato L, Garbarino G, Taliano M, Ponti R, Sala G, Ghidoni R, Ghigo E** 2004 Dual effects of IGFBP-3 on endothelial cell apoptosis and survival: involvement of the sphingolipid signaling pathways. FASEB J 18:1456–1458
53. **Chang KH, Chan-Ling T, McFarland EL, Afzal A, Pan H, Baxter LC, Shaw LC, Caballero S, Sengupta N, Li Calzi S, Sullivan SM, Grant MB** 2007 IGF binding protein-3 regulates hematopoietic stem cell and endothelial precursor

cell function during vascular development. Proc Natl Acad Sci USA 104:10595–10600
54. **Lofqvist C, Chen J, Connor KM, Smith AC, Aderman CM, Liu N, Pintar JE, Ludwig T, Hellstrom A, Smith LE** 2007 IGFBP3 suppresses retinopathy through suppression of oxygen-induced vessel loss and promotion of vascular regrowth. Proc Natl Acad Sci USA 104:10589–10594
55. **Oh Y, Müller HL, Pham H, Rosenfeld RG** 1993 Demonstration of receptors for insulin-like growth factor binding protein-3 on Hs578T human breast cancer cells. J Biol Chem 268:26045–26048
56. **ten Dijke P, Miyazono K, Heldin CH** 1996 Signaling via hetero-oligomeric complexes of type I and type II serine/threonine kinase receptors. Curr Opin Cell Biol 8:139–145
57. **Wu RY, Zhang Y, Feng XH, Derynck R** 1997 Heteromeric and homomeric interactions correlate with signaling activity and functional cooperativity of Smad 3 and Smad 4/DPC4. Mol Cell Biol 17:2521–2528
58. **Lagna G, Hata A, Hemmati-Brivanlou A, Massagué J** 1996 Partnership between DPC4 and SMAD proteins in TGF-$\beta$ signaling pathways. Nature 383:832–836
59. **Fanayan S, Firth SM, Butt AJ, Baxter RC** 2000 Growth inhibition by insulin-like growth factor binding protein-3 in T47D breast cancer cells requires transforming growth factor-$\beta$ (TGF-$\beta$) and the type II TGF-$\beta$ receptor. J Biol Chem 275:39146–39151
60. **Leal SM, Huang SS, Huang JS** 1999 Interactions of high affinity insulin-like growth factor binding proteins with the type V transforming growth factor-$\beta$ receptor in mink lung epithelial cells. J Biol Chem 274:6711–6717
61. **Huang SS, Ling TY, Tseng WF, Huang YH, Tang FM, Leal SM, Huang JS** 2003 Cellular growth inhibition by IGFBP-3 and TGF-$\beta$ I requires LRP-I. FASEB J 17:2068–2081
62. **Butt AJ, Firth SM, King MA, Baxter RC** 2000 Insulin-like growth factor binding protein-3 modulates expression of Bax and Bcl-2 and potentiates p53-independent radiation-induced apoptosis in human breast cancer cells. J Biol Chem 275:39174–39181
63. **Fowler CA, Perks CM, Newcomb PV, Savage PB, Farndon JR, Holly JM** 2000 Insulin-like growth factor binding protein-3 potentiates paclitaxel-induced apoptosis in human breast cancer cells. Int J Cancer 88:448–453
64. **Fraser A, Evan G** 1996 A license to kill. Cell 85:781–784
65. **Li J, Yuan J** 2008 Caspases in apoptosis and beyond. Oncogene 27:6194–6206
66. **Kim HS, Ingermann AR, Tsubaki J, Twigg SM, Walker GE, Oh Y** 2004 Insulin-like growth factor binding protein-3 induces caspase-dependent apoptosis through a death receptor-mediated pathway in MCF-7 human breast cancer cells. Cancer Res 64:2229–2237
67. **Rajah R, Lee KW, Cohen P** 2002 Insulin-like growth factor binding protein-3 mediates tumor necrosis factor-$\alpha$-induced apoptosis: role of Bcl-2 phosphorylation. Cell Growth Differ 13:163–171
68. **Sovak MA, Bellas RE, Kim DW, Zanieski GJ, Rogers AE, Traish AM, Sonenshein GE** 1997 Aberrant nuclear factor-$\kappa$B//Rel expression and the pathogenesis of breast cancer. J Clin Invest 100:2952–2960
69. **Palayoor ST, Youmell MY, Calderwood SK, Coleman CN, Price BD** 1999 Constitutive activation of I$\kappa$B kinase $\alpha$ and NF-$\kappa$B in prostate cancer cells in inhibited by ibuprofen. Oncogene 18:7389–7394
70. **Baldwin Jr AS** 1996 The NF-$\kappa$B and I$\kappa$B proteins: new discoveries and insights. Annu Rev Immunol 14:649–683
71. **Pahl HL** 1999 Activators and target genes of Rel/NF-$\kappa$B transcription factors. Oncogene 18:6853–6866
72. **Karin M** 1999 The beginning of the end: I$\kappa$B kinase (IKK) and NF-$\kappa$B activation. J Biol Chem 274:27339–27342
73. **Van Antwerp DJ, Martin SJ, Kafri T, Green DR, Verma IM** 1996 Suppression of TNF-$\alpha$-induced apoptosis by NF-$\kappa$B. Science 274:787–789
74. **Giri DK, Aggarwal BB** 1998 Constitutive activation of NF-$\kappa\kappa$B causes resistance to apoptosis in human cutaneous T cell lymphoma HuT-78 cells. Autocrine role of tumor necrosis factor and reactive oxygen intermediates. J Biol Chem 273:14008–14014
75. **Butt AJ, Dickson KA, Jambazov S, Baxter RC** 2005 Enhancement of tumor necrosis factor-$\alpha$-induced growth inhibition by insulin-like growth factor-binding protein-5 (IGFBP-5), but not IGFBP-3 in human breast cancer cells. Endocrinology 146:3113–3122
76. **H-Zadeh AM, Collard TJ, Malik K, Hicks DJ, Paraskeva C, Williams AC** 2006 Induction of apoptosis by the 16-kDa amino-terminal fragment of the insulin-like growth factor binding protein 3 in human colonic carcinoma cells. Int J Oncol 29:1279–1286
