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Nuclear actions of insulin-like growth factor binding protein-3

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ABSTRACT

In addition to its actions outside the cell, cellular uptake and nuclear import of insulin-like growth factor binding protein-3 (IGFBP-3) has been recognized for almost two decades, but knowledge of its nuclear actions has been slow to emerge. IGFBP-3 has a functional nuclear localization signal and interacts with the nuclear transport protein importin-β. Within the nucleus IGFBP-3 appears to have a role in transcriptional regulation. It can bind to the nuclear receptor, retinoid X receptor-α and several of its dimerization partners, including retinoic acid receptor, vitamin D receptor (VDR), and peroxisome proliferator-activated receptor-γ (PPARγ). These interactions modulate the functions of these receptors, for example inhibiting VDR-dependent transcription in osteoblasts and PPARγ-dependent transcription in adipocytes. Nuclear IGFBP-3 can be detected by immunohistochemistry in cancer and other tissues, and its presence in the nucleus has been shown in many cell culture studies to be necessary for its pro-apoptotic effect, which may also involve interaction with the nuclear receptor Nur77, and export from the nucleus. IGFBP-3 is p53-inducible and in response to DNA damage, forms a complex with the epidermal growth factor receptor (EGFR), translocating to the nucleus to interact with DNA-dependent protein kinase. Inhibition of EGFR kinase activity or downregulation of IGFBP-3 can inhibit DNA double strand-break repair by nonhomologous end joining. IGFBP-3 thus has the ability to influence many cell functions through its interactions with intranuclear pathways, but the importance of these interactions *in vivo*, and their potential to be targeted for therapeutic benefit, require further investigation.

Contents

Abbreviations: IGFBP-3, insulin-like growth factor binding protein-3; ALS, acid-labile subunit; IGF1R, type 1 IGF receptor; GRP78, glucose-regulated protein 78; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; EGFR, epidermal growth factor receptor; NLS, nuclear localization signal; NES, nuclear export signal; RXRα, retinoid X receptor-α; NR, nuclear receptor; RARα, retinoic acid receptor-α; VDR, vitamin D receptor; PPARγ, peroxisome proliferator-activated receptor-γ; atRA, all-*trans* retinoic acid; 15dPG, 15-deoxy$\Delta^{12,14}$prostaglandin J_2; ATM, ataxia-telangiectasia mutated; HDAC, histone deacetylase; SphK1, sphingosine kinase 1; S1P, sphingosine-1-phosphate.

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1. Key features of IGFBP-3

Insulin-like growth factor binding protein-3 (IGFBP-3) is a multifunctional protein with a strong evolutionary link to the five other members of the IGFBP family (Daza et al., 2011). These proteins share the properties of high-affinity IGF binding and a high degree of structural conservation in their cysteine-rich amino- and carboxyterminal domains (Forbes et al., 2012). The terminal domains each comprise about one-third of the mature 28.7-kDa IGFBP-3 protein (Baxter, 2000). The central or linker domain shows little or no structural similarity among the six IGFBPs.

Like the other IGFBPs, IGFBP-3 is secreted by many cell types and is found in the circulation where, in adults, it is by far the most abundant IGFBP, with typical levels of 3–5 mg/L (Baxter, 1993; Friedrich et al., 2014). IGFBP-3 is the main circulating transport protein for IGF-I and IGF-II, which compete for a single binding site with similar affinities around 10^{10} L/mol (Martin and Baxter, 1986). Circulating IGF–IGFBP-3 complexes are found almost entirely bound to another protein, the acid-labile subunit (ALS), to form ternary complexes (Baxter et al., 1989). Among the other IGFBPs, only IGFBP-5 forms similar complexes with ALS (Twigg and Baxter, 1998). The site of interaction of IGFBP-3 with ALS includes a basic motif in the carboxyterminal domain (Fig. 1); mutation of residues 228–232 to the corresponding residues of IGFBP-1 decreased ALS affinity by >90% while having little effect on IGF-binding affinity (Firth et al., 1998). Note: in this review, amino acid residues are numbered for mature IGFBP-3 containing 264 amino acids, excluding the 27-residue signal peptide.

By binding the anabolic and mitogenic peptides IGF-I and IGF-II with high affinity and restricting their access to their shared receptor, the type 1 IGF receptor (IGF1R), IGFBP-3 is growth-inhibitory in many systems *in vitro* and *in vivo* (Firth and Baxter, 2002). High-affinity binding of the IGFs appears to be achieved by their interaction with hydrophobic residues in the aminoterminal domain (Fig. 1), as well as carboxyterminal residues (Payet et al., 2003; Forbes et al., 2012), which may act cooperatively to maintain IGF binding even if the protein is partially proteolyzed (Yan et al., 2009), as observed in pregnancy and some other conditions (Hughes et al., 1995). Although isolated IGFBP-3 fragments have greatly reduced IGF-binding affinity, there are reports that they retain growth-inhibitory activity in a variety of cell systems (Lalou et al., 1997; Booth et al., 1999).

