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Structure and actions of insulin-like growth factor binding protein-2 (IGFBP-2)

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Abstract

The insulin-like growth factor (IGF) system consists of growth factors, receptors and binding proteins, and overlaps significantly with insulin signalling pathways. IGF-1 and IGF-2 are essential for growth and development and have also been shown to play a key role in insulin sensitivity, obesity, endothelial dysfunction, angiogenesis and tumorigenesis. They are tightly regulated by a family of six binding proteins (IGFBP) which form complexes with IGF, with only 1% of IGF free in the circulation. Despite this apparently predominantly inhibitory role, IGFBPs have been shown to have a variety of effects on cell growth, proliferation, angiogenesis and metabolism, with both IGF dependent and independent effects observed. IGFBP-2 in particular has been shown in several studies to be linked to tumour metastasis and poorer outcomes in cancer, and has been shown to promote tube formation, proliferation, migration and angiogenesis *in vitro*, through a variety of pathways. Higher circulating levels of IGFBP-2 have also been shown to protect against obesity and improve insulin sensitivity. In this brief review we will look at some of these actions in more detail, and describe a potential future role for IGFBP-2 incorporation in cell based therapies.

Introduction

The insulin-like growth factor (IGF) system is a complex hierarchy of growth factors, receptors and binding proteins, and exhibits significant overlap with traditional insulin signalling pathways. IGF-I share common ancestry with insulin and have a nearly homologous structure [1]. They therefore not only interact with IGF receptors, but also insulin and hybrid receptors [1]. IGF synthesis is primarily mediated by growth hormone rather than blood glucose, though free IGF-1 does play a role in blood glucose homeostasis [2]. Through these interactions, they promote cell growth and survival and are indispensable in normal development and whole-body metabolism [3]. However, dysregulation of both IGF activity and IGF-1 receptor expression has also been implicated in tumorigenesis, cardiovascular disease and diabetes mellitus [4,5].

Less than 1% of IGF is unbound in plasma, with activity tightly modulated by the insulin-like growth factor binding proteins (IGFBPs). These are a family of six structurally similar proteins, acting primarily to regulate the activity of IGFs through the binding and transport of these proteins within the vasculature and into peripheral tissues [6]. Through this binding, they increase IGF half-life and inhibit receptor interactions. They may additionally stimulate IGF activity by transporting directly to the IGF-1 receptor [7], and certain IGFBPs have reduced affinity for IGF when they are bound to cellular membrane receptors, increasing IGF bioavailability in the pericellular environment [8]. Although each binding protein has complementary structure and binding domains, this apparent paradoxical activity relates to the differing nature of interactions between the IGFs and each binding protein [9].

Evidence is also emerging that the IGFBPs can both potentiate the action of IGF and act independently [10,11], and the dependent and independent effects of IGFBP-2 in particular will be explored further in this mini review.

IGFBP-2 is the second most abundant of the IGFBPs, and has been strongly associated with metastasis and angiogenesis in the context of malignancies [12]. It has been shown to promote glioma progression and invasion [13] and is positively associated with both prostate and breast cancer grade and metastasis [14,15]. Furthermore, knockdown of IGFBP-2 in mice has been shown to reduce breast cancer metastatic colonisation of the lungs as well as tumour vascular density[16]. However, IGFBP-2 has also been shown to have protective effects against diet-induced obesity and enhance insulin sensitivity, independent of IGF-1 activity [17].

Structure

IGFBP-2 is a 36kDa protein with three distinct structural regions in common with the other IGFBPs: a N-terminal cysteine rich region, C-terminal cysteine rich region and link region [18]. Both the N and C terminals bind IGFs, with affinity of each to IGF varying between the IGFBPs [18]. In addition, IGFBP-2 possesses a heparin binding domain (HBD) and integrin binding domain (RGD), which are found in the link region and C-terminal region respectively. Both of these motifs are functional and mediate both IGF-dependent and -independent actions, with the RGD motif predominantly mediating cell membrane integrin interactions [19]. In contrast, the HBD motif in the link region interacts with components of the extra cellular matrix (ECM) and promotes cell proliferation and migration [20]. An additional HBD domain has been identified in the C-terminal region, although less is known about its interactions [21].