77. **Williams AC, Smartt H, H-Zadeh AM, Macfarlane M, Paraskeva C, Collard TJ** 2007 Insulin-like growth factor binding protein 3 (IGFBP-3) potentiates TRAIL-induced apoptosis of human colorectal carcinoma cells through inhibition of NF-$\kappa$B. Cell Death Differ 14:137–145
78. **Nakshatri H, Bhat-Nakshatri P, Martin DA, Goulet Jr RJ, Sledge Jr GW** 1997 Constitutive activation of NF-$\kappa$B during progression of breast cancer to hormone-independent growth. Mol Cell Biol 17:3629–3639
79. **Wang CY, Cusack Jr JC, Liu R, Baldwin Jr AS** 1999 Control of inducible chemoresistance: enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-$\kappa$B. Nat Med 5:412–417
80. **Fulda S, Debatin KM** 2004 Targeting apoptosis pathways in cancer therapy. Curr Cancer Drug Targets 4:569–576
81. **Kim BY, Kim KA, Kwon O, Kim SO, Kim MS, Kim BS, Oh WK, Kim GD, Jung M, Ahn JS** 2005 NF-$\kappa$B inhibition radiosensitizes Ki-Ras-transformed cells to ionizing radiation. Carcinogenesis 26:1395–1403
82. **Mishra S, Murphy LJ** 2003 Phosphorylation of insulin-like growth factor (IGF) binding protein-3 by breast cancer cell membranes enhances IGF-I binding. Endocrinology 144: 4042–4050
83. **Mishra S, Murphy LJ** 2004 Tissue transglutaminase has intrinsic kinase activity. Identification of transglutaminase 2 as an insulin-like growth factor binding protein-3 kinase. J Biol Chem 279:23863–23868
84. **Mishra S, Raz A, Murphy LJ** 2004 Insulin-like growth factor binding protein-3 interacts with autocrine motility factor/phosphoglucose isomerase (AMF/PGI) and inhibits the AMF/PGI function. Cancer Res 64:2516–2522
85. **Schedlich LJ, Young TF, Firth SM, Baxter RC,** 1998 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
86. **Li W, Fawcett J, Widmer HR, Fielder PJ, Rabkin R, Keller GA** 1997 Nuclear transport of insulin-like growth factor-I

and insulin-like growth factor binding protein-3 in opossum kidney cells. Endocrinology 138:1763–1766

87. **Jaques G, Noll K, Wegmann B, Witten S, Kogan E, Radulescu RT, Havemann K** 1997 Nuclear localization of insulin-like growth factor binding protein 3 in a lung cancer cell line. Endocrinology 138:1767–1770
88. **Wraight CJ, Liepe IJ, White PJ, Hibbs AR, Werther GA** 1998 Intranuclear localization of insulin-like growth factor binding protein-3 (IGFBP-3) during cell division in human keratinocytes. J Investig Dermatol 111:239–242
89. **Radulescu RT** 1994 Nuclear localization signal in insulin-like growth factor-binding protein type 3. Trends Biochem Sci 19:278
90. **Schedlich LJ, O'Han MK, Leong GM, Baxter RC** 2004 Insulin-like growth factor binding protein-3 prevents retinoid receptor heterodimerization: implications for retinoic acid-sensitivity in human breast cancer cells. Biochem Biophys Res Commun 314:83–88
91. **Kastner P, Mark M, Chambon P** 1995 Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life? Cell 83:859–869
92. **Mangelsdorf DJ, Evans RM** 1995 The RXR heterodimers and orphan receptors. Cell 83:841–850
93. **Hazel TG, Nathans D, Lau LF** 1988 A gene inducible by serum growth factors encodes a member of the steroid and thyroid hormone receptor superfamily. Proc Natl Acad Sci USA 85:8444–8448
94. **Zhang Y, Dawson MI, Mohammad R, Rishi AK, Farhana L, Feng KC, Leid M, Peterson V, Zhang XK, Edelstein M, Eilander D, Biggar S, Wall N, Reichert U, Fontana JA** 2002 Induction of apoptosis of human B-CLL and ALL cells by a novel retinoid and its nonretinoidal analog. Blood 100: 2917–2925
95. **Dawson MI, Hobbs PD, Peterson VJ, Leid M, Lange CW, Feng KC, Chen Gq, Gu J, Li H, Kolluri SK, Zhang Xk, Zhang Y, Fontana JA** 2001 Apoptosis induction in cancer cells by a novel analogue of 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalenecarboxylic acid lacking retinoid receptor transcriptional activation activity. Cancer Res 61:4723–4730
96. **Holmes WF, Soprano DR, Soprano KJ** 2003 Comparison of the mechanism of induction of apoptosis in ovarian carcinoma cells by the conformationally restricted synthetic retinoids CD437 and 4-HPR. J Cell Biochem 89:262–278
97. **Wu Q, Liu S, Ye XF, Huang ZW, Su WJ** 2002 Dual roles of Nur77 in selective regulation of apoptosis and cell cycle by TPA and ATRA in gastric cancer cells. Carcinogenesis 23:1583–1592
98. **Wilson AJ, Arango D, Mariadason JM, Heerdt BG, Augenlicht LH** 2003 TR3/Nur77 in colon cancer cell apoptosis. Cancer Res 63:5401–5407
99. **Li H, Kolluri SK, Gu J, Dawson MI, Cao X, Hobbs PD, Lin B, Chen G, Lu J, Lin F, Xie Z, Fontana JA, Reed JC, Zhang X** 2000 Cytochrome C release and apoptosis induced by mitochondrial targeting of nuclear orphan receptor TR3. Science 289:1159–1164
100. **Forman BM, Umesono K, Chen J, Evans RM** 1995 Unique response pathways are established by allosteric interactions among nuclear hormone receptors. Cell 81:541–550
