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

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-II, type II IGF receptor; IκBα, inhibitory protein IκBα; 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.

A. Evidence from epidemiologic studies
B. Genetic polymorphisms of IGFBP-3
X. Concluding Comment

1. Introduction

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

2. IGF-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 α-subunits and two β-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-IR), which is identical to the cation-independent mannose-6-phosphate receptor, binds IGF-II with 500-fold increased affinity over IGF-I (6). IGF-II/IR does not bind insulin. Most of the biological actions of IGF-II are thought to be mediated via IGF-IR (6). IGF-IR 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 IGFBPs 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 IGFBPs 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 IGFBPs 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 IGFBPs.

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).
Glycosylation sites at Asn89, Asn109, and Asn172, the first forms estimated to be 40–43 kDa. Of the three potential proteolysis can affect both IGF/IGF-IR-dependent and IGF-binding affinity and susceptibility to proteases, and can affect cell interactions, phosphorylation can affect tissues depending upon both their primary structure and terminal domains. IGFBPs might be differentially targeted to with other IGFBPs. Intriguingly, posttranslational modifi-
cation such as potential phos-
phoacceptor 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-resi-
due 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-resi-
due basic motif. Furthermore, IGFBP-3 as well as IGFBP-
3-IGF-I complexes bind fibrinogen, fibrin (31), and plas-
minogen (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-β 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 and β1, caveolin-1, and transferrin receptor by unmasking of metal-binding do-

epinephrine, guanylyl cyclase, MAPK, and other pro-
eductive actions of IGFBP-3 involve the N-terminal and C-terminal regions (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 Asn89, Asn109, and Asn172, 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 charac-
teristic doublet form of the protein (24). Other sites of posttranslational modification such as potential phos-
phoacceptor sites also reside in this central domain. Studies conducted by Yamanaka et al. (25) suggest that IGFBP-3 binds human breast cancer cell surface with typical receptor-ligand interaction and the midregion of the IGFBP-3 molecule is responsible for the interaction.

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—GGCGC-
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-bind-
ing 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 ob-
servation 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 modifi-
cations (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 Asn89, Asn109, and Asn172, the first
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 IGFBP-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.

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-β signaling pathways as well as an interaction with a TGF-β receptor. TGF-β is a multifunctional growth factor secreted by many types of cells. TGF-β signaling from the cell surface to the nucleus requires a series of interdependent events. It is initiated by the association between TGF-β and the type II TGF-β receptor (TGF-βRII), resulting in the recruitment of the type I TGF-β receptor (TGF-βRI) into a heteromeric complex, which allows TGF-βRII to phosphorylate and activate TGF-βRI (56). Signaling intermediates Smad2 and 3 are phosphorylated by active TGF-βRII 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-β 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-β receptor (TGF-βRV) and interaction of IGFBP-3 with TGF-βRV causes cell growth inhibition (44, 60).

The cell signaling pathways activated by IGFBP-3/TGF-βRV interactions are distinct from those TGF-βRII/TGF-βRII 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-βRV remain to be elucidated. Recent structural and functional analyses of purified TGF-βRV have revealed that TGF-βRV is identical to the low-density lipoprotein receptor-related protein-1/activated α3M receptor, which is known as an endocytic receptor and has been shown to mediate the biological functions of the ligands, TGF-β1 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-Xl) 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-α 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-α-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-α 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 IkBα, 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 IkBα 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 IkBα is a critical step in the pathway leading to NF-κB activation, which is catalyzed by the IkBα 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 colon cancer cells. In this report, they further demonstrated that the secreted 16-kDa: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 activity. 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 anti-apoptotic 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-βR 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 D₃, 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). 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 RXRα 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 RXRα is required in this process (101). Because IGFBP-3 is a binding partner of RXRα, Lee et al. (101) proposed that IGFBP-3 modifies the RXRα/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 RXRα (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 RXRα 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.

| i) IGFBP-3 endocytic entry into cell via transferrin and caveolin |
| ii) Phosphorylation of IGFBP-3 at S156 by DNA-PK |
| iii) NLS on IGFBP-3 allows importin β to transport IGFBP-3 to the nucleus |

(A) Heterodimerization of RXRα/Nur77 regulates transcriptional activities in the nucleus

(B) IGFBP-3 binds to RXRα and induces translocation of Nur77 to the mitochondria
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-β1 (113–115), retinoic acid (114, 116, 117), TNF-α (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-α-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 fragmenting 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 g-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.

Grzmił 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-β. 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. (203), 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.
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