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IGF-dependent actions

IGFBP-2 has been shown as a major regulator of angiogenesis in malignant melanomas. It appears to be induced by the expression of mda-9/syntenin, a protein associated with melanoma progression and metastasis [22]. In the context of human umbilical vein endothelial cells (HUVECs), addition of recombinant human IGFBP-2 augments cell proliferation and tube formation *in vitro*, and this effect is negated by an IGFBP-2 neutralising antibody [23]. Although this pro-angiogenic effect appears to be mediated by interaction with the integrin receptor aVβ3, IGF-1 receptor knockdown significantly attenuates the effects of IGFBP-2, suggesting an inter-dependent relationship between IGF-1 and IGF-1 receptor [23]. Interestingly, IGFBP-2 downregulates IGF-mediated proliferation of cells in the context of breast carcinoma through interactions with the same integrin receptor, suggesting that observed effects may be determined by environmental milieu [24].

Others have shown complementary findings, with IGFBP-2 shown to bind to receptor protein tyrosine phosphatase β (RPTPβ) via its HBD domain in the link region [25]. This caused inactivation of RPTPβ and inhibited transcription of the tumour suppressor gene PTEN. Inhibition of PTEN enables downstream activation of the PI3K/AKT pathway and promotes vascular smooth muscle cell (VSMC) proliferation. However, inhibition of IGF-1 receptor expression prevented RPTPβ inactivation, suggesting that effects of IGFBP-2 on PTEN phosphorylation require coordination of IGFBP-2, IGF-1 and its receptor [25].

The role of microRNA-126 in silencing IGFBP-2 induced metastatic angiogenesis has also been demonstrated in endothelial cells [16]. IGFBP-2 secreted by breast carcinoma cells positively modulated IGF-1 mediated activation of IGF-1 receptors on endothelial cells, promoting their recruitment. This interaction was suppressed by miRNA-126, and enhanced with miRNA-126 knockdown.

IGF-independent actions

The interaction of IGFBP-2 with integrins and ECM components is well established [26]. However, others have shown IGFBP-2 to localise to the nuclei of neuroblastoma cells, subsequently promoting angiogenesis through upregulation of VEGF mRNA transcription as well as upregulating activation of other protumorigenic genes [27]. This upregulation was only seen in the presence of intracellular IGFBP-2, with no role observed for IGF or its receptor. The same group later showed that nuclear translocation was mediated through a nuclear localisation signal sequence within the IGFBP-2 link domain, and that an IGFBP-2 mutant that could not enter the nucleus was unable to upregulate VEGF expression in the same fashion [28]. Nuclear translocation of IGFBP-2 was also seen in breast and prostate cancer cells in addition to neuroblastoma, indicating the potential for a similar role across several cancer types [28].

IGFBP-2 levels also appear to be regulated by hypoxia-inducible factor-1α (HIF-1α) [29], a growth factor strongly associated with the metastatic phenotype [30]. This association would be consistent with the role of IGFBP-2 in tumour angiogenesis.

A key role for IGBP-2 in the development and progression of lymphangioleiomyomatosis (LAM) has also been demonstrated [31]. This is an unusual disease affecting mainly young women, in which LAM cells – a histologically benign appearing smooth muscle-like cell - proliferate and metastasize to the lungs, causing progressive remodelling and emphysematous-like lung disease [32]. IGFBP-2 was found to accumulate in the nucleus of these cells independently of IGF-1, with nuclear translocation mediated by estrogen-receptor alpha (ER-α). Knockdown of IGFBP-2 by siRNA reduced LAM cell proliferation, migration and invasiveness, indicating the importance of IGFBP-2 in these tumorigenic actions. Additionally, IGFBP-2 knockdown abrogated mitogen-activated protein kinase (MAPK) phosphorylation, explaining a potential mechanism for its tumorigenic effects [31].

Summary

IGFBP-2 exhibits significant effects in a wide range of cell types, although the majority of studies thus far have assessed its role in the context of tumorigenesis. Effects appear to be both IGF-dependent and -independent, with function and activity affected by cell type as well as receptor activation and nuclear localisation. Its pro-angiogenic potential and upregulation in hypoxic conditions lends IGFBP-2 to be potentially reparative in disorders such as ischaemic heart disease or peripheral arterial disease. Nonetheless, further studies to scrutinise this potential are warranted.

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