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IGFBPs and Neoplastic Models

New Concepts for Roles of IGFBPs in Regulation of Cancer Cell Growth

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The insulin-like growth factor binding proteins (IGFBPs) are a family of seven structurally homologous proteins that bind insulin-like growth factor 1 (IGF-I) and IGF-II with high affinity, thereby modu-lating the actions of IGFs. Several lines of recent evidence from various cell systems have suggested that IGFBPs, especially IGFBP-3, may play more active, IGF-independent, roles in growth regulation of cancer cells. In support of this hypothesis, the author has recently shown that IGFBP-3 binds specifically and with high affinity to the surface of various cell types and directly inhibits monolayer growth of these cells in an IGF-independent manner, presumably by specific interaction with cell membrane proteins that function as an IGFBP-3 receptor. The author's current studies demonstrate that a new class of IGFBP, IGFBP-7, constitutes a low affinity member of the IGFBP family, but primarily functions as a modulator of cell growth in an IGF-independent manner, similar to the action observed with IGFBP-3 in breast cancer cells. The author's studies on the mechanisms of action of the low affinity IGFBPs will provide insight into the IGF-independent actions of the classical high affinity IGFBPs and their impact on cancer cell growth.

Key Words: Antiproliferation; breast cancer; antiestrogen; TGF-β; retinoic acid.

The insulin-like growth factor binding proteins (IGFBPs) are members of the IGF signaling system, includes the ligands (insulin, IGF-I and IGF-II), and a family of transmembrane receptors (the insulin, type 1 IGF and type 2 IGF receptors) *(1–9)*. The human IGFBP family consists of at least seven proteins, designated as IGFBP-1,-2, -3, -4, -5, -6, and -7 *(10–13)*. IGFBPs 1–6 bind IGF-I and IGF-II with high affinity and serve to transport the IGFs, prolong their half-lives, and modulate their proliferative and anabolic effects on target cells. The molecular mechanisms involved in the interaction of the IGFBPs, with the IGFs and their receptors remain unclear, but these molecules appear, at least, to regulate the availability of free IGFs for interaction with IGF receptors *(10,11)*. Recently, the author has identified mac25 as IGFBP-7, which binds IGFs with lower affinity than do the other IGFBPs, and which appears to be a low-affinity member of the IGFBP family *(13)*. Taken together the multiplicity of regulatory factors and variations in tissue distribution, it suggests that there is biological redundancy in IGFBPs.

The IGF system plays an important role in the neoplastic process in various tissues. Aberrant expression of IGFs and type 1 IGF receptor involved in deregulation of cell growth and, to some extent, transformation and tumorigenesis *(14,15)*. Many tumors and neoplastic cells also produce IGFBPs, which can regulate the biological activity of the IGFs in those cells. Breast cancer cells also secrete various types of IGFBPs. The predominant secreted IGFBP appears to correlate with the estrogen receptor status of the cell *(16)*. Estrogen-nonresponsive (ER-negative) cells predominantly secrete IGFBP-3 and IGFBP-4 as major species and IGFBP-6 as a minor binding protein, whereas estrogen-responsive (ER-positive) cells secrete IGFBP-2 and IGFBP-4 as major species, and IGFBP-3 and IGFBP-5 as minor proteins. These different patterns of IGFBP secretion in two different classes of breast cancer cells imply that the IGF system in breast cancer is complex, and that the biological significance and determination of the cellular response of IGFBPs to autocrine, paracrine, or endocrine-derived IGFs may be significantly different, depending on estrogen responsiveness.

IGFBP-3 is the principal IGFBP in adult serum, where it circulates as a 150 kDa-complex consisting of IGFBP-3, an acid-labile subunit (ALS), and IGF peptide *(17–19)*. Its principal role has been postulated to be the transport of IGFs, protecting them from rapid clearance and/or degradation *(20–22)*. In human breast cancer cells, expression of IGFBP-3 is hormonally regulated: Estrogen inhibits expression of IGFBP-3 in ER-positive cells, whereas antiestrogens stimulate production of IGFBP-3, suggesting

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that it may exert an important role in estrogen-induced cell proliferation *(23)*. Furthermore, post-translational modification of IGFBP-3 has been observed: IGFBP-3 can be proteolyzed by proteases such as cathepsin D, prostate-specific antigen (PSA), and plasmin, all of which can be detected in human breast cancer cells *(24–29)*. In general, IGFBP-3 proteases are postulated to play a role in altering tissue IGF availability by lowering the affinity of IGFBP-3 for its ligand, thereby increasing the availability of IGFs to cell-membrane receptors. PSA, for example, has been shown to reverse the inhibitory effect of IGFBP-3 on IGF-stimulated prostate cell growth by cleaving IGFBP-3 and generating IGFBP-3 fragments with lower affinity for IGFs *(30)*. Although the biological significance and molecular actions of IGFBP-3 proteolysis are unclear in human breast cancer cells, recent reports have demonstrated that IGFBP-3 fragments generated by limited plasmin digestion inhibit the mitogenic effects of IGF-I and insulin, despite the significantly reduced affinities of those fragments for IGF-I, suggesting that IGFBP-3 fragments are bioactive.

