

IGFBP-2 - taking the lead in growth, metabolism and cancer

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Abstract The activity of the Insulin-like Growth Factors (IGFs) ligands elicited via their receptors and transduced by various intracellular signal pathways is modulated by the IGF Binding Proteins (IGFBPs). Among all the IGFBPs, IGFBP-2 has been implicated in the regulation of IGF activity in most tissue and organs. Besides binding to IGFs in the circulation these IGF-regulatory activities of IGFBP-2 involve interactions with components of the extracellular matrix, cell surface proteoglycans and integrin receptors. In addition to these local peri-cellular activities, IGFBP-2 exerts other key functions within the nucleus, where IGFBP-2 directly or indirectly promotes transcriptional activation of specific genes. All of these IGFBP-2 activities, intrinsic or dependent on IGFs, contribute to its functional roles in growth/development, metabolism and malignancy as evidenced by studies in IGFBP-2 animal models and also by many in vitro studies. Finally, preclinical studies have demonstrated that IGFBP-2 administration can be beneficial in improving metabolic responses (inhibition of

adipogenesis and enhanced insulin sensitivity), while blockade of IGFBP-2 appears to be an effective approach to inhibiting tumour growth and metastasis.

Keywords IGF system · IGFBP-2 · Growth · Metabolism · Obesity · Diabetes · Leukaemia · Lung cancer · Colon cancer

The IGF system (Fig. 1)

The insulin-like growth factors I and II (IGF-I and IGF-II) are growth-promoting peptides (Zapf and Froesch 1986; Daughaday and Rotwein 1989), that are expressed in most tissues from early in embryonic development, with IGF-II being highly expressed in utero and IGF-I prevalently expressed during postnatal life (Daughaday and Rotwein 1989; Han et al. 1987; Murphy et al. 1987).

The biological action of the IGFs are mainly mediated by the type I IGF-receptor (IGF1R) (Sepp-Lorenzino 1998) (Fig. 1a). The IGF1R receptor and insulin receptor (IR) are very similar in structure and function (Sepp-Lorenzino 1998), however, the insulin receptor mediates insulin specific metabolic functions, whereas the IGF-I receptor mediates IGF-I mitogenic and differentiation functions.

There are also various IGF-I receptor subtypes (Milazzo et al. 1992), which have one $\alpha_{\text{ins}}\text{-}\beta_{\text{ins}}$ IR dimer and one $\alpha_{\text{I}}\text{-}\beta_{\text{I}}$ IGF1R dimer (Moxham et al. 1989) and bind insulin as well as IGFs with relatively high affinity (Siddle et al. 1994) (Fig. 1a). IGF-II exerts insulin-like effects via the insulin and the IGF1R. However, IGF-II has great affinity for a distinct receptor, the IGF-II receptor (Sakano et al. 1991), which is identical to the cation-independent mannose-6 phosphate receptor (M6P) (Bräulke 1999) (Fig. 1b).

S. W. Yau and W. J. Azar contributed equally to this work.

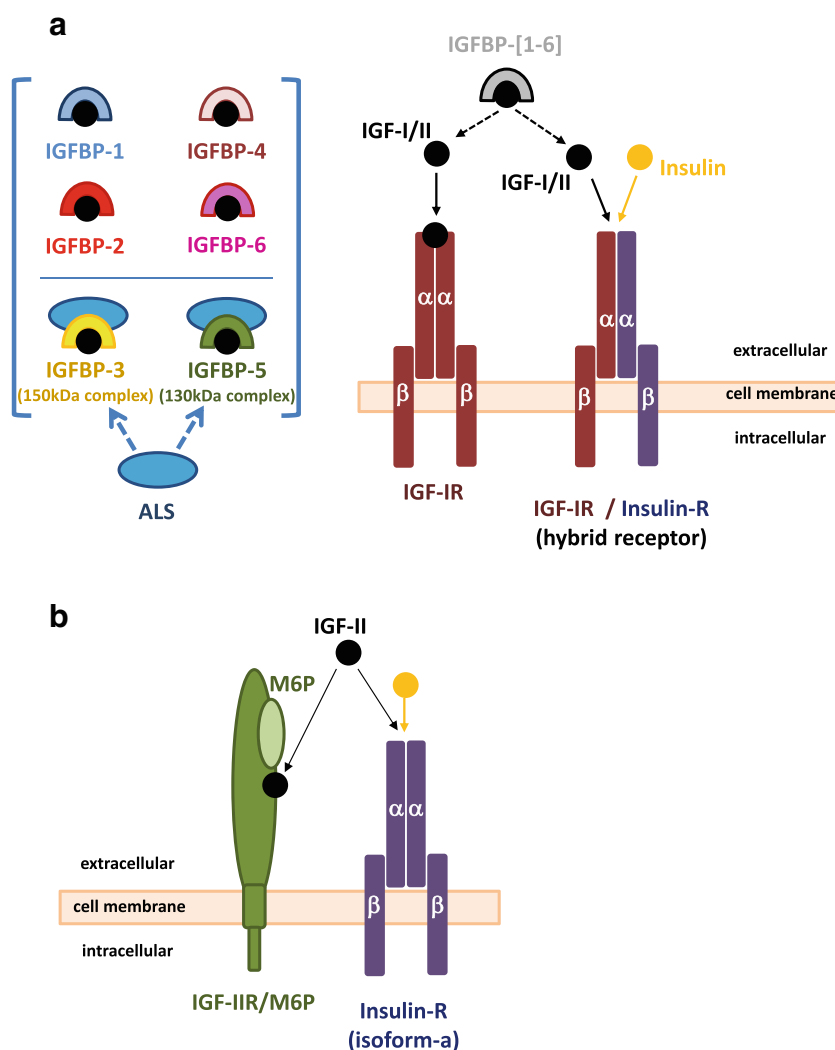
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Fig. 1 IGF System. The IGF system comprises of the IGF-I and IGF-II ligands, cell-membrane receptors, IGF1R and IGF-IIR/M6P (*lower panel*), insulin receptor isoform-a (IRa; *lower panel*) and IGF1R/IR hybrid receptors. Insulin is also included in these schematic cartoons to illustrate cross modulation of IRa and IGF1R/IR hybrid receptors by the three ligands. Six high affinity IGF binding proteins (IGFBPs; IGFBP-[1–6]) modulate IGF-I/II activities, with IGFBP-3 and IGFBP-5 also forming complexes with the acid labile subunit (ALS). IGFBPs in addition to modulating IGFs bioavailability to their receptors with involvement of specific IGFBP-proteases, possess intrinsic activities that are exerted via cell membrane receptors or via translocation into the cell and nuclear compartment (shown for IGFBP-2 in Fig. 3). These activities are exerted in the presence or absence of the IGF ligands (see also Fig. 3)



There are six high affinity insulin-like growth factor binding proteins (IGFBPs) (Firth and Baxter 2002), which coordinate and regulate IGFs bioavailability and activity (Firth and Baxter 2002) (Fig. 1a). The IGFBPs bind IGF-I and IGF-II, but do not bind insulin. IGFBP-3 and IGFBP-5 can also form a ternary complex with IGF-I and acid-labile subunit (ALS) (Twigg et al. 2000) (Fig. 1a).

This review focuses on IGFBP-2 physiology and particularly discusses the role of IGFBP-2 in growth (bone), metabolism and cancer.

IGFBP-2 protein structure (Fig. 2)

Similarly to the other IGFBPs (Firth and Baxter 2002), three structural regions can be identified in the IGFBP-2 mature protein: N-terminal cysteine-rich region; link region; C-terminal cysteine-rich region (Firth and Baxter 2002; Galea et al. 2012; Forbes et al. 1998), with the N- and C-terminal

regions of IGFBP-2 involved in IGF-I/II binding (Ho and Baxter 1997a; Russo et al. 1999). IGFBP-2 is not glycosylated (Firth and Baxter 2002), and even though IGFBP-2 has a phosphorylation site (Graham et al. 2007), it is mainly found in a non phosphorylated form (Coverley and Baxter 1997).

