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Review article

Current ideas on the biology of IGFBP-6: More than an IGF-II inhibitor?

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ABSTRACT

IGFBP-6 binds IGF-II with higher affinity than IGF-I and it is a relatively specific inhibitor of IGF-II actions. More recently, IGFBP-6 has also been reported to have IGF-independent effects on cell proliferation, survival, differentiation and migration. IGFBP-6 binds to several ligands in the extracellular space, cytoplasm and nucleus. These interactions, together with activation of distinct intracellular signaling pathways, may contribute to its IGF-independent actions; for example, IGF-independent migration induced by IGFBP-6 involves interaction with prohibitin-2 and activation of MAP kinase pathways. A major challenge for the future is delineating the relative roles of the IGF-dependent and –independent actions of IGFBP-6, which may lead to the development of therapeutic approaches for diseases including cancer.

Regulation of insulin-like growth factor (IGF) actions is critically important because of their essential physiological roles and the potential contribution of abnormally high or low IGF levels to a range of common disease processes including cancer and atherosclerosis [1]. Not surprisingly, this regulation takes place at a number of levels. Expression of both IGF-I and IGF-II is transcriptionally regulated and tissue-specific. IGFs act predominantly through the IGF-I receptor but some actions are mediated by insulin and IGF-II/mannose 6-phosphate receptors [2–4]. Expression of each of these receptors is also tissue-specific and regulated transcriptionally. In addition, IGF actions are regulated by the family of six high-affinity IGF binding proteins (IGFBPs) [5,6]. Each of these IGFBPs inhibits IGF actions, whereas some potentiate IGF actions under some circumstances. IGFBPs differ in their IGF binding affinities, sites of expression, transcriptional regulation and susceptibility to specific proteases, resulting in a complex system that finely regulates IGF actions. Most actions of IGFBPs are extracellular, but recent evidence suggests they also have intracellular actions, including actions within the nucleus [6].

IGFBPs share a common three-domain structure, with sequences within their conserved N-terminal and C-terminal domains both contributing to high affinity IGF binding [5]. The linker domains joining these domains are not conserved between IGFBPs, are thought to be unstructured, and are sites of post-translational modifications including glycosylation, phosphorylation and proteolysis. IGFBPs are effective competitive inhibitors of IGF actions because they bind IGFs with higher affinity than IGF receptors and their binding sites overlap with or are adjacent to IGF receptor binding sites, precluding simultaneous binding [7]. Proteolysis of IGFBPs is one mechanism for release of IGFs from IGFBP complexes [8]. Additionally, some IGFBPs have been shown to associate with cell surfaces and this may result in enhancement of IGF actions by concentrating and localizing IGFs close to IGF receptors [8]. Binding of IGFBPs to glycosaminoglycans within cell membranes and extracellular matrix is one mechanism of cell association which also results in decreased IGF binding affinity and therefore may facilitate release of free IGFs for receptor binding.

Over the last two decades, a number of IGFBPs have also been shown to have IGF-independent actions [8]. Most studies demonstrating these effects have been performed *in vitro*, but *in vivo* evidence has also recently emerged. At least some of these IGF-independent actions are mediated by binding of IGFBPs to a number of non-IGF ligands, including extracellular proteins and proteoglycans, cell surface proteins and receptors, intracellular proteins, and nuclear receptors [6]. Additionally, IGFBPs may modulate gene transcription by intranuclear interactions. Most of these non-IGF ligands interact with the C-terminal domains of IGFBPs [5]; in particular, some IGFBPs have a sequence of highly basic amino acids that mediate binding to a range of ligands including importins, resulting in nuclear localization, and glycosaminoglycans.

1. IGFBP-6

IGFBPs 1–3 were the first to be identified and characterized, beginning in the 1970s and continuing into the mid-1980s. A few years later, three more IGFBPs, including IGFBP-6, were identified and cloned [9–13].