Several recent lines of evidence have indicated that IGFBP-3 potently inhibits breast cancer cell growth in an IGF-independent manner. We have recently shown that IGFBP-3 binds specifically to the surface of human breast cancer cells and directly inhibits monolayer growth of these cells *(31)*. The interaction of IGFBP-3 with the breast cancer cell surface and its subsequent biological effects are mediated through IGFBP-3-specific cell-surface association proteins; these cell-surface proteins are putative IGFBP-3-specific receptors that mediate the direct inhibitory effect of IGFBP-3 on monolayer growth of cells *(32)*. Further studies from our laboratory and others have confirmed our hypothesis that the IGFBP-3/IGFBP-3 receptor complex functions as a major growth-suppressing factor in various cell systems by showing that;

1. Transforming growth factor-β (TGF-β)- and retinoic acid (RA)-induced cell growth inhibition in estrogen receptor (ER)-negative human breast cancer cells is mediated through IGFBP-3 action *(33,34)*,
2. The mitogenic effect of estrogen is accompanied by inhibition of IGFBP-3 expression, whereas antiestrogens such as tamoxifen and ICI 182, 780 stimulate expression of IGFBP-3 in ER-positive breast cancer cells *(23)*,
3. IGFBP-3 mediates antiestrogen-induced growth inhibition in ER-positive breast cancer cells *(35)*,
4. The growth rate is significantly reduced in human IGFBP-3-transfected fibroblast cells derived from mouse embryos homozygous for a targeted disruption of the type 1 IGF receptor gene *(36)*,
5. Purified mouse IGFBP-3 binds to the chick embryo fibroblast cell surface and inhibits cell growth *(37)*,
6 Up-regulation of IGFBP-3 expression by RA is correlated with RA-induced cell growth inhibition in cervical epithelial cells *(38)*, and

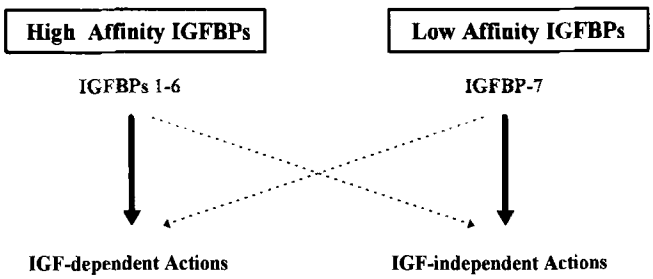

Fig. 1. IGFBPs and their biological actions.

7. Regulation of IGFBP-3 gene expression has been shown to play a role in signaling by p53, a potent tumor suppressor protein *(39)*.

Thus, IGFBP-3 appears to be a major factor in a negative control system involved in regulating cell growth in various systems including human breast cancer cells in vitro.

To extend the understanding of the IGF-independent action of IGFBPs, the author has investigated the biological action of IGFBP-7 in human breast cancer cells. Using baculovirus-expressed human IGFBP-7 (IGFBP-7^{bac}) and polyclonal antibodies specific for IGFBP-7, the author has found that expression of IGFBP-7 mRNA and protein is up-regulated by TGF-β in Hs578T breast cancer cells. Intriguingly, treatment with IGFBP-7^{bac} resulted in inhibition of DNA synthesis and cell proliferation in a dose-dependent manner in human breast cancer cells, even in the absence of IGF peptides. Even though IGFBP-7 exhibits low affinity for IGFs, its biological actions are predominantly IGF-independent. Overall structural similarity between IGFBP-7 and classical high affinity IGFBPs 1-6 suggests that the mechanisms of action and signaling pathways used by IGFBP-7 may provide insight into the IGF-independent actions of the high affinity IGFBPs (Fig. 1).

In conclusion, IGFBPs, especially IGFBP-3 and -7, play important roles in breast cancer cell proliferation not only by regulating IGF access to IGF receptors (IGF-dependent mechanism) but also by their direct actions (IGF-independent mechanism). Therefore, a fuller understanding of the IGF-independent action of IGFBPs will allow us to understand how the growth of neoplastic cells can be modulated by the IGF/IGFBP system, and how other growth factors or pharmacological agents can interface with this system.

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