- i) ***IGFBP-2 integrin binding domain (RGD)***. Similar to IGFBP-1, IGFBP-2 possesses an RGD sequence (Arg-Gly-Asp) at position 265–267 in human and 246–248 in rat (Firth and Baxter 2002) and other species (Daza et al. 2011). The IGFBP-2 RGD motif is functional and mediates IGFBP-2 association with cell membrane integrins (Song et al. 2003; Frommer et al. 2006; Pereira et al. 2004; Perks et al. 2007) including the integrins $\alpha V\beta 3$ or $\alpha 5\beta 1$ (Song et al. 2003; Frommer et al. 2006; Pereira et al. 2004; Perks et al. 2007; Foulstone et al. 2013) (Fig. 3, right side).
- ii) ***IGFBP-2 heparin binding domain (HBD)***. The middle region of IGFBP-2 presents a heparin binding domain (HBD) represented by the sequence $_{179}\text{PKKLRP}_{184}$ in

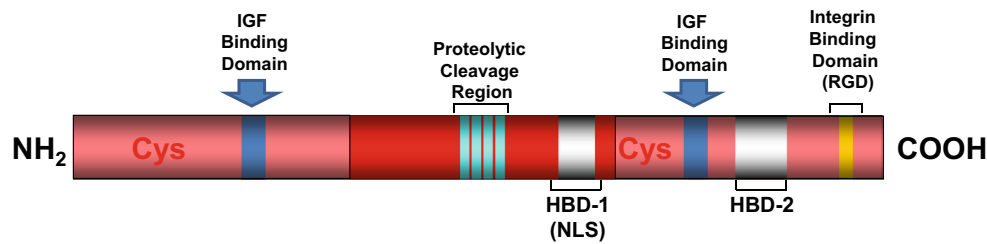


Fig. 2 IGFBP-2 structure. Similarly to the other IGFBPs, mature IGFBP-2 protein has three structural regions: N-terminal cysteine-rich region (12 Cys); middle or link region; C-terminal cysteine-rich region (6 Cys). The cysteine-rich region of N- and C-terminal have IGF binding domains. A protease sensitive region is located within the link-region. A heparin

binding domain (HBD-1) is located within the link-region. Another heparin binding domain (HBD-2) is located within the C-terminal regions. This region is also involved with integrin binding via the RGD domain

human IGFBP-2 mature protein. Using a site directed mutagenesis approach, we have demonstrated that the HBD motif is required for IGFBP-2 binding to components of the ECM, and IGFBP-2 mediated cell proliferation and migration (Russo et al. 2005) (Fig. 3, left side).

Recent work from Shen et al. (2012) demonstrated that the HBD motif in IGFBP-2, also reported as HBD1 (Fig. 2), mediates the binding of IGFBP-2 to the cell surface receptor protein tyrosine phosphatase β (RPTP β) (Fig. 3 left), an event leading to inhibition of the RPTP β phosphatase activity (Shen et al. 2012). An

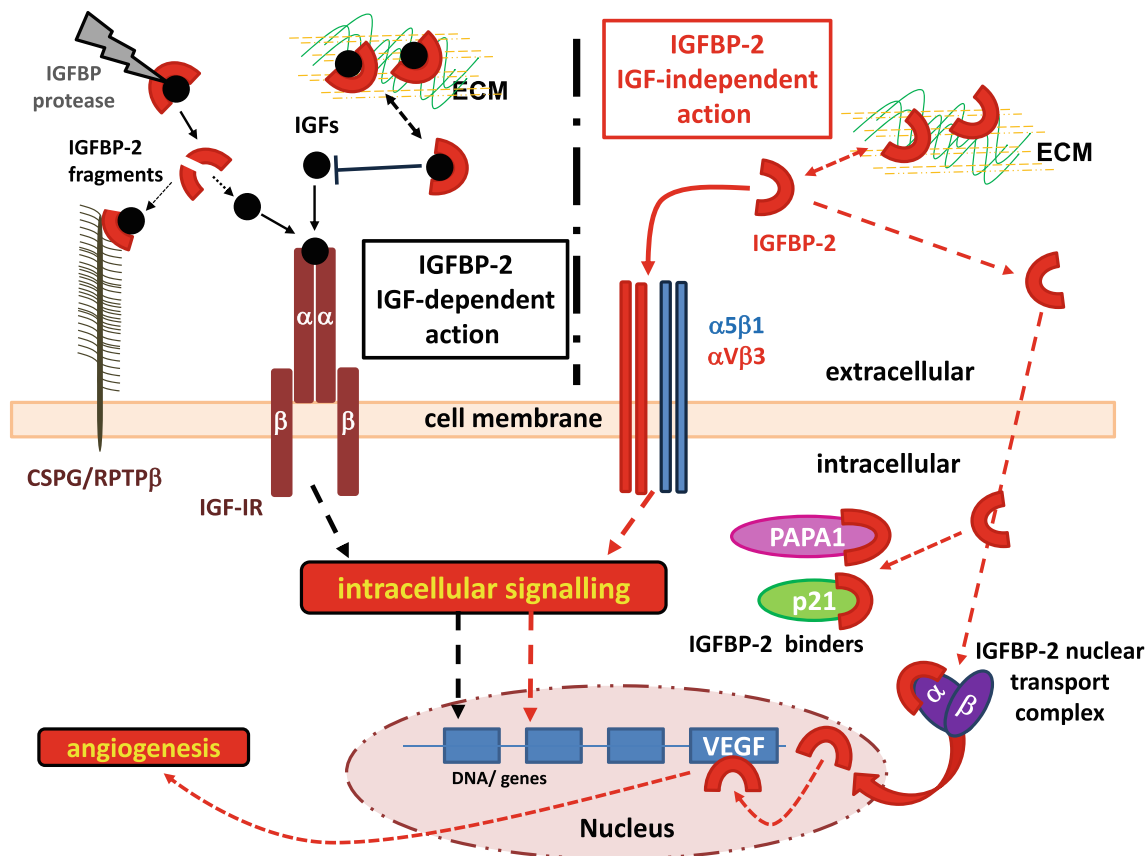


Fig. 3 IGFBP-2 cellular activities, **IGFBP-2 IGF-dependent action:** IGFBP-2 modulates IGFs bioavailability to their receptors, with involvement of specific IGFBP-2 protease. IGFBP-2 also interacts with components of the extracellular matrix (ECM) and cell surface proteoglycan receptor (CSPG; RPTP β), thus providing a peri-cellular reservoir of bioactive IGFs. **IGFBP-2 IGF-independent action:** IGFBP-2 also possesses intrinsic activities that are exerted via interaction with integrin receptors (α V β 3; α 5 β 1) or via translocation into the cell and binding to specific binders (p21; PAPA1) or to nuclear

transportation complexes (importin α and β) to enter the nuclear compartment. Within the nucleus IGFBP-2 directly or indirectly activates the expression of a number of genes involved tumourigenesis including angiogenesis. This IGFBP-2 nuclear activity might be also implicated in growth and metabolic action of IGFBP-2 as described in the main text. Although all these IGFBP-2 activities are likely to be exerted in the absence of the IGF ligands, as we suggest in this schematic cartoon, we cannot exclude the involvement of IGF-I/II in these IGFBP-2 intrinsic activities

additional pH-dependent heparin-binding site, reported as HBD2 (Fig. 2), has been located within the carboxyl-terminal region and thyroglobulin type-1 domain of IGFBP-2 (Kuang et al. 2006).