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Although IGFBP-6 is a member of the IGFBP family and shares most of its common properties, it has a number of distinct functional and structural characteristics [14–16]. The most prominent of these is its ~ 50-fold binding preference for IGF-II over IGF-I [9–11,17], compared with the equal or slight IGF-II preference of IGFBPs 1–5. All six IGFBPs have three conserved disulphide bonds in their C-terminal domains, and a high affinity IGF binding region with two conserved disulphide bonds in the second half of their N-terminal domains. However, IGFBP-6 differs from IGFBPs 1–5 in the first half of the N-terminal domain. The latter have four conserved disulphide bonds in this region, but IGFBP-6 has only three that differ [18]. Additionally, this region of IGFBP-6 has a different three-dimensional structure than that of the other IGFBPs [19].

Like the other IGFBPs, IGFBP-6 has a number of post-translational modifications. It is O-glycosylated in its linker domain [20]; the addition of carbohydrate inhibits binding of IGFBP-6 to glycosaminoglycans, decreases proteolysis, and extends its circulating half-life, but it has no direct effect on IGF binding affinity [21]. Nevertheless, these effects of O-glycosylation may contribute to the predominantly inhibitory effects of IGFBP-6 on IGF-II actions. IGFBP-6 may also be phosphorylated and sulfated, although the biological significance of these modifications has not been defined [22]. IGFBP-6 is susceptible to proteolysis in its linker domain by a range of specific proteases [23].

At least in part because of its IGF-II binding preference, IGFBP-6 is a relatively specific inhibitor of IGF-II actions [14–16]. This has been demonstrated for many actions such as proliferation, survival, migration and differentiation of many cell types (Figs. 1–4), including normal and cancer cells. As is the case for all IGFBPs, IGFBP-6 is expressed widely in many tissues. These include lung, liver, gut and the central nervous system for this particular IGFBP. Its expression is regulated by a number of physiological effectors including cAMP, IGFs, retinoic acid, vitamin D, p53 and glucocorticoids. Wnt and Hedgehog signaling pathways are also involved in IGFBP-6 regulation. In keeping with the role of dysregulation of the IGF system in many diseases, IGFBP-6 levels are also altered in many of these. In particular, IGFBP-6 levels are decreased in many but not all cancer cells as discussed below [23].

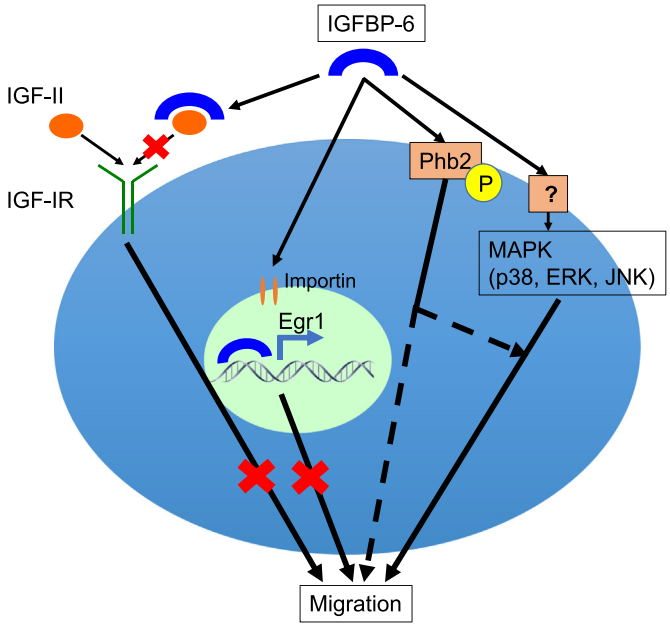

Fig. 1. Effects of IGFBP-6 on cell migration. In some cells, IGFBP-6 impairs migration by inhibiting IGF-II actions (left) and by promoting EGR-1 transcription in an IGF-independent manner (middle). In contrast, IGFBP-6 promotes migration of cells by pathways involving binding to Phb2 and MAP kinase activation (right). Phb2 acts downstream and/or independently of MAP kinase pathways.