101. **Lee KW, Ma L, Yan X, Liu B, Zhang XK, Cohen P** 2005 Rapid apoptosis induction by IGFBP-3 involves an insulin-like growth factor-independent nucleomitochondrial translocation of RXR$\alpha$/Nur77. J Biol Chem 280:16942–16948
102. **Lee KW, Cobb LJ, Paharkova-Vatchkova V, Liu B, Milbrandt J, Cohen P** 2007 Contribution of the orphan nuclear receptor Nur77 to the apoptotic action of IGFBP-3. Carcinogenesis 28:1653–1658
103. **Cobb LJ, Liu B, Lee KW, Cohen P** 2006 Phosphorylation by DNA-dependent protein kinase is critical for apoptosis induction by insulin-like growth factor binding protein-3. Cancer Res 66:10878–10884
104. **Lee KW, Liu B, Ma L, Li H, Bang P, Koeffler HP, Cohen P** 2004 Cellular Internalization of Insulin-like growth factor binding protein-3— distinct endocytic pathways facilitate re-uptake and nuclear localization. J Biol Chem 279: 469–476
105. **Butt AJ, Fraley KA, Firth SM, Baxter RC** 2002 Insulin-like growth factor binding protein-3-induced growth inhibition and apoptosis do not require cell-surface binding and nuclear translocation in human breast cancer cells. Endocrinology 143:2693–2699
106. **Bhattacharyya N, Pechhold K, Shahjee H, Zappala G, Elbi C, Raaka B, Wiench M, Hong J, Rechler MM** 2006 Nonsecreted insulin-like growth factor binding protein-3 (IGFBP-3) can induce apoptosis in human prostate cancer cells by IGF-independent mechanisms without being concentrated in the nucleus. J Biol Chem 281:24588–24601
107. **Zappala G, Elbi C, Edwards J, Gorenstein J, Rechler MM, Bhattacharyya N** 2008 Induction of apoptosis in human prostate cancer cells by insulin-like growth factor binding protein-3 does not require binding to retinoid X receptor-$\alpha$. Endocrinology 149:1802–1812
108. **Liao L, Dearth RK, Zhou S, Britton OL, Lee AV, Xu J** 2006 Liver-specific overexpression of the insulin-like growth factor-I enhances somatic growth and partially prevents the effects of growth hormone deficiency. Endocrinology 147:3877–3888
109. **DiGirolamo DJ, Mukherjee A, Fulzele K, Gan Y, Cao X, Frank SJ, Clemens TL** 2007 Mode of growth hormone action in osteoblasts. J Biol Chem 282:31666–31674
110. **Blum WF** 1996 Insulin-like growth factors and IGF-binding proteins: their use for diagnosis of growth hormone deficiency. In: Juul A, Jorgensen JOL, eds. Growth hormone in adults: physiological and clinical aspects. Cambridge, UK: Cambridge University Press; 48–74
111. **Marzullo P, Di Somma C, Pratt KL, Khosravi J, Diamandis A, Lombardi G, Colao A, Rosenfeld RG** 2001 Usefulness of different biochemical markers of the insulin-like growth factor (IGF) family in diagnosing growth hormone excess and deficiency in adults. J Clin Endocrinol Metab 86:3001–3008
112. **Arosio M, Garrone S, Bruzzi P, Faglia G, Minuto F, Barreca A** 2001 Diagnostic value of the acid-labile subunit in acromegaly: evaluation in comparison with insulin-like growth factor (IGF) I, and IGF-binding protein-1, -2, and -3. J Clin Endocrinol Metab 86:1091–1098
113. **Oh Y, Müller HL, Ng L, Rosenfeld RG** 1995 Transforming growth factor-$\beta$-induced cell growth inhibition in human breast cancer cells is mediated through insulin-like growth factor-binding protein-3 action. J Biol Chem 270:13589–13592
114. **Gucev ZS, Oh Y, Kelley KM, Rosenfeld RG** 1996 Insulin-like growth factor binding protein-3 mediates retinoic acid-

and transforming growth factor β2-induced growth inhibition in human breast cancer cells. Cancer Res 56:1545–1550
115. **Martin JL, Ballesteros M, Baxter RC** 1992 Insulin-like growth factor-I (IGF-I) and transforming growth factor-β1 release IGF-binding protein-3 from human fibroblasts by different mechanisms. Endocrinology 131:1703–1710
116. **Yateman ME, Claffey DC, Cwyfan Hughes SC, Frost VJ, Wass JA, Holly JM** 1993 Cytokines modulate the sensitivity of human fibroblasts to stimulation with insulin-like growth factor-I (IGF-I) by altering endogenous IGF-binding protein production. J Endocrinol 137:151–159
117. **Besset V, Le Magueresse-Battistoni B, Collette J, Benahmed M** 1996 Tumor necrosis factor α stimulates insulin-like growth factor binding protein 3 expression in cultured porcine Sertoli cells. Endocrinology 137:296–303
118. **Olney RC, Wilson DM, Mohtai M, Fielder PJ, Smith RL** 1995 Interleukin-1 and tumor necrosis factor-α increase insulin-like growth factor binding protein-3 production and IGFBP-3 protease activity in human articular chondrocytes. J Endocrinol 146:279–286
119. **Nickerson T, Huynh H** 1999 Vitamin D analogue EB1089-induced prostate regression is associated with increased gene expression of insulin-like growth factor binding proteins. J Endocrinol 160:223–229
120. **Huynh H, Yang X, Pollak M** 1996 Estradiol and antiestrogens regulate a growth inhibitory insulin-like growth factor binding protein 3 autocrine loop in human breast cancer cells. J Biol Chem 271:1016–1021