- iii) **IGFBP-2 nuclear localization signal (NLS) motif.** Nuclear entry of IGFBP-2 (Besnard et al. 2001; Terrien et al. 2005; Miyako et al. 2009; Azar et al. 2011) has only been recently demonstrated by our laboratory, with the presence of a nuclear localization signal (NLS) (Azar et al. 2014). In contrast to IGFBP-3, -5, -6 (Schedlich et al. 2000; Iosef et al. 2008), which possess bi-partite NLS motifs in their C-terminal domains (Schedlich et al. 2000; Iosef et al. 2008), IGFBP-2 has a classic mono-partite NLS sequence (X-K-K/R-X-K/R-X), within its link/middle domain (Azar et al. 2014). This NLS domain is required for interaction with Importin- α (Chelsky et al. 1989) and corresponds exactly with the HBD1 sequence at $_{179}\text{PKKLRP}_{184}$ (Russo et al. 2005; Azar et al. 2014) (Fig. 2).

IGFBP-2 - the knockout (KO) and transgenic (Tg) mice models

The IGFBP-2 KO mouse – effects on bone

Despite the widespread pattern of expression of IGFBP-2 during fetal development and the fact that IGFBP-2 exerts key endocrine, autocrine and paracrine functions such as transport and modulation of the IGFs in the pericellular space (Fig. 3 left), ablation of the IGFBP-2 gene has generated a phenotype surprisingly less dramatic than that initially predicted (Pintar et al. 1996; Wood et al. 2000). Initial analysis of IGFBP-2 null mice only identified alterations in spleen and liver size (Wood et al. 2000).

Critical clues about which tissue / organ might be affected by *IGFBP2* gene ablation have come from studies regarding either IGFBP-2 expression in osteoblasts (Chen et al. 1993; Palermo et al. 2004; Wang et al. 1995), IGFBP-2 involvement in bone formation and density (Conover et al. 2002) or the growth of bones (Fisher et al. 2005).

In a follow up analysis of the Wood's IGFBP-2 null mice (Wood et al. 2000) by DeMambro et al. (2008), it was demonstrated that these mice have gender specific changes in bone. DeMambro et al. (2008) reported that the *IGFBP2* (–/–) male mice had shorter femora and were heavier than controls, while *IGFBP2* (–/–) female mice had increased cortical thickness with a greater periosteal circumference compared with controls (DeMambro et al. 2008). Male *IGFBP2*(–/–) mice had instead reduced cortical bone area and reduction in the trabecular bone volume fraction due to thinner trabeculae than *IGFBP2* (+/+) controls (DeMambro et al. 2008). While the cognate increase in the serum levels of IGF-I and liver

expression of IGFBP-3 and IGFBP-5 (DeMambro et al. 2008) might have balanced the loss of circulating IGFBP-2, it appeared not to be effective in compensating the loss of local IGFBP-2 expression in bone.

The effects of IGFBP-2 ablation on bone have indirectly suggested that IGFBP-2 might play a key role in the regulation of bone turnover, and that this might be exerted via both IGF-dependent and IGF-independent mechanisms (Fig. 3). Investigation of these mechanisms by Kawai et al. (2011) then demonstrated that the HBD (Russo et al. 2005) of IGFBP-2 is a key determinant of IGFBP-2 action in bone and that IGFBP-2 functions are coordinated with IGF-I to stimulate growth and skeletal acquisition (Kawai et al. 2011). These findings were further supported in later work by DeMambro et al. (2012) where it was demonstrated that IGFBP-2 is a regulator of osteoclastogenesis and that the HBD (Russo et al. 2005) and the IGF-binding domains of IGFBP-2 (Fig. 2) are essential for the formation of fully differentiated and functional osteoclasts (DeMambro et al. 2012).

Furthermore, recent studies by Xi et al. (2014) have demonstrated that an IGFBP-2 based peptide containing the HBD1 sequence can rescue, presumably via interaction with RPTP β , the differentiation and the expression of osteocalcin in calvarial osteoblast from the IGFBP-2 –/– mice. Although the IGFBP-2 –/– mouse model has greatly contributed to our understanding of the role IGFBP-2 in bone, the development of an animal model with osteoblast-specific deletion of IGFBP-2 should overcome some of the confounding intrinsic issues of *Igfbp2* global deletion.

IGFBP-2 Tg mouse – effects on growth, metabolisms and bone

- i) **Effects on growth.** The IGFBP-2 transgenic mouse model developed by Hoeflich et al. (1999) has clearly demonstrated that IGFBP-2 is a negative regulator of postnatal growth in mice, an effect potentially mediated via reducing local IGF-I bioavailability. In these studies, the body weights of the IGFBP-2 Tg mice were substantially reduced, an effect attributable to the significant reduction in carcass weight, with absolute organ weights (except the spleen) not affected (Hoeflich et al. 1999). Similar effects were seen even when hemizygous CMV-IGFBP-2 transgenic mice were crossed with hemizygous PEPCK-bGH transgenic mice (with very high GH and IGF-I serum levels) (Hoeflich et al. 2001), thus supporting further the inhibitory role of IGFBP-2 in vivo.

Whether these effects are exclusively occurring as a result of IGFBP-2 sequestration of circulating and/or local IGF-I, or more likely involving both canonical and non-canonical actions of IGFBP-2 (Fig. 3 IGF-dependent and IGF-independent

action), including IGFBP-2 interactions at the cell surface (Russo et al. 1995, 2005; Shen et al. 2012; Kawai et al. 2011; DeMambro et al. 2012) (Fig. 3) and intracellular / nuclear (Terrien et al. 2005; Miyako et al. 2009; Azar et al. 2011, 2014) (Fig. 3), is unclear. However, it is important to highlight that in both studies (Hoeftlich et al. 1999, 2001), IGFBP-2 was constitutively over-expressed in most tissues resulting in very high local levels of IGFBP-2, such that overexpression of IGFBP-2 at supraphysiological levels may be different from more physiological alterations and therefore more profoundly affected local activities of IGFBP-2.

Further examination of these IGFBP-2 Tg mice (Rehfeldt et al. 2010) suggested that the reduced post-natal growth was also attributable to the observed reduced body lean protein, reduced water content and reduced muscle weight, although this was associated with increased fat percentage. Interestingly, the reduction in muscle weight was a result of smaller myofiber size, rather than reduced myofiber number, and in older mice, these myofibers showed elevated white glycolytic fibers and lactate dehydrogenase activity (Rehfeldt et al. 2010).

As seen in the studies by DeMambro (2008), most of the differences observed between transgenic and non-transgenic mice were more pronounced in males. These studies further support a functional role for IGFBP-2 in in vivo skeletal muscle growth and function, skeletal muscle metabolism as well as body growth and composition.

- ii) **The effects on bone.** Overexpression of IGFBP-2 also affects bone size and mass but not bone density (Eckstein et al. 2002), effects seen in either the presence or absence of growth hormone (GH) excess. These effects appear to be in contrast with those studies described by Conover et al. (2002), which demonstrated that administration of IGFBP-2 and IGF-II stimulated bone formation in rats.

Overexpression of IGFBP-2 in these mice (Eckstein et al. 2002) resembles, to some extent, the phenotype of the *IGFBP2* ^{-/-} mice, while on the other hand the IGFBP-2 Tg mice bone phenotype is in part consistent with that describe for the *IGF* ^{-/-} mice.

These obvious discrepancies between the effects of IGFBP-2 overexpression in vivo (ie. increased fat mass, reduced skeletal muscle metabolism, body growth / composition and bone formation) and the effects of “add-back” or elevated circulating IGFBP-2 (e.g., inhibition of adipogenesis, anti-diabetic properties, bone formation and anorexia nervosa), suggest a potential dysregulation of normal physiological activities of IGFBP-2 in the IGFBP-2 Tg mice.

- iii) **Effects on metabolism.** In the above studies by Rehfeldt et al. (2010), the IGFBP-2 Tg mice also

demonstrated a significant increase in fat proportion, which was confirmed by biochemical analysis of body composition indicating that the IGFBP-2 Tg mice contained a higher percentage of fat. Conversely, as mentioned above, these mice, showed lower percentages of water content and protein, but with ash percentage (bone) being equal to that of non-transgenic mice (Rehfeldt et al. 2010). Once again the differences in body fat and water content were more pronounced in males than in females.