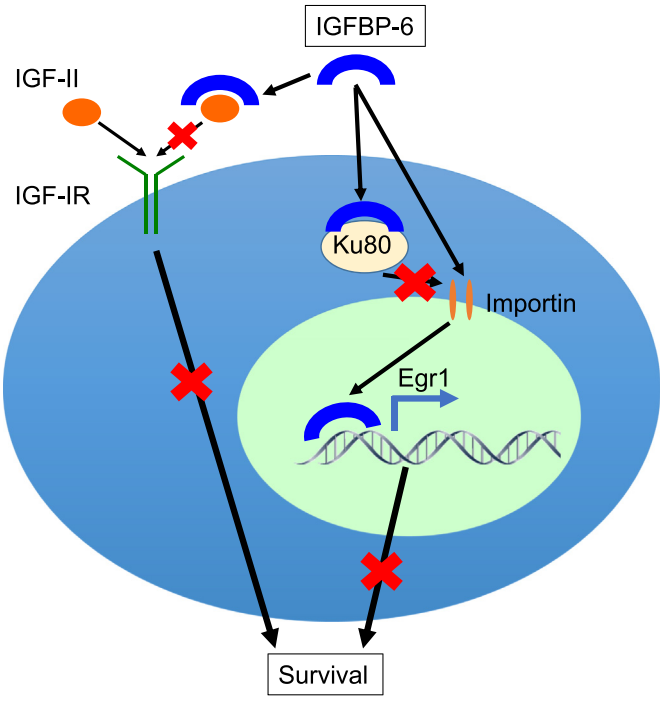

Fig. 2. Effects of IGFBP-6 on cell survival. IGFBP-6 impairs survival by inhibiting IGF-II actions (left) and modulating cytoplasmic-nuclear translocation of Ku80 to increase EGR-1 transcription in an IGF-independent manner (right).

2. IGF-independent actions of IGFBP-6

In recent years, a number of studies have demonstrated IGF-independent actions of IGFBP-6. Although IGFBP-6 decreases proliferation of many cell types by inhibiting IGF-II [14], it was more recently shown to inhibit fibroblast proliferation by both IGF-dependent and –

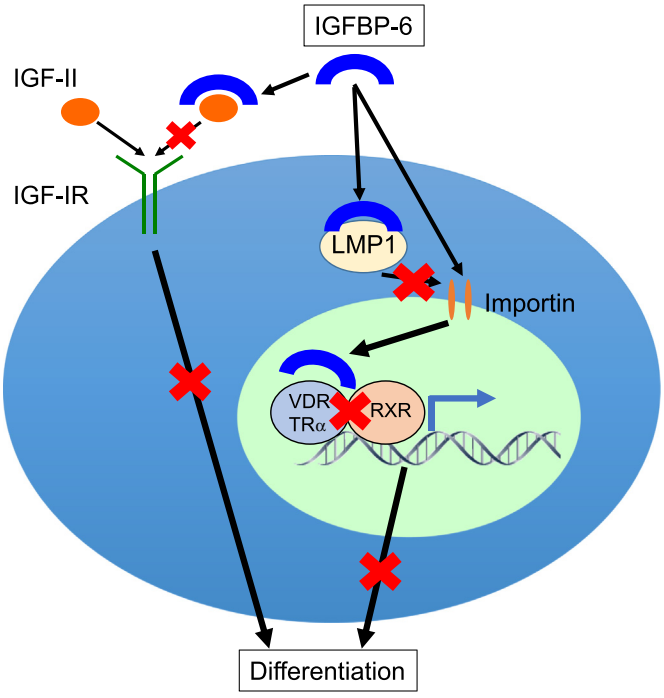

Fig. 3. Effects of IGFBP-6 on cell differentiation. IGFBP-6 impairs differentiation by inhibiting IGF-II actions (left) and by modulating cytoplasmic-nuclear translocation of LMP-1 and nuclear receptor-mediated transcription in an IGF-independent manner (right).

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