121. **Nickerson T, Pollak M** 1999 Bicalutamide (Casodex)-induced prostate regression involves increased expression of genes encoding insulin-like growth factor binding protein. Urology 54:1120–1125
122. **Boyle BJ, Zhao XY, Cohen P, Feldman D** 2001 Insulin-like growth factor binding protein-3 mediates 1 α,25-dihydroxyvitamin d(3) growth inhibition in the LNCaP prostate cancer cell line through p21/WAF1. J Urol 165:1319–1324
123. **Peng L, Malloy PJ, Feldman D** 2004 Identification of a functional vitamin D response element in the human insulin-like growth factor binding protein-3 promoter. Mol Endocrinol 18:1109–1119
124. **Peng L, Malloy PJ, Wang J, Feldman D** 2006 Growth inhibitory concentrations of androgens up-regulate insulin-like growth factor binding protein-3 expression via an androgen response element in LNCaP human prostate cancer cells. Endocrinology 147:4599–4607
125. **Krishnan AV, Moreno J, Nonn L, Malloy P, Swami S, Peng L, Peehl DM, Feldman D** 2007 Novel pathways that contribute to the antiproliferative and chemopreventive activities of calcitriol in prostate cancer. J Steroid Biochem Mol Biol 103:694–702
126. **Zhao XY, Ly LH, Peehl DM, Feldman D** 1997 1α,25-Dihydroxyvitamin D3 actions in LNCaP human prostate cancer cells are androgen-dependent. Endocrinology 138:3290–3298
127. **Peng L, Wang J, Malloy PJ, Feldman D** 2008 The role of insulin-like growth factor binding protein-3 (IGFBP-3) in the growth inhibitory actions of androgens in LNCaP human prostate cancer cells. Int J Cancer 122:558–566
128. **Sonnenschein C, Olea N, Pasanen ME, Soto AM** 1989 Negative controls of cell proliferation: human prostate cancer cells and androgens. Cancer Res 49:3474–3481
129. **Lee C, Sutkowski DM, Sensibar JA, Zelner D, Kim I, Amsel I, Shaw N, Prins GS, Kozlowski JM** 1995 Regulation of proliferation and production of prostate-specific antigen in androgen-sensitive prostatic cancer cells, LNCaP, by dihydrotestosterone. Endocrinology 136:796–803
130. **Buckbinder L, Talbott R, Velasco-Miguel S, Takenaka I, Faha B, Seizinger BR, Kley N** 1995 Induction of the growth inhibitor IGF-binding protein-3 by p53. Nature 377:646–649
131. **Bourdon JC, Deguin-Chambon V, Lelong JC, Dessen P, May P, Debuire B, May E** 1997 Further characterization of the p53 responsive element-identification of new candidate genes for trans-activation by p53. Oncogene 14:85–94
132. **Grimberg A, Coleman CM, Burns TF, Himelstein BP, Koch CJ, Cohen P, El-Deiry WS** 2005 P53-Dependent and p53-independent induction of insulin-like growth factor binding protein-3 by deoxyribonucleic acid damage and hypoxia. J Clin Endocrinol Metab 90:3568–3574
133. **Yi HK, Kim SY, Hwang PH, Kim CY, Yang DH, Oh Y, Lee DY** 2005 Impact of PTEN on the expression of insulin-like growth factors (IGFs) and IGF binding proteins in human gastric adenocarcinoma cells. Biochem Biophys Res Commun 330:760–767
134. **Yin Y, Ni J, Chen M, DiMaggio MA, Guo Y, Yeh S** 2007 The therapeutic and preventive effect of RRR-α-vitamin E succinate on prostate cancer via induction of insulin-like growth factor binding protein-3. Clin Cancer Res 13:2271–2280
135. **Jenuwein T, Allis CD** 2001 Translating the histone code. Science 293:1074–1080
136. **Feinberg AP, Vogelstein B** 1983 Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature 301:89–92
137. **Jones PA, Laird PW** 1999 Cancer epigenetics comes of age. Nat Genet 21:163–167
138. **Baylin SB, Esteller M, Rountree MR, Bachman KE, Schuebel K, Herman JG** 2001 Aberrant patterns of DNA methylation, chromatin formation and gene expression in cancer. Hum Mol Genet 10:687–692
139. **Esteller M, Corn PG, Baylin SB, Herman JG** 2001 A gene hypermethylation profile of human cancer. Cancer Res 61:3225–3229
140. **Herman JG, Baylin SB** 2003 Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med 349:2042–2054
141. **Suzuki H, Gabrielson E, Chen W, Anbazhagan R, van Engeland M, Weijenberg MP, Herman JG, Baylin SB** 2002 A genomic screen for genes upregulated by demethylation and histone deacetylase inhibition in human colorectal cancer. Nat Genet 31:141–149
142. **Baylin SB** 2004 Reversal of gene silencing as a therapeutic target for cancer—roles for DNA methylation and its interdigitation with chromatin. Novartis Found Symp 259:226–233; discussion 234–237, 285–288
143. **Tomii K, Tsukuda K, Toyooka S, Dote H, Hanafusa T, Asano H, Naitou M, Doihara H, Kisimoto T, Katayama H, Pass HI, Date H, Shimizu N** 2007 Aberrant promoter methylation of insulin-like growth factor binding protein-3 gene in human cancers. Int J Cancer 120:566–573
144. **Wiley A, Katsaros D, Fracchioli S, Yu H** 2006 Methylation of the insulin-like growth factor binding protein-3 gene and prognosis of epithelial ovarian cancer. Int J Gynecol Cancer 16:210–218

145. **Ibanez de Caceres I, Dulaimi E, Hoffman AM, Al-Saleem T, Uzzo RG, Cairns P** 2006 Identification of novel target genes by an epigenetic reactivation screen of renal cancer. Cancer Res 66:5021–5028