In contrast are the findings from another study by Hoeftlich et al. (1998) in non-transgenic mice selected for body weight (e.g., control [C] out-bred stock versus, high [H] or low [L] body weight) where only male L mice have significantly increased serum levels of IGFBP-2. The hepatic *Igfbp2* mRNA levels were significantly increased in L mice, while they were decreased in H mice as compared to C mice. This increase in IGFBP-2 levels in the lower weight mice is consistent with the observation by Barrios et al. (2000), Hall et al. (1988) and Hotta et al. (2000) reporting elevated circulating levels of IGFBP-2 in patients with anorexia nervosa.

In agreement are the studies in human subjects by Wheatcroft et al. (2007a), Hedbacker et al. (2010), (Claudio et al. 2010a), Ballerini et al. (2004) and Frystyk et al. (1999), which report low circulating IGFBP-2 levels in obesity (Wheatcroft et al. 2007a; Hedbacker et al. 2010; Claudio et al. 2010a) and that administration of IGFBP-2 can prevent adipogenesis (Wheatcroft et al. 2007a) and has anti-diabetic properties (Hedbacker et al. 2010).

Modulation of IGFBP-2 levels

IGFBP-2 and PTEN cross-modulation

At the cellular level, the expression of IGFBP-2 is influenced by the functional status of the tumour suppressor gene phosphatase and tensin homolog (PTEN) (Levitt et al. 2005; Mehrian-Shai et al. 2007). Loss of function of PTEN, reported in 50 % of human cancers, is often cognate with high levels of IGFBP-2 expression (Levitt et al. 2005). On the other hand, induction of PTEN in cancer cells results in a substantial suppression of IGFBP-2 levels (Levitt et al. 2005). The involvement of PTEN in IGFBP-2 down-regulation, is also substantiated by chemical blockade of phosphatidylinositol 3 kinase (PI3K) / protein kinase B (Akt) (Levitt et al. 2005), (ie. mimicking PTEN activation) or that of the downstream signalling intermediate mammalian target of rapamycin (mTOR), both resulting in markedly reduced expression of IGFBP-2 (Mehrian-Shai et al. 2007; Martin and Baxter 2007).

A study by Perks et al. (2007) provides in vitro evidence for modulation of PTEN activity by IGFBP-2 via an integrin mediated mechanism. Subsequent work by Dean et al. (2014) in

triple-negative breast cancer subjects, has reported that high levels of expression of IGFBP-2 are associated with loss of PTEN in these aggressive tumours, with this data suggesting that elevated IGFBP-2 is a potential prognostic marker for loss of PTEN in these tumours. It is thus reasonable to argue that activation of aggressive behavior in cancer cell might depend on the balance between IGFBP-2 and PTEN activation.

IGFBP-2 and p53

Another tumour suppressor gene involved in modulation of IGFBP-2 levels is p53 (Grimberg et al. 2006). As for IGFBP-3, IGFBP-2 mRNA is induced in a p53-dependent manner, with p53 shown to bind to an intronic regulative sequence of the IGFBP-2 gene (Grimberg et al. 2006). IGFBP-2 is a direct target of p53, and mediates p53 inhibition of IGF-I signalling (Grimberg et al. 2006), while the suppression of p53 results in reduction of IGFBP-2 expression, and activation ERK-1 in the presence of IGF-I (Grimberg et al. 2006). These findings suggested that the mitogenic activity of IGFs can be switched to growth inhibition and apoptosis via p53 activation of IGFBP-2.

IGFBP-2 and Ptc1

In a recent study by Villani et al. (2010) in basal cell carcinoma (BCC), it was demonstrated that the Hedgehog (Hh) signalling pathway, which is also implicated in many tumour types (e.g., brain, gastrointestinal, lung, breast and prostate cancers), plays a role in modulation of IGFBP-2 activities. These studies have shown that in the absence of Patched1 (Ptc1; trans-membrane receptor and negative regulator of Hh signalling), IGFBP-2 expression is enhanced and this led to the expansion of the epidermal basal cells in the proliferative layer of the skin, an event required to promote BCC development (Villani et al. 2010).

Thus Ptc1 inhibits epidermal progenitor cell expansion and basal cell carcinoma formation by limiting IGFBP-2 activities (e.g., IGFBP-2 mediated hair follicle progenitor cell progression into anagen) (Villani et al. 2010). Although synergism between the IGF1R and Hh signalling has been implicated in another cancer type (Rao et al. 2004), the expression and action of IGFBP-2 in BCC appears to be IGF-independent in nature (Villani et al. 2010).

IGFBP-2 and HIF-1 α

IGFBP-2 expression is also regulated by the transcription factor hypoxia-inducible factor 1 α (HIF-1 α) (Feldser et al. 1999), the expression of which is in turn regulated by cellular O₂ concentrations. Exposure to hypoxic conditions activates a number of adaptive cellular responses (Harris 2002; Semenza 1999). This is the case in solid tumours that become hypoxic

because of tumour overgrowth and consequent aberrant development of new blood vessels within the tumour mass leading to inefficient local circulation (Harris 2002; Semenza 2003). Although hypoxia is toxic to both cancer cells and normal cells, cancer cells undergo genetic and adaptive changes that allow them to survive and even proliferate in a hypoxic environment (Harris 2002; Semenza 2003). These processes contribute to the malignant and aggressive phenotype (Poomthavorn et al. 2009). The upregulation of IGFBP-2 expression by HIF1 α is therefore consistent with the crucial role of IGFBP-2 in tumourigenesis.

IGFBP-2 proteolysis

Providing another level of regulation of IGFBP-2 abundance and functionality is proteolysis of IGFBP-2. IGFBP-2 proteolysis has been reported under physiological conditions in vivo, in biological fluids (Matsumoto et al. 1996; Ishikawa et al. 1995), including human serum (Mark et al. 2005), human breast milk (Ho and Baxter 1997b), maternal serum during and post gestation (Giudice et al. 1990). Proteolysis or fragmentation of IGFBP-2 has also been exhaustively reported in many pathophysiological conditions, including nutritional deprivation or starvation (Pucilowska et al. 1993), inflammation (Brandt et al. 2011) and cancer (Russo et al. 1999; DeGraff et al. 2007).

Proteolysis of IGFBP-2 generates fragments of variable sizes (Fig. 3, left), depending on the cell line and species (Russo et al. 1999; Ishikawa et al. 1995; Ho and Baxter 1997b), including a small fragment of about 14–18 kDa and a larger one of about 21–25 kDa. These fragments have been reported either not to bind or to have markedly reduced binding affinity for the IGFs (Russo et al. 1999; Ho and Baxter 1997b). We have described, in differentiating neuroblastoma cells (Russo et al. 2004) (1999), an IGFBP-2 fragment that binds IGF-I while it also associated to the cell surface (Fig. 3, left). This IGFBP-2 fragment most likely represents the carboxy-terminal portion of IGFBP-2 containing the HBD domain (Ishikawa et al. 1995; Ho and Baxter 1997b) (Fig. 3, left). A similar IGFBP-2 fragment was also detected perinuclearly in vivo in various tissues of the IGFBP-2 Tg mouse (Hoefflich et al. 2004). The role of this peri-nuclear IGFBP-2 fragment is yet to be determined.

In most cases, the activity of the IGFBP-2 proteases (e.g., MMPs, plasmin, calpain, kallikrein) can be activated or induced by hormones, cytokines and drugs (Russo et al. 2004; Si et al. 2007), but also modulated by protease inhibitors such as α 2-macroglobulin (α 2M), which enable the formation of an IGFBP-2/ α 2M complex resistant to proteolysis (Sunderic et al. 2013). Although formation of the IGFBP-2/IGF-I or -II complex might show an acquired resistance to proteolysis in some cases, a major function of IGFBP proteases is to cleave IGFBP and release IGFs from the IGFBP/IGF complex, an

event likely to enhance local IGF action (Gerard et al. 2004; Qin et al. 2006). This has been exhaustively described in cancer and cancer cell models where proteolysis of IGFBP-2 might be regarded as an important post-translational event contributing to increased tumour aggressiveness.

