



Can [IGF-I] & [IGFBP-3] be Diagnostic for Cancer?

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There is evidence from epidemiological studies in humans, from studies with transgenic animals, as well as *in vitro* and *in vivo* studies, that the Insulin-like Growth Factors (IGF-I and IGF-II), their six binding proteins (IGFBPs 1-6 (GroPep Technical Bulletin No. 8)) and the IGF Receptors (IGF1R and IGF2R (GroPep Technical Bulletin No. 7)) are important mediators of normal growth and development. However, it is increasingly recognised that they can also play key roles in cellular transformation, and the survival and growth of malignant cells (GroPep Technical Bulletin No. 9).

The IGF System and Cancer

Evidence is accumulating that the progression of most cancers is dependent more on epigenetic influences than on primary gene mutation. Only 5-15% of all cancers may have a major genetic component (18). Western lifestyle, in particular western diet, has been most heavily implicated in epithelial breast, prostate and colorectal cancers (6). As serum [IGFs] are nutritionally dependent(6), further impetus has been given to determining the role of the IGF system in cancer.

There is epidemiological evidence that high circulating levels of GH / IGF-I are a risk factor in hormonally responsive epithelial cancers - breast, prostate, colon (3,7). High levels of IGFs, some IGFBPs and IGF1R are associated with many cancers (1). This suggests that intervention to control the availability of IGFs and IGFBPs (e.g. protease resistant IGFBPs), selective inhibition of IGF1R or increased levels of soluble IGF2R may be of therapeutic value in the treatment of a number of cancers.

Can serum [IGF-I] and [IGFBP-3] be used as prospective markers for epithelial cancers?

To date there is inadequate evidence to determine the potential role of serum IGF and IGFBP levels as prospective markers for cancer detection. Three large prospective studies have identified high levels of serum IGF-I and low levels of serum IGFBP-3 as risk factors in the development of prostate (10), colorectal (10) and breast (11) cancer. However other studies in breast (1) and prostate cancer (26) have given ambiguous / conflicting results e.g. serum IGFBP-3 levels in breast cancer have been shown to be decreased, increased and unchanged (1). It may be relevant that the major studies have predominantly used a commercial Kit that measures 'Total' IGFBP-3 (i.e. including fragments). A better correlation may be found if intact (i.e. IGF-binding) IGFBP-3 is measured. The correlation between high serum IGF levels and cancer progression remains (17).

Cancer	Ref.	Serum [IGF]	Serum [IGFBP]
Breast	(11)	IGF-I ↑	IGFBP-3 ↓
Prostate	(10)	IGF-I ↑	IGFBP-3 ↓
	(23)	IGF-I ↑	IGFBP-3 ↓
	(26)	IGF-I ↑	IGFBP-3 no association
	(25)		IGFBP-2 inversely related to progression
Colon	(20)	IGF-I ↑	IGFBP-3 ↓
Bladder	(28)	IGF-I ↑	IGFBP-3 ↓ (predictive)
Adenomas	(24)	IGF-II ↑	
Acute childhood leukemia	(22)		IGFBP-3 ↓ predicts survival
Lung	(19)	no association	IGFBP-1, 2, 3 no association

Support from studies in mice with a disrupted liver IGF-I gene

In mice with a disrupted IGF-I gene in the liver, serum IGF-I levels are 25% of normal without significantly affecting normal growth and development (27). Grafting of mouse adenocarcinomas resulted in tumors in 31% of these mice compared to 57% in control mice, along with a longer latency period and fewer hepatic metastases (27). However, replacement of serum [IGF-I] led to a 64.5% incidence of tumors in these mice. This study indicates that dietary restriction to reduce IGF-I levels may be a preventative measure and nutritional restriction experiments in animals support this hypothesis.

Specific Inhibition of the Type 1 IGF Receptor (IGF1R)

As the mitogenic effects of IGF-I require signalling through its Receptor, and IGF1R is over-expressed in many primary cancers, targeted disruption / inhibition of IGF1R as a cancer treatment has been investigated by many groups (2). Studies from knock-out mice have shown that low levels of IGF-I and IGF1R are associated with animal longevity (13).

IGFs bind to IGFBPs with higher affinity than the IGF Receptors.

Both the N- and C-terminal domains of all 6 IGFBPs are highly conserved, cysteine rich and appear to be required for high affinity IGF binding (4). The binding affinity of the IGFBPs for IGFs is greater than that of the IGF Receptors. Proteolysis of the IGFBPs appears to be required for IGF release (1).