146. **Hanafusa T, Yumoto Y, Nouso K, Nakatsukasa H, Onishi T, Fujikawa T, Taniyama M, Nakamura S, Uemura M, Takuma Y, Yumoto E, Higashi T, Tsuji T** 2002 Reduced expression of insulin-like growth factor binding protein-3 and its promoter hypermethylation in human hepatocellular carcinoma. Cancer Lett 176:149–158
147. **Chang YS, Wang L, Liu D, Mao L, Hong WK, Khuri FR, Lee HY** 2002 Correlation between insulin-like growth factor binding protein-3 promoter methylation and prognosis of patients with Stage I non-small cell lung cancer. Clin Cancer Res 8:3669–3675
148. **Chang YS, Wang L, Suh YA, Mao L, Karpen SJ, Khuri FR, Hong WK, Lee HY** 2004 Mechanisms underlying lack of insulin-like growth factor binding protein-3 expression in non-small-cell lung cancer. Oncogene 23:6569–6580
149. **Yamashita S, Tsujino Y, Moriguchi K, Tatematsu M, Ushijima T** 2006 Chemical genomic screening for methylation-silenced genes in gastric cancer cell lines using 5-aza-2′-deoxycytidine treatment and oligonucleotide microarrayed. Cancer Sci 97:64–71
150. **Paquette J, Bessette B, Ledru E, Deal C** 2007 Identification of upstream stimulatory factor binding sites in the human IGFBP3 promoter and potential implication of adjacent single-nucleotide polymorphisms and responsiveness to insulin. Endocrinology 148:6007–6018
151. **Gray SG, Kytola S, Lui WO, Larsson C, Ekstrom TJ** 2000 Modulating IGFBP-3 expression by trichostatin A: potential therapeutic role in the treatment of hepatocellular carcinoma. Int J Mol Med 5:33–41
152. **Walker GE, Wilson EM, Powell D, Oh Y** 2001 Butyrate, a histone deacetylase inhibitor, activates the human IGF binding protein-3 promoter in breast cancer cells: molecular mechanism involves an Sp1/Sp3 multiprotein complex. Endocrinology 142:3817–3827
153. **Tsubaki J, Choi WK, Ingermann AR, Twigg SM, Kim HS, Rosenfeld RG, Oh Y** 2001 Effects of sodium butyrate on expression of members of the IGF-binding protein superfamily in human mammary epithelial cells. J Endocrinol 169:97–110
154. **Thelen P, Schweyer S, Hemmerlein B, Wuttke W, Seseke F, Ringert RH** 2004 Expressional changes after histone deacetylase inhibition by valproic acid in LNCaP human prostate cancer cells. Int J Oncol 24:25–31
155. **Chun SY, Chen F, Washburn JG, MacDonald JW, Innes KL, Zhao R, Cruz-Correa MR, Dang LH, Dang DT** 2007 CDX2 promotes anchorage-independent growth by transcriptional repression of IGFBP-3. Oncogene 26:4725–4729
156. **Niwa H, Toyooka Y, Shimosato D, Strumpf D, Takahashi K, Yagi R, Rossant J** 2005 Interaction between Oct3/4 and Cdx2 determines trophectoderm differentiation. Cell 123:917–929
157. **Irwin JC, Suen LF, Cheng BH, Martin R, Cannon P, Deal CL, Giudice LC** 2000 Human placental trophoblasts secrete a disintegrin metalloproteinase very similar to the insulin-like growth factor binding protein-3 protease in human pregnancy serum. Endocrinology 141:666–674
158. **Prieur A, Tirode F, Cohen P, Delattre O** 2004 EWS/FLI-1 silencing and gene profiling of Ewing cells reveal downstream oncogenic pathways and a crucial role for repression of insulin-like growth factor binding protein-3. Mol Cell Biol 24:7275–7283
159. **Benini S, Zuntini M, Manara MC, Cohen P, Nicoletti G, Nanni P, Oh Y, Picci P, Scotlandi K** 2006 Insulin-like growth factor binding protein 3 as an anticancer molecule in Ewing's sarcoma. Int J Cancer 119:1039–1046
160. **Cohen P, Graves HC, Peehl DM, Kamarei M, Giudice LC, Rosenfeld RG** 1992 Prostate-specific antigen (PSA) is an insulin-like growth factor binding protein-3 protease found in seminal plasma. J Clin Endocrinol Metab 75:1046–1053
161. **Cohen P, Peehl DM, Graves HC, Rosenfeld RG** 1994 Biological effects of prostate specific antigen as an insulin-like growth factor binding protein-3 protease. J Endocrinol 142:407–415
162. **Rajah R, Bhala A, Nunn SE, Peehl DM, Cohen P** 1996 7S Nerve growth factor is an insulin-like growth factor binding protein protease. Endocrinology 137:2676–2682
163. **Angelloz-Nicoud P, Harel L, Binoux M** 1996 Recombinant human insulin-like growth factor (IGF) binding protein-3 stimulates prostate carcinoma cell proliferation via an IGF-independent mechanism: role of serine proteases. Growth Regul 6:130–136
164. **Sano A, Sangai T, Maeda H, Nakamura M, Hasebe T, Ochiai A** 2007 Kallikrein 11 expressed in human breast cancer cells releases insulin-like growth factor through degradation of IGFBP-3. Int J Oncol 30:1493–1498
165. **Gibson TL, Cohen P** 1999 Inflammation-related neutrophil proteases, cathepsin G and elastase, function as insulin-like growth factor binding protein proteases. Growth Horm IGF Res 9:241–253
166. **Claussen M, Kübler B, Wendland M, Neifer K, Schmidt B, Zapf J, Braulke T** 1997 Proteolysis of insulin-like growth factors (IGF) and IGF binding proteins by cathepsin D. Endocrinology 138:3797–3803