IGFBP-2 cellular activities

IGFBP-2 action at the cell surface mediated via integrin receptors

Integrins represent a family of transmembrane α/β heterodimers receptors that mediate cell-cell and cell-extracellular matrix (ECM) interaction and play a key role in the regulation of cell cycle, cell shape and adhesion and cell motility (Ruoslahti 1996). Integrin interaction with the components of the ECM (e.g., fibronectin, vitronectin, laminin) occurs via a conserved integrin attachment recognition site containing the Arg-Gly-Asp (RGD) motif (Ruoslahti 1996). As mentioned earlier, this RGD motif is present in IGFBP-1 and IGFBP-2 (Fig. 2) and is required for their interactions with cell membrane integrin receptors.

The effects mediated by the interaction of IGFBP-2 with the $\alpha V\beta 3$ or $\alpha 5\beta 1$ integrins remain controversial. A study by Pereira and co-workers investigated the interaction of IGFBP-2 with $\alpha V\beta 3$ integrin by over-expressing the $\alpha V\beta 3$ in MCF-7 breast cancer cells (Pereira et al. 2004). It was demonstrated that $\alpha V\beta 3$ integrin and IGFBP-2 act cooperatively in a negative regulatory manner to reduce tumour growth (MCF-7/ $\alpha V\beta 3$ cell xenografts in nude mice) and the migratory potential of breast cancer cells (Pereira et al. 2004).

In other studies by Schutt et al. (2004) and Frommer et al. (2006) investigating the effects of IGFBP-2 interaction with the $\alpha 5\beta 1$ integrin in the breast cancer cell line Hs578T, it was shown that IGFBP-2 binding to integrin led to dephosphorylation of the focal adhesion-kinase (FAK) and p42/44 MAP-kinases, reducing cell proliferation and activating pro-apoptotic events in Hs578T cells.

Conversely, studies by Mendes et al. (2010) demonstrated in glioma cells that the interaction of IGFBP-2 with the $\alpha 5\beta 1$ integrin is required for IGFBP-2 induced motility of glioma cells and activation of invasion pathways, all events mediated via the RGD motif of IGFBP-2 and leading to tumour progression. Similarly, in work from Perks et al. (2007) in MCF-7 breast cancer cells and by Uzoh et al. (2011) in prostate cancer cells, an integrin-mediated pro-tumourigenic action of IGFBP-2, which occurs via modulation of PTEN has also been suggested.

More recently, Foulstone et al. (2013) demonstrated that IGFBP-2/ $\alpha 5\beta 1$ -mediated PTEN suppression in MCF-7 breast cancer cells, resulted in protection of MCF-7 cells from chemotherapy induced cell death and this requires a functional oestrogen receptor- α (ER- α) (Foulstone et al. 2013).

Silencing of IGFBP-2, leading to loss of the ER- α abolished cell response to estrogen while exogenous IGFBP-2, acting through the $\alpha 5\beta 1$ integrin, increased ER- α (Foulstone et al. 2013). This is interesting because IGFBP-2 not only modulates IGFs and directly regulates PTEN via the $\alpha 5\beta 1$ integrin, but also has a role in maintaining ER- α expression.

Consistent with this pro-tumourigenic action of IGFBP-2 exerted via integrin binding, is the work from Song et al. (2003), who used a yeast two-hybrid system and demonstrated in glioblastoma multiforme (GBM), that the invasion inhibitory protein-45 (Iip45) binds to IGFBP-2 via the thyroglobulin-RGD region of the C terminus of IGFBP-2. In these cells, Iip45 inhibited the expression of cell invasion-associated genes and invasion of GBM cells both in vitro and in xenograft models (Song et al. 2003). Their studies showed that Iip45 binding to IGFBP-2 prevented glioma cell invasion (Song et al. 2003), thus demonstrating that the IGFBP-2 interaction with integrins is required for an enhancing role for IGFBP-2 in GBM invasion.

A potential explanation for the observed integrin-mediated inhibitory activities of IGFBP-2, described less commonly, is that the cell lines used in those studies do not normally express $\alpha V\beta 3$ integrin (Pereira et al. 2004), do not express IGFBP-2 and/or key components of the IGF system (Frommer et al. 2006; Schutt et al. 2004). It is therefore possible that the antagonistic and inhibitory activities of IGFBP-2 observed in these studies might reflect specific cell types and experimental conditions.

IGFBP-2 binding to ECM components and cell membrane proteoglycans

There is strong consensus that ECM bound IGFBP-2 contributes to modulation of IGF bioavailability in the peri-cellular space (Fig. 3 left), and the affinity of IGFs for ECM-bound IGFBP-2 is likely to be significantly reduced compared to that enabling the formation of the IGFBP-2/IGF complex in solution (Arai et al. 1996). Therefore ECM-bound IGFBP-2 acts as an active reservoir for the local IGFs, which in turn may also prevent IGF clearance from the peri-cellular space.

These peri-cellular or cell surface storage sites for bioactive IGFs, can be affected by IGFBP-2 proteases (Russo et al. 1999) (Ishikawa et al. 1995), which by cleaving IGFBP-2 augment local release of IGFs (Firth and Baxter 2002) (Fig. 3 left).

We have identified an IGFBP-2 fragment on the cell surface of neuroblastoma cells (Russo et al. 1999), and demonstrated that such IGFBP-2 fragment can hold IGF-I onto the cell surface (Fig. 3 left). Thus proteolysis of membrane-bound IGFBP-2 provides a mechanism for creating low affinity peri-receptor IGF-I binding sites in neuroblastoma cells (Russo et al. 1999), and most likely in other cancer cells.

Of interest is a study by Kiepe et al. (2008), which demonstrated that human haemo-filtrate derived IGFBP-2 fragments possess intrinsic mitogenic activities in growth plate chondrocytes. These IGFBP-2 fragments overlap the middle and carboxyl terminal region of IGFBP-2 (e.g., variably extending within the amino-acid stretch Gly₁₀₄-Gln₂₈₉ of the mature protein) and have a size comparable to that described for proteolytic fragments of IGFBP-2 (Kiepe et al. 2008). Interestingly, in the studies by Kiepe et al., it was shown that the mitogenic effects of these fragments were comparable to those seen with equimolar concentrations of IGF-I, although there was no IGF1R signalling associated with the activity of the haemo-filtrate derived IGFBP-2 fragments (Kiepe et al. 2008).

The biological activity of these IGFBP-2 fragments, described to occur independently of IGF-I, was associated with their ability to bind to the cell surface (Kiepe et al. 2008). Another interesting observation in these studies was that intact IGFBP-2 did not enhance chondrocyte proliferation (presumably by complexing with the IGFs), while partially reduced full-length IGFBP-2 (e.g., DTT-mediated disruption of the Cys-Cys bonds, leading to loss of IGF binding) stimulated chondrocyte proliferation to a comparable extent to that observed for the IGFBP-2 fragments (Kiepe et al. 2008). Although the putative cell membrane binding site was not identified in this study, activation of the mitogen-activated protein kinase (MAPK) pathway was shown to be involved in these events.

Of relevance to the findings of Kiepe et al., is the study by Kawai et al. (2011) demonstrating that an IGFBP-2 based HBD1 peptide (Russo et al. 2005; Azar et al. 2014) was able to produce skeletal rescue in the *Igfbp2*^{-/-} mice (DeMambro et al. 2008), via activation of the IGF-I/Akt and β -catenin signalling pathways (Kawai et al. 2011). Kawai et al. demonstrated that the HBD1 of IGFBP-2 has IGF binding-independent biological activity in the growing skeleton (Kawai et al. 2011). These findings suggest, as we have described in another cell system (Russo et al. 2005; Azar et al. 2014), that the HBD1 motif present in the Kawai HBD peptide and within IGFBP-2 fragments (Russo et al. 1999, 2005; Kiepe et al. 2008), has a key functional role and is likely to be involved in recognition of a cell surface binding site. This putative IGFBP-2 receptor might be responsible for activation of specific intracellular signalling pathways as described by Kiepe et al. (2008) and Kawai et al. (2011).