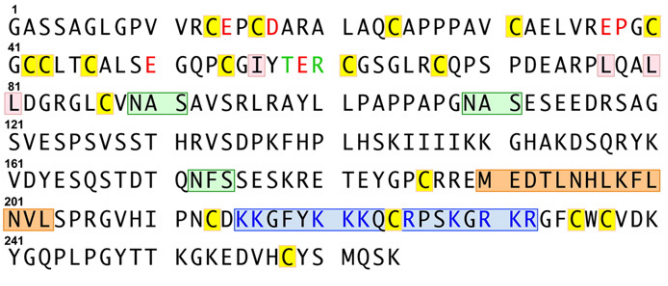

Fig. 1. Aminoacid sequence of mature human IGFBP-3 (residues 1–264), omitting the 27-residue signal peptide. Conserved cysteine residues are boxed in yellow, and hydrophobic residues involved in IGF binding, in pink. Residues assumed (by comparison with IGFBP-5) to be involved in transcriptional regulation (Zhao et al., 2006), are in red font; residues shown by mutagenesis to be involved in nuclear receptor binding (Schedlich et al., 2007a), in green font. The bipartite nuclear localization signal that includes residues that bind ALS and RXRα is boxed in blue, and its basic residues are shown in blue font. The three N-glycosylation motifs are boxed in green. Putative overlapping hydrophobic nuclear export sequences are boxed in orange. The canonical 264-residue mature sequence shown above has been termed *isoform 1* or *isoform b* (NCBI Reference Sequence: NP_000589.2, UniProt: P17936-1). The 270-residue mature product of an alternatively spliced transcript, termed *isoform 2* or *isoform a* (NCBI Reference Sequence: NP_001013416.1, UniProt: P17936-2) has a 6-residue insertion after Pro107.

1.1. Post-translational modification

IGFBP-3 has three potential sites of N-linked glycosylation (Fig. 1), of which either two (Asn89, Asn109) or all three are normally occupied by glycans, resulting in a doublet of approximately 40 kDa when analyzed by SDS-PAGE (Martin and Baxter, 1986; Firth and Baxter, 1999). Decreased glycosylation has little effect on IGF or ALS binding by IGFBP-3 but enhances its cell-surface binding (Firth and Baxter, 1999), as well as its interaction with glucose-regulated protein 78 (GRP78) (Grkovic et al., 2013). Since this interaction has been implicated in the induction of autophagy by IGFBP-3, it has been proposed that IGFBP-3 hypoglycosylation under conditions of nutritional deprivation might be a signal for enhanced autophagy (Grkovic et al., 2013).

The other well-characterized post-translational modification of IGFBP-3 is phosphorylation. The IGFBP-3 sequence contains multiple consensus sites for Ser/Thr phosphorylation (Coverley and Baxter, 1997), and appears to be constitutively phosphorylated at the protein kinase CK2 sites, Ser111 and Ser113 (Hoeck and Mukku, 1994). CK2 phosphorylation of IGFBP-3 has no effect on IGF binding but decreases its binding to ALS and the cell surface, and makes it relatively resistant to proteolysis (Coverley et al., 2000). In contrast to the lack of effect of CK2, and several other Ser/Thr kinases, on IGF binding, phosphorylation of IGFBP-3 by DNA-dependent protein kinase, catalytic subunit (DNA-PKcs) abolishes IGF binding (Schedlich et al., 2003). The role of this phosphorylation is further discussed below. IGFBP-3 also contains several predicted tyrosine kinase consensus sites (http://www.hprd.org/PhosphoMotif_finder), including sites for Src kinase and EGF receptor (EGFR) kinase. Phosphotyrosine residues identified by mass spectrometry include the putative EGFR kinase sites Tyr163 and Tyr183 (http://www.phosphosite.org/).

This review will focus on nuclear actions of IGFBP-3, and takes many examples from cancer cell biology, where most of the relevant discoveries have been made. For a broader view of IGFBP-3 physiology, genetics, and signaling, the reader is referred to other recent reviews (Jogie-Brahim et al., 2009; Yamada and Lee, 2009; Baxter, 2014; Johnson and Firth, 2014).

2. Nuclear import and export of IGFBP-3

2.1. Cell uptake and nuclear import

Radulescu (Radulescu, 1994) first identified a putative nuclear localization signal (NLS) in the IGFBP-3 sequence, although its activity was not tested. It was postulated that IGFBP-3 might associate in the nucleus with IGF-I, which was shown in an earlier electron microscopy study to translocate to the nucleus of chicken lens epithelial cells (Soler et al., 1990). Subsequently it was shown that cells at the growing edge of a monolayer could internalize both IGF-I and IGFBP-3 to the nucleus, where they co-localized (Li et al., 1997). In contrast, in resting cells the two proteins co-localized in endosome-like structures. IGF-I nuclear transport, demonstrated in digitonin-permeabilized Chinese hamster ovary cells, was dependent on IGFBP binding, since LR3-IGF-I, an IGF-I analog with greatly reduced IGFBP interaction, showed little nuclear translocation (Schedlich et al., 2003). The presence of nuclear IGFBP-3 is well-recognized in clinical histopathology (Hunziker et al., 2008; Seligson et al., 2013) and in cell lines (Jaques et al., 1997; Wraight et al., 1998), and extensive cell biology studies suggest important biological roles for IGFBP-3 in the nucleus.

Extracellular IGFBP-3 has been shown to enter the cell through a variety of endocytic mechanisms that may involve both caveolin 1 and clathrin-coated pits (Lee et al., 2004; Micutkova et al., 2012). Its nuclear import appears to share a common pathway with IGFBP-5 (Schedlich et al., 1998), both mediated by binding to importin-β (also known as karyopherin-β) (Schedlich et al., 2000; Micutkova et al., 2012), part of the importin-α/β nuclear transport complex. This contrasts with IGFBP-6, which interacts more strongly with importin-α (Iosef et al.,