167. **Conover CA, De Leon DD** 1994 Acid-activated insulin-like growth factor-binding protein-3 proteolysis in normal and transformed cells. Role of cathepsin D. J Biol Chem 269:7076–7080
168. **Zwad O, Kübler B, Roth W, Scharf JG, Saftig P, Peters C, Braulke T** 2002 Decreased intracellular degradation of insulin-like growth factor binding protein-3 in cathepsin L-deficient fibroblasts. FEBS Lett 510:211–215
169. **Visse R, Nagase H** 2003 Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 92:827–839
170. **Lijnen HR** 2001 Plasmin and matrix metalloproteinases in vascular remodeling. Thromb Haemost 86:324–333
171. **Birkedal-Hansen H** 1995 Proteolytic remodeling of extracellular matrix. Curr Opin Cell Biol 7:728–735
172. **Lynch CC, Matrisian LM** 2002 Matrix metalloproteinases in tumor-host cell communication. Differentiation 70:561–573
173. **Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T** 2003 Regulation of matrix metalloproteinases: an overview. Mol Cell Biochem 253:269–285
174. **Yu WH, Woessner Jr JF, McNeish JD, Stamenkovic I** 2002 CD44 anchors the assembly of matrilysin/MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling. Genes Dev 16:307–323

175. **Gearing AJ, Beckett P, Christodoulou M, Churchill M, Clements J, Davidson AH, Drummond AH, Galloway WA, Gilbert R, Gordon JL, Leber TM, Mangan M, Miller K, Nayee P, Owen K, Patel S, Thomas W, Wells G, Wood LM, Woolley K** 1994 Processing of tumour necrosis factor-$\alpha$ precursor by metalloproteinases. Nature 370:555–557
176. **Hashimoto G, Inoki I, Fujii Y, Aoki T, Ikeda E, Okada Y** 2002 Matrix metalloproteinases cleave connective tissue growth factor and reactivate angiogenic activity of vascular endothelial growth factor 165. J Biol Chem 277:36288–36295
177. **Miyamoto S, Yano K, Sugimoto S, Ishii G, Hasebe T, Endoh Y, Kodama K, Goya M, Chiba T, Ochiai A** 2004 Matrix metalloproteinase-7 facilitates insulin-like growth factor bioavailability through its proteinase activity on insulin-like growth factor binding protein 3. Cancer Res 64:665–671
178. **Mochizuki S, Shimoda M, Shiomi T, Fujii Y, Okada Y** 2004 ADAM28 is activated by MMP-7 (matrilysin-1) and cleaves insulin-like growth factor binding protein-3. Biochem Biophys Res Commun 315:79–84
179. **Mitsui Y, Mochizuki S, Kodama T, Shimoda M, Ohtsuka T, Shiomi T, Chijiiwa M, Ikeda T, Kitajima M, Okada Y** 2006 ADAM28 is overexpressed in human breast carcinomas: implications for carcinoma cell proliferation through cleavage of insulin-like growth factor binding protein-3. Cancer Res 66:9913–9920
180. **Grzmil M, Hemmerlein B, Thelen P, Schweyer S, Burfeind P** 2004 Blockade of the type I IGF receptor expression in human prostate cancer cells inhibits proliferation and invasion, up-regulates IGF binding protein-3, and suppresses MMP-2 expression. J Pathol 202:50–59
181. **Sadowski T, Dietrich S, Koschinsky F, Sedlacek R** 2003 Matrix metalloproteinase 19 regulates insulin-like growth factor-mediated proliferation, migration, and adhesion in human keratinocytes through proteolysis of insulin-like growth factor binding protein-3. Mol Biol Cell 14:4569–4580
182. **Rajah R, Nachajon RV, Collins MH, Hakonarson H, Grunstein MM, Cohen P** 1999 Elevated levels of the IGF-binding protein protease MMP-1 in asthmatic airway smooth muscle. Am J Respir Cell Mol Biol 20:199–208
183. **Fowlkes JL, Thrailkill KM, Serra DM, Suzuki K, Nagase H** 1995 Matrix metalloproteinases as insulin-like growth factor binding protein-degrading proteinases. Prog Growth Factor Res 6:255–263
184. **Ikonen M, Liu B, Hashimoto Y, Ma L, Lee KW, Niikura T, Nishimoto I, Cohen P** 2003 Interaction between the Alzheimer's survival peptide humanin and insulin-like growth factor-binding protein 3 regulates cell survival and apoptosis. Proc Natl Acad Sci USA 100:13042-13047
185. **Oufattole M, Lin SW, Liu B, Mascarenhas D, Cohen P, Rodgers BD** 2006 Ribonucleic acid polymerase II binding subunit 3 (Rpb3), a potential nuclear target of insulin-like growth factor binding protein-3. Endocrinology 147:2138–2146
186. **Wu C, Yao G, Zou M, Chen G, Wang M, Liu J, Wang J, Xu D** 2007 N-Acetylgalactosaminyltransferase 14, a novel insulin-like growth factor binding protein-3 binding partner. Biochem Biophys Res Commun 357:360–365
187. **Grimberg A** 2000 p53 and IGFBP-3: apoptosis and cancer protection. Mol Genet Metab 70:85–98
188. **Sherr CJ** 2004 Principles of tumor suppression. Cell 116:235–246
189. **Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, Hennekens CH, Pollak M** 1998 Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science 279:563–566
190. **Wang L, Habuchi T, Tsuchiya N, Mitsumori K, Ohyama C, Sato K, Kinoshita H, Kamoto T, Nakamura A, Ogawa O, Kato T** 2003 Insulin-like growth factor-binding protein-3 gene -202A/C polymorphism is correlated with advanced disease status in prostate cancer. Cancer Res 63:4407–4411