In another study by Xi et al. (2013), peptides spanning the HBD1 or HBD2 motif (Fig. 2) were used to determine whether these domains were involved in the observed IGFBP-2-mediated resistance to high fat feeding and inhibition of preadipocyte differentiation (Wheatcroft et al. 2007a). The HBD2 motif containing peptide was more effective in mediating inhibition of adipogenesis *in vitro* and reduction of total fat mass and increase serum leptin *in vivo* in IGFBP-2^{-/-}

mice. The activity of the HBD2 peptide was comparable to that demonstrated by native full-length IGFBP-2 (Xi et al. 2013).

As mentioned earlier, studies by Shen et al. (2012) and DeMambro et al. (2012) have shown that IGFBP-2 in the presence or absence of IGFs, interacts with the cell surface receptor RPTP β (Shen et al. 2012) (Fig. 3, left). The characterisation of this RPTP β receptor was previously reported from work in the human brain by Shitara et al. (1994). It was demonstrated that RPTP β was a chondroitin-sulfate proteoglycan with a molecular weight of about 350–500 kDa, and that the extracellular region of human RPTP β contained a classic Fibronectin type III module (Shitara et al. 1994).

Shen et al. (2012) demonstrated that IGFBP-2 via its HBD (Russo et al. 2005), interacts with the cell surface receptor RPTP β leading to dimerization and inactivation of this receptor, an event which in turn enhances PTEN tyrosine phosphorylation and inhibits PTEN activity. Interestingly, it was shown that an antibody against the Fibronectin motif (Fn3), prevented the interaction of IGFBP-2 with RPTP β , suggesting that IGFBP-2 binds to RPTP β via the Fn3 motif, previously described by Shitara et al. (1994).

Intracellular and intranuclear activities of IGFBP-2

IGFBP-2 is present within the cell (Terrien et al. 2005; Miyako et al. 2009; Hoeflich et al. 2004), where it interact with cell cycle inhibitor p21 or Pim-1-associated protein-1 (PAP-1)-associated protein-1 (PAPA-1), with both of these interactions affecting IGFBP-2 growth-promoting actions (Terrien et al. 2005; Miyako et al. 2009; Hoeflich et al. 2004) (Fig. 3, left). Recent work from our laboratory has provided strong biochemical evidence for IGFBP-2 nuclear localization in cancer cells (Azar et al. 2011, 2014). Nuclear translocation of IGFBP-2 is an active import process (Azar et al. 2011, 2014), mediated by importin- α and involves a nuclear localization signal (NLS) motif located within the link-region of human IGFBP-2 at ₁₇₉P-K-K-L-R-PP₁₈₅ (Azar et al. 2014) (Fig. 3, left). This NLS motif overlaps exactly with that of our previously described IGFBP-2 HBD motif (Russo et al. 2005) (Fig. 2), and this is also a common feature described for other IGFBPs (Firth and Baxter 2002). A functional role for the NLS motif of IGFBP-2 was then demonstrated via an NLS mutant form of IGFBP-2 (NLS_{mut}IGFBP-2), which did not interact with importin- α and was not transported / translocated into the nucleus (Azar et al. 2014).

We have previously described that IGFBP-2 overexpression enhances neuroblastoma cell proliferation and migration (Russo et al. 2005), events suggesting that IGFBP-2 activates a pro-tumourigenic gene expression program in these cells. Overexpression of IGFBP-2 leads to induction of genes regulating cell proliferation, migration and invasion, while tumour suppressor genes are down-regulated (Azar et al. 2011, 2014).

Of these genes, vascular endothelial growth factor-A (VEGF-A) was markedly upregulated by the Wt-IGFBP-2, but not by the NLSmut-IGFBP-2 (Azar et al. 2014), suggesting a potential role for nuclear IGFBP-2 in transactivation of the VEGF gene (Fig. 3, bottom). This was confirmed via VEGF-A-promoter-luciferase assays. Furthermore, as we predicted the NLSmut-IGFBP-2 did not enter the nucleus, so failing to elicit luciferase activity. These findings provide strong support for IGFBP-2 as a transactivator of the VEGF-A gene (Fig. 3, bottom) and likely other growth promoting genes.

In further experiments, it was then demonstrated that IGFBP-2-mediated elevation of VEGF-A in neuroblastoma cells, led to activation of angiogenesis *in vivo* as determined by the chick embryo chorio-allantoic membrane (CAM) assay. No angiogenesis was seen in the presence of NLSmut-IGFBP-2.

Initiation of angiogenesis is an important early event in tumour progression, and the studies above demonstrate that IGFBP-2 enhances the expression of the angiogenic growth factor VEGF-A, an event that requires intracellular / nuclear localization of IGFBP-2. It is thus clear that nuclear IGFBP-2 directly or indirectly contributes to the development of a pro-metastatic milieu consistent with the enhancement of an aggressive cancer phenotype.

IGFBP-2 and metabolism

There is increasing evidence for IGFBP-2 regulation by metabolism and in metabolic disorders, while there is also evidence for IGFBP-2 contribution to regulation of normal metabolism (Sabin et al. 2011a). In obesity, circulating levels of IGFBP-2 are suppressed (Wheatcroft et al. 2007a; Hedbacker et al. 2010; Claudio et al. 2010a; Ballerini et al. 2004; Frystyk et al. 1999; Nam et al. 1997a), and low levels of IGFBP-2 are associated with Type 2 diabetes mellitus (T2DM) (Frystyk et al. 1999), and metabolic syndrome (Heald et al. 2006). On the other hand, IGFBP-2 overexpression protects against obesity and diabetes (Wheatcroft et al. 2007a; Hedbacker et al. 2010), via mechanisms involving IGFBP-2 mediated inhibition of adipogenesis and modulation of insulin sensitivity (Wheatcroft et al. 2007a; Hedbacker et al. 2010).

In a study in obese mice and men by Li and Picard (2010a), it was demonstrated that IGFBP-2 mRNA levels were significantly lower in visceral white adipose tissue (WAT) than in subcutaneous WAT. This differs from findings in individuals with anorexia nervosa, who have high IGFBP-2 levels and significantly reduced levels of total percentage fat and visceral adipose tissue (Krassas 2003; Counts et al. 1992). This suggests that IGFBP-2 gene might be modulated in a depot-specific fashion. Furthermore, studies from our laboratory (Sabin et al. 2011a), have demonstrated that increased consumption of dietary fat or sugar in early life differentially

impact upon adiposity (increased) and insulin sensitivity (decreased). These changes were associated with alterations in IGFBP-2 mRNA expression in muscle and fat (Sabin et al. 2011a), with monounsaturated fatty acid affecting IGFBP-2 mRNA in *in vitro* cultures of skeletal myotubes (Sabin et al. 2011a).

A direct correlation between low circulating levels of IGFBP-2 and obesity has been described by Nam et al. (1997a) in obese males. IGFBP-2 levels were found to be suppressed and inversely related to levels of free IGF-I, which were elevated (Nam et al. 1997a) and positively correlated with elevated fasting serum insulin levels (Nam et al. 1997a). However, in obese subjects the levels of total IGF-I and those of IGFBP-3, despite suppressed GH levels, were not significantly different between obese and control subjects (Nam et al. 1997a). These findings suggest a potential role for IGFBP-2 in modulation of IGF-I bioavailability and action (e.g., abnormal low IGFBP-2 leading to abnormal elevated free IGF-I) and in turn, IGFBP-2 contribution to maintenance of metabolic function (Nam et al. 1997a).

A study by Frystyk et al. (1999), investigated potential alterations in the GH/IGF axis following overnight fasting in matched groups of lean subjects, obese subjects with or without T2DM, and subjects with type 1 diabetes mellitus (T1DM). In obese subjects without T2DM, IGFBP-2 levels were reduced and they were further reduced in those patients that also had T2DM. Interestingly, IGFBP-2 was actually increased in patients with T1DM (Frystyk et al. 1999), consistent with findings by others in T1DM (Berek et al. 1999) and diabetic retinopathy (Frystyk et al. 2003), suggesting that T1DM and T2DM have a different impact on IGFBP-2 levels, perhaps related to differences in insulin sensitivity.