Modulation of IGF and IGFBP Activity by Proteases:

Proteolytic cleavage of IGFBPs increases the availability of autocrine and paracrine IGFs for proliferative and invasive purposes. IGFBPs are cleaved at specific sites by a range of proteases including Prostate-specific antigen (PSA), Pregnancy associated plasma protein (PAPP-A), matrix metalloproteases (MMPs), cathepsin D, plasmin, thrombin and serine proteases. The majority of protease-sensitive sites are localized in the central non-conserved domain of the IGFBPs. For example, the central domain of IGFBP-3 is susceptible to proteolysis by plasmin, thrombin, cathepsin L and MMPs 1,2,3 (1). Following limited proteolysis, IGFBPs exhibit a dramatically reduced affinity for IGFs and some IGFBP fragments have IGF independent activities (1).

What to measure - total or intact (IGF-binding) IGFBP-3?

IGFBP-3 (264 aa, 43-45 kDa glycoprotein) is the major carrier of IGFs in serum and forms a 150 kDa ternary complex with an acid labile, leucine-rich glycoprotein, ALS, which binds to the IGFBP C-terminal domain (aa 216-231). This ternary complex does not traverse cell membranes (1).

Most epidemiological studies have used a Kit that measures 'Total IGFBP-3' i.e. including non-IGF-binding fragments. In serum from healthy individuals, 25.7 to 29.3% of serum IGFBP-3 is proteolysed (16). This % is unchanged in GH deficiency, despite very low [IGF], but is elevated (31.3 - 36.7%) in acromegalic, IDDM and NIDDM patients (16). The major IGFBP-3 proteolytic fragments detected comprise aa residues 1-160 (30 kDa) and 1-95, (20 kDa (glycosylated) or 16 kDa (nonglycosylated)). Studies of human serum IGFBP-3 fragments obtained by hemofiltration have indicated aa residues 1-98, (16 kDa and 11kDa (nonglycosylated), 1-172 (34kDa), and 139-157 or 139-159 (2-2.5kDa) (15). Studies determining both intact and total IGFBP-3 levels may indicate whether there is a better correlation between intact (IGF-binding) IGFBP-3 and IGF levels.

Biological Activity of the N- and C- terminal domains of IGFBP-3

Most IGFBP-3 fragments, including the N-terminal domain, have reduced or little affinity for the IGFs, although the C-terminal domain has moderate affinity (1nM) (21). An equimolar mixture of the N- and C-terminal domains binds IGF-I with a 13 fold reduced affinity (3.2 nM) and IGF-II with a 4 fold reduced affinity (12.2 nM). The trimeric complex of IGF and the N- and C-terminal domains of IGFBP-3 still inhibits IGF binding to IGF1R and IGF-II to IGF2R (21).

IGFBP-3 fragments - IGF independent actions of IGFBP-3 fragments

A 15 amino acid peptide sequence spanning the two serines (S111, S113) in the central domain of IGFBP-3, can mimic the UV-induced apoptosis by IGFBP-3 in KYSE190 oesophageal carcinoma cell line (12). This activity can be negated by phosphorylation of the serines (12). The C-terminal domain of IGFBP-3 contains a cell binding site and a nuclear localization signal, co-sited with a heparin binding site (aa 220-225). Via Importin B, this fragment can be transported into the nucleus where it is able to cross-signal with pathways used by receptors for retinoids, steroids, EGF, TGF β , TNF- α , HIF (6).

Properties of Non-IGF binding IGFBP-3

Non-IGF binding IGFBP-3, mutated by alanine substitution for 5 aa in the IGF binding site in the N-terminal domain - Ile56/Tyr57/Arg75/Leu77/Leu81 - has equal ability to induce apoptosis in prostate cancer PC3 cells (14). A similar situation occurs in breast cancer T47D cells, where aa 228-232 KGRKR mutated to MDGEA in the basic C-terminal region of IGFBP-3 has no effect on apoptosis, but blocks IGF binding, and renders the IGFBP-3 unable to associate with the cell surface receptor or translocate to the nucleus (9). This mutation is also within the region of the ALS binding site.

A Role for Intervention in the IGF System for Cancer Treatment?

The above studies give some indications of the multiple activities of the IGFBP family, and suggest it is highly likely that approaches targeting the IGF-I system at multiple sites - IGFBPs and IGF1R, as well as the use of agents that up-regulate IGFBP-3, such as anti-estrogens, anti-androgens, vitamin D, retinoic acid analogs may prove useful in cancer treatment (5).

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