191. **Ma J, Pollak MN, Giovannucci E, Chan JM, Tao Y, Hennekens CH, Stampfer MJ** 1999 Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor(IGF)-1 and IGF-binding protein-3. J Natl Cancer Inst 91:620–625
192. **Yu H, Spitz MR, Mistry J, Gu J, Hong WK, Wu X** 1999 Plasma levels of insulin-like growth factor-I and lung cancer risk: a case-control analysis. J Natl Cancer Inst 91:151–156
193. **Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, Rosner B, Speizer FE, Pollak M** 1998 Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 351:1393–1396
194. **Toniolo P, Bruning PF, Akhmedkhanov A, Bonfrer JM, Koenig KL, Lukanova A, Shore RE, Zeleniuch-Jacquotte A** 2000 Serum insulin-like growth factor-I and breast cancer. Int J Cancer 88:828–832
195. **Kaaks R, Lundin E, Rinaldi S, Manjer J, Biessy C, Söderberg S, Lenner P, Janzon L, Riboli E, Berglund G, Hallmans G** 2002 Prospective study of IGF-I, IGF-binding proteins and breast cancer risk in northern and southern Sweden. Cancer Causes Control 13:307–316
196. **Keinan-Boker L, Bueno De Mesquita HB, Kaaks R, Van Gils CH, Van Noord PA, Rinaldi S, Riboli E, Seidell JC, Grobbee DE, Peeters PH** 2003 Circulating levels of insulin-like growth factor I, its binding proteins -1, -2, -3 C peptide and risk of postmenopausal breast cancer. Int J Cancer 106:90–95
197. **Byrne C, Colditz GA, Willett WC, Speizer FE, Pollak M, Hankinson SE** 2000 Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. Cancer Res 60:3744–3748
198. **Becker S, Kaaks R** 2009 Exogenous and endogenous hormones, mammographic density and breast cancer risk: can mammographic density be considered an intermediate marker of risk? Recent Results Cancer Res 181:135–157
199. **Weiss JM, Huang WY, Rinaldi S, Fears TR, Chatterjee N, Chia D, Crawford ED, Kaaks R, Hayes RB** 2007 IGF-1 and IGFBP-3: risk of prostate cancer among men in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. Int J Cancer 121:2267–2273
200. **Allen NE, Key TJ, Appleby PN, Travis RC, Roddam AW, Rinaldi S, Egevad L, Rohrmann S, Linseisen J, Pischon T, Boeing H, Johnsen NF, Tjønneland A, Grønbaek H, Overvad K, Kiemeney L, Bueno-de-Mesquita HB, Bingham S, Khaw KT, Tumino R, Berrino F, Mattiello A, Sacerdote C, Palli D, Quirós JR, Ardanaz E, Navarro C, Larrañaga N, Gonzalez C, Sanchez MJ, Trichopoulou A, Travezea C, Trichopoulos D, Jenab M, Ferrari P, Riboli E, Kaaks R** 2007 Serum insulin-like growth factor (IGF)-I and IGF-

binding protein-3 concentrations and prostate cancer risk: results from the European Prospective Investigation into Cancer and Nutrition. Cancer Epidemiol Biomarkers Prev 16:1121–1127

201. **Severi G, Morris HA, MacInnis RJ, English DR, Tilley WD, Hopper JL, Boyle P, Giles GG** 2006 Circulating insulin-like growth factor-I and binding protein-3 and risk of prostate cancer. Cancer Epidemiol Biomarkers Prev 15:1137–1141
202. **Chen C, Lewis SK, Voigt L, Fitzpatrick A, Plymate SR, Weiss NS** 2005 Prostate carcinoma incidence in relation to prediagnostic circulating levels of insulin-like growth factor I, insulin-like growth factor binding protein 3, and insulin. Cancer 103:76–84
203. **Baglietto L, English DR, Hopper JL, Morris HA, Tilley WD, Giles GG** 2007 Circulating insulin-like growth factor-I and binding protein-3 and the risk of breast cancer. Cancer Epidemiol Biomarkers Prev 16:763–768
204. **Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M** 2004 Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 363:1346–1353
205. **Fletcher O, Gibson L, Johnson N, Altmann DR, Holly JM, Ashworth A, Peto J, Silva Idos S** 2005 Polymorphisms and circulating levels in the insulin-like growth factor system and risk of breast cancer: a systematic review. Cancer Epidemiol Biomarkers Prev 14:2–19
206. **Sugumar A, Liu YC, Xia Q, Koh YS, Matsuo K** 2004 Insulin-like growth factor (IGF)-I and IGF-binding protein 3 and the risk of premenopausal breast cancer: a meta-analysis of literature. Int J Cancer 111:293–297
207. **Rinaldi S, Peeters PH, Berrino F, Dossus L, Biessy C, Olsen A, Tjonneland A, Overvad K, Clavel-Chapelon F, Boutron-Ruault MC, Téhard B, Nagel G, Linseisen J, Boeing H, Lahmann PH, Trichopoulou A, Trichopoulos D, Koliva M, Palli D, Panico S, Tumino R, Sacerdote C, van Gils CH, van Noord P, Grobbee DE, Bueno-de-Mesquita HB, Gonzalez CA, Agudo A, Chirlaque MD, Barricarte A, Larrañaga N, Quiros JR, Bingham S, Khaw KT, Key T, Allen NE, Lukanova A, Slimani N, Saracci R, Riboli E, Kaaks R** 2006 IGF-I, IGFBP-3 and breast cancer risk in women: The European Prospective Investigation into Cancer and Nutrition (EPIC). Endocr Relat Cancer 13:593–605
208. **Friedrich N, Haring R, Nauck M, Lüdemann J, Rosskopf D, Spilcke-Liss E, Felix SB, Dörr M, Brabant G, Völzke H, Wallaschofski H** 2009 Mortality and serum insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. J Clin Endocrinol Metab 94:1732–1739