Low circulating levels of IGFBP-2 have also been reported in lifestyle-related childhood obesity (with normal growth in spite of reduced GH secretion) (Ballerini et al. 2004) and pre-pubertal obese subjects (Street et al. 2013). In childhood obesity, the reduction in IGFBP-2 serum levels coupled with a lower ratio of IGFBP-2/IGF-I, suggest an increase of tissue IGF-I bioavailability which in turn might be contributing to the observed accelerated growth seen in the pre-pubertal years (Ballerini et al. 2004).

Furthermore, in intra-uterine growth retardation (IUGR) (Smerieri et al. 2011; Giudice et al. 1995), cord serum levels of IGFBP-2 are elevated and negatively correlate with both birth length and weight (Smerieri et al. 2011; Giudice et al. 1995). Conversely, circulating levels of IGFBP-2 are low in pre-pubertal and pubertal subjects that were born small for gestational age (IUGR/SGA) (Ko et al. 2012; de Kort et al. 2010). These changes in IGFBP-2 levels, were investigated by Ko et al. (2012), and de Kort et al. (2010); they assessed growth pattern in relation to insulin resistance, body composition and IGFBP-2 (potential modulator of insulin sensitivity) in individuals born small for gestational age (SGA; pre-

pubertal and pubertal subjects). IGFBP-2 levels were inversely correlated with BMI, body fat mass, percentage body fat, insulin and leptin levels in both pre-pubertal and pubertal groups (Ko et al. 2012) (de Kort et al. 2010), while being positively correlated with insulin sensitivity (de Kort et al. 2010). In young adults who were born SGA, reduced serum IGFBP-2 levels also associated with increased cardiovascular risk markers (de Kort et al. 2010). These studies (Ko et al. 2012) (de Kort et al. 2010) therefore suggest that IGFBP-2 could be a potential marker for early recognition of insulin resistance, particularly in SGA children.

Increasing evidence has indicated that leptin, the product of the obesity gene (*Ob*), is a major metabolic regulator of circulating IGFBP-2 (Wheatcroft et al. 2007a; Hedbacker et al. 2010). Obesity is associated with leptin resistance and increased circulating levels of leptin (a regulator of energy balance produced from white adipose tissue) (Friedman and Halaas 1998). Studies in rodents have shown that leptin regulates hepatic IGFBP-2 expression (Hedbacker et al. 2010) and that IGFBP-2 levels are closely linked with body weight and glucose metabolism (Wheatcroft et al. 2007a; Sabin et al. 2011a). In recent studies from our laboratory (Yau et al. 2014a), we demonstrate that leptin increases skeletal muscle IGFBP-2 by directly activating leptin signalling pathways in the periphery, as well as activating central mechanisms which communicate to peripheral tissues via the sympathetic nervous system (SNS) through sympathetic activation of β -adrenergic receptors (Yau et al. 2014a). We also showed, in sheep, that central infusion of leptin improves glucose tolerance and reduces circulating insulin levels in vivo, while silencing of IGFBP-2 in human skeletal muscle reduces insulin signalling, as well as leptin- and insulin-stimulated glucose uptake (Yau et al. 2014a). The direct and indirect regulation of skeletal muscle IGFBP-2 by leptin, mediated via STAT-3 and PI3K/AKT signalling pathways, suggest that leptin appears to regulate peripheral insulin sensitivity via IGFBP-2 (Yau et al. 2014a). These findings also suggest that leptin may improve insulin sensitivity by directly and indirectly regulating peripheral IGFBP-2 expression.

As mentioned earlier, low circulating levels of IGFBP-2 are associated with adiposity (Sabin et al. 2011a, b; Nam et al. 1997b; Ballerini et al. 2004; Claudio et al. 2010b) and particularly with visceral adiposity rather than subcutaneous adiposity (Li and Picard 2010b). Conversely, high IGFBP-2 levels as seen by overexpression of IGFBP-2 in mice appears to prevent diet induced obesity and insulin resistance, with IGFBP-2 also preventing murine adipocytes differentiation in vitro (Wheatcroft et al. 2007b). Interestingly, recent studies by Xi et al. (2013) have shown that IGFBP-2-based peptides containing the HBD motifs, efficiently inhibited preadipocyte differentiation in male mice, suggesting that IGFBP-2 cell surface interactions via these motifs might be implicated in the modulation of adipogenesis.

Recent studies from our laboratory (Yau et al. 2014b), investigating the effects of IGFBP-2 on visceral versus subcutaneous adipocytes, have demonstrated that exogenously added human IGFBP-2 significantly reduced adipo/lipogenesis in visceral adipocytes, but not in subcutaneous adipocytes. In fact, silencing IGFBP-2 resulted in exaggerated adipo/lipogenesis in visceral adipocytes, but not in subcutaneous adipocytes, an effect ablated when IGFBP-2 was added-back.

In agreement with Xi et al. (2013) studies with peptides containing the HBD motifs, our HBD-1 mutant IGFBP-2 had reduced inhibitory effects on adipogenesis, when compared with wild-type IGFBP-2 (Yau et al. 2014b). Wild-type IGFBP-2 increased phosphorylation of focal adhesion kinase (FAK) and decreased phosphatase and tensin homolog (PTEN) levels, suggestive of integrin-mediated signalling (Yau et al. 2014b). Blockade of this signalling, using Echistatin, completely negated the effects of IGFBP-2 on visceral adipo/lipogenesis (Yau et al. 2014b). These studies have thus provided strong evidence that IGFBP-2 inhibits both adipogenesis and lipogenesis in visceral, but not subcutaneous, adipocytes (Yau et al. 2014b). This depot-specific impairment appears to be independent of IGF-I and involves cell-surface association of IGFBP-2 and activation of integrin signalling pathways (Yau et al. 2014b).

IGFBP-2 and cancer

IGFBP-2 is one of the most commonly and abundantly expressed IGFBPs in a broad range of human cancers, often reaching the high abundance levels seen in fetal life (Reeve et al. 1992a). IGFBP-2 expression positively correlates with tumour aggressiveness and the expression of known tumour markers (Tennant et al. 1996; Wex et al. 1998). The role of IGFBP-2 in glioma (Wang et al. 2003; Fukushima and Kataoka 2007; Becher et al. 2008; Moore et al. 2009; Fukushima et al. 2007), prostate (Ho and Baxter 1997a; Heinlein and Chang 2004; Karantanos et al. 2013; Kimura et al. 1996; Cohen et al. 1993; Degraff et al. 2009), breast (Perks et al. 2007; Foulstone et al. 2013; Martin and Baxter 2007; Wang et al. 2008; Juncker-Jensen et al. 2006; Sohn et al. 2013; Mireuta et al. 2010) and ovarian (Karasik et al. 1994; Kanety et al. 1996; Wang et al. 2006; Lee et al. 2005) cancers has been exhaustively discussed and therefore we wish to focus on other major malignancies in which the role of IGFBP-2 remains still unclear.

Leukaemia

Leukaemias are cancer of the white blood cells (lymphoid or myeloid; acute and chronic), which begin in the bone marrow, with four major types including acute lymphocytic leukaemia (ALL) (Inaba et al. 2013; Lo Nigro 2013; Carroll and Raetz

2012; Rivera et al. 1994), chronic lymphocytic leukaemia (CLL) (Rodríguez-Vicente et al. 2013; Shanshal and Haddad 2012; Hillmen 2011), acute myeloid leukaemia (AML) (Estey 2013; Puumala et al. 2013; Gamis et al. 2013) and chronic myeloid leukaemia (CML) (Jabbour and Kantarjian 2012; Maru 2012; Zhang and Rowley 2011).