209. **Fuchs CS, Goldberg RM, Sargent DJ, Meyerhardt JA, Wolpin BM, Green EM, Pitot HC, Pollak M** 2008 Plasma insulin-like growth factors, insulin-like binding protein-3, and outcome in metastatic colorectal cancer: results from intergroup trial N9741. Clin Cancer Res 14:8263–8269
210. **Wolpin BM, Meyerhardt JA, Chan AT, Ng K, Chan JA, Wu K, Pollak MN, Giovannucci EL, Fuchs CS** 2009 Insulin, the insulin-like growth factor axis, and mortality in patients with nonmetastatic colorectal cancer. J Clin Oncol 27:176–185
211. **Roddam AW, Allen NE, Appleby P, Key TJ, Ferrucci L, Carter HB, Metter EJ, Chen C, Weiss NS, Fitzpatrick A, Hsing AW, Lacey JV Jr, Helzlsouer K, Rinaldi S, Riboli E, Kaaks R, Janssen JA, Wildhagen MF, Schröder FH, Platz EA, Pollak M, Giovannucci E, Schaefer C, Quesenberry CP Jr, Vogelman JH, Severi G, English DR, Giles GG, Stattin P, Hallmans G, Johansson M, Chan JM, Gann P, Oliver SE, Holly JM, Donovan J, Meyer F, Bairati I, Galan P** 2008 Insulin-like growth factors, their binding proteins, and prostate cancer risk: analysis of individual patient data from 12 prospective studies. Ann Intern Med 149:461–471, W83–W88
212. **Rowlands MA, Gunnell D, Harris R, Vatten LJ, Holly JM, Martin RM** 2009 Circulating insulin-like growth factor peptides and prostate cancer risk: a systematic review and meta-analysis. Int J Cancer 124:2416–2429
213. **Neuhouser ML, Schenk J, Song YJ, Tangen CM, Goodman PJ, Pollak M, Penson DF, Thompson IM, Kristal AR** 2008 Insulin-like growth factor-I, insulin-like growth factor binding protein-3 and risk of benign prostate hyperplasia in the prostate cancer prevention trial. Prostate 68:1477–1486
214. **Saydah S, Graubard B, Ballard-Barbash R, Berrigan D** 2007 Insulin-like growth factors and subsequent risk of mortality in the United States. Am J Epidemiol 166:518–526
215. **Missmer SA, Spiegelman D, Bertone-Johnson ER, Barbieri RL, Pollak MN, Hankinson SE** 2006 Reproducibility of plasma steroid hormones, prolactin, and insulin-like growth factor levels among premenopausal women over a 2- to 3-year period. Cancer Epidemiol Biomarkers Prev 15:972–978
216. **Rinaldi S, Kaaks R, Zeleniuch-Jacquotte A, Arslan AA, Shore RE, Koenig KL, Dossus L, Riboli E, Stattin P, Lukanova A, Toniolo P** 2005 Insulin-like growth factor-I, IGF binding protein-3, and breast cancer in young women: a comparison of risk estimates using different peptide assays. Cancer Epidemiol Biomarkers Prev 14:48–52
217. **Berrigan D, Potischman N, Dodd KW, Nicar M, McQuillan G, Lavigne JA, Barrett JC, Ballard-Barbash R** 2007 Serum levels of insulin-like growth factor-I and insulin-like growth factor-I binding protein-3: quality control for studies of stored serum. Cancer Epidemiol Biomarkers Prev 16:1017–1022
218. **Deal C, Ma J, Wilkin F, Paquette J, Rozen F, Ge B, Hudson T, Stampfer M, Pollak M** 2001 Novel promoter polymorphism in insulin-like growth factor-binding protein-3: correlation with serum levels and interaction with known regulators. J Clin Endocrinol Metab 86:1274–1280
219. **Kao PC, Matheny Jr AP, Lang CA** 1994 Insulin-like growth factor-I comparisons in healthy twin children. J Clin Endocrinol Metab 78:310–312
220. **Harrela M, Koistinen H, Kaprio J, Lehtovirta M, Tuomilehto J, Eriksson J, Toivanen L, Koskenvuo M, Leinonen P, Koistinen R, Seppälä M** 1996 Genetic and environmental components of interindividual variation in circulating levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-3. J Clin Invest 98:2612–2615
221. **Schernhammer ES, Hankinson SE, Hunter DJ, Blouin MJ, Pollak MN** 2003 Polymorphic variation at the −202 locus in IGFBP3: influence on serum levels of insulin-like growth factors, interaction with plasma retinol and vitamin D and breast cancer risk. Int J Cancer 107:60–64
222. **Tamimi RM, Cox DG, Kraft P, Pollak MN, Haiman CA,**

**Cheng I, Freedman ML, Hankinson SE, Hunter DJ, Colditz GA** 2007 Common genetic variation in IGF1, IGFBP-1, and IGFBP-3 in relation to mammographic density: a cross-sectional study. Breast Cancer Res 9:R18

223. **Cheng I, DeLellis Henderson K, Haiman CA, Kolonel LN, Henderson BE, Freedman ML, Le Marchand L** 2007 Genetic determinants of circulating insulin-like growth factor (IGF)-I, IGF binding protein (BP)-1, and IGFBP-3 levels in a multiethnic population. J Clin Endocrinol Metab 92:3660–3666
224. **Patel AV, Cheng I, Canzian F, Le Marchand L, Thun MJ, Berg CD, Buring J, Calle EE, Chanock S, Clavel-Chapelon F, Cox DG, Dorronsoro M, Dossus L, Haiman CA, Hankinson SE, Henderson BE, Hoover R, Hunter DJ, Kaaks R, Kolonel LN, Kraft P, Linseisen J, Lund E, Manjer J, McCarty C, Peeters PH, Pike MC, Pollak M, Riboli E, Stram DO, Tjonneland A, Travis RC, Trichopoulos D, Tumino R, Yeager M, Ziegler RG, Feigelson HS** 2008 Breast and Prostate Cancer Cohort Consortium. IGF-1, IGFBP-1, and IGFBP-3 polymorphisms predict circulating IGF levels but not breast cancer risk: findings from the Breast and Prostate Cancer Cohort Consortium (BPC3). PLoS ONE 3:e2578