A number of studies have associated IGFBP-2 levels to the proliferation of lymphoblasts (Barrios et al. 2000; Wex et al. 1998; Mohnike et al. 1996; Dawczynski et al. 2003), and high levels of IGFBP-2 have been associated with or predicted adverse outcomes in childhood leukaemia (Barrios et al. 2000; Wex et al. 1998; Elmlinger et al. 1996; Attard-Montalto et al. 1998; Vorwerk et al. 2005; Dawczynski et al. 2006, 2008; Hattori et al. 2006; Kuhn et al. 2011; Kitszel and Krawczuk-Rybak 2007).

IGFBP-2 appears to be differentially expressed between leukaemic B- and T-lymphocyte cell lines (Elmlinger et al. 1996), with the latter showing higher levels of IGFBP-2 mRNA (Elmlinger et al. 1996, 1998). The levels of IGFBP-2 secreted by these cells are regulated by the IGFs, mostly IGF-II, via pathways involving activation of the IGF1R (Elmlinger et al. 1998).

In a study by Mohnike et al. (1996) it was shown that in children with ALL the levels of IGF-I, -II and IGFBP-3 are significantly decreased, while the levels of IGFBP-2 are markedly elevated (+4.0/-0.45 to +7.4 SDS). Similar regulation of IGF system components in ALL patients, including the increase in IGFBP-2 levels, was also reported by Wex et al. (1998). Interestingly, these studies showed that the high serum levels of IGFBP-2 correlated with the detection rate of IGFBP-2 mRNA in the tumour cells. During chemotherapy, IGFBP-2 mRNA levels decreased from 72 % at diagnosis to 35 % post-chemotherapy. These findings suggest that the increased serum levels of IGFBP-2, measured in children suffering from ALL, mainly originated from the tumour clone itself (Wex et al. 1998).

Studies by Dawczynski et al. (2003) report steady and significant increases in IGFBP-2 levels in patients with either ALL or AML following hematological stem cell transplantation (HSCT) (Dawczynski et al. 2003). This observed elevation in serum IGFBP-2, in childhood AML after HSCT, was associated with a high relapse risk (Dawczynski et al. 2006). In fact, most AML-patients with evidence of relapse showed a continuous increase of IGFBP-2 during 100 days follow-up. After HSCT, as above, IGFBP-2 concentrations were significantly higher in patients with relapse than in children without relapse, and patients with IGFBP-2 concentrations up to 4.5 SDS were more likely to relapse and demonstrate poorer clinical outcome (Dawczynski et al. 2006).

In summary, there is a strong consensus that serum and mRNA levels of IGFBP-2 are independent risk factors for the prediction of chemo-resistance and relapse in ALL and AML patients (Dawczynski et al. 2006, 2008; Hattori et al.

2006; Kuhn et al. 2011; Kitszel and Krawczuk-Rybak 2007). It could be speculated that molecular therapies targeting the IGF pathway, and in particularly IGFBP-2, might assist with the management of these difficult to cure malignancies.

Lung cancer

Lung cancers are classified according to the type of cell affected and include the most common non-small cell lung cancer (NSCLC), also classified as squamous cell carcinoma, small cell lung cancer (SCLC) and mesothelioma. IGFBP-2 is consistently expressed in small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC) cell lines and primary NSCLC tumours (Jaques et al. 1992; Reeve et al. 1992b; Hu et al. 2014) (Reeve et al. 1993), while the pattern of expression for all the other IGFBPs differ significantly between cell lines and primary tumours.

Interesting studies from Reeve and co-workers in SCLC and NSCLC (Reeve et al. 1993), showed that the secreted IGFBP-2 can also associate with the cell surface of SCLC cells, but not with that of the NSCLC cells. As a result of this, when the SCLC and NSCLC cells were exposed to iodinated IGF-I or -II, most of the IGF binding was found associated with cell surface bound IGFBP-2 in the SCLC cells, while it was associated with the IGF1R in the NSCLC cells (Reeve et al. 1993). These findings suggest for the first time that in addition to the canonical regulatory activities of soluble IGFBP-2, cell surface associated IGFBP-2 might also play a key role in regulating IGF responsiveness in these type of tumours (Reeve et al. 1993).

A later report from Dong et al. (2002) provided evidence for soluble IGFBP-2 inhibiting DNA synthesis in Mv1 mink lung epithelial cells (CCL64), an event that was attenuated in the presence of IGF-I. As shown by Reeve et al. in SCLC cells (Reeve et al. 1993), Dong and co-workers also showed that IGFBP-2 was associated with the CCL64 cell surface and that this association was blunted in the presence of IGF-I (Dong et al. 2002). The use of IGF analogs, with selective high binding affinity for IGFBPs or for the IGF1R, indicated that the observed IGFBP-2-mediated inhibition of DNA synthesis in CCL64 cells only occurs when IGFBP-2 binds to the cell surface of CCL64 cells (Dong et al. 2002), where it presumably activates specific signalling.

SCLC cells express high levels of IGFBP-2 and this expression appears to be mediated via NeuroD, a neuroendocrine cell-specific transcription factor (Yazawa et al. 2009). SCLC is the most aggressive *in vivo* lung cancer, whereas *in vitro* growth of SCLC is paradoxically slow as compared with that of NSCLC (Yazawa et al. 2009). A further observation by Yazawa et al., that might provide an explanation for this intriguing behavior of SCLC cell *in vitro*, is that the IGFBP-2 promoter is rarely methylated in tumour samples, whereas it is hyper-methylated in most adenocarcinomas and

squamous cell carcinoma lines. It is thus likely that epigenetic alterations at the IGFBP-2 promoter are responsible for the striking differences in IGFBP-2 expression between SCLC and NSCLC in vivo and growth of SCLC and NSCLC cells in vitro.

Further studies in lung adenocarcinoma cells suggest that the elevated expression of IGFBP-2 enhances aggressive behavior of cancer cells via inhibition of apoptosis (Migita et al. 2010). This was shown to occur via an IGFBP-2 mediated decrease of pro-caspase-3 levels, supported by the observation that intracellular IGFBP-2 and procaspase-3 are expressed in a mutually exclusive manner (Migita et al. 2010).

The dramatic increase in circulating levels of IGFBP-2 observed in many cancers, suggests that IGFBP-2 might be considered as a tumour antigen and as such, it could activate an IGFBP-2 tumour-specific immunity. This appears to be the case in lung cancer where serum levels of IGFBP-2 autoantibody are significantly elevated (Zhang et al. 2013). Whether the high circulating levels of IGFBP-2 (Lee et al. 1999; Guo et al. 2013) and anti-IGFBP-2 autoantibodies are appropriate diagnostic biomarkers for lung cancer (Zhang et al. 2013) remains to be determined.

Colon cancer

Colon or colorectal cancer (CRC) originates in the mucosal surface of the large bowel, where dysregulated cell growth can lead to pre-cancerous formations such as intestinal polyposis including hyperplastic or inflammatory polyps or serrate adenomas (Moser et al. 1992). Common sites of CRC development are the sigmoid colon (25 %), rectum (21 %), caecum (20 %), rectosigmoid junction (20 %), followed by transverse colon (15 %) and the ascending colon (10 %).

A large proportion (80 %) of CRCs carries a mutation in the tumour suppressor gene APC (Adenomatous Polyposis Coli; e.g., Familial Adenomatous Polyposis). This mutation produces a truncated and inactive APC protein, an event leading to dysregulated cell growth and oncogenic transformation (Burgess 1998) (Rowan et al. 2000). Mutations in the APC gene affect regulation and degradation of β -catenin, a protein with multiple functions including regulation of cell-cell adhesion and gene transcription (Valenta et al. 2012). Overexpression and/or cytoplasm accumulation of β -catenin is often associated with colorectal carcinomas, which might lead to its translocation into the nucleus where β -catenin acts as a proto-oncogene modulating the expression of a number genes (Valenta et al. 2012), including up-regulation of IGFBP-2 gene (Naishiro et al. 2005).

Although the IGF system is significantly expressed in CRC, the role of IGFBP-2 in CRC is unclear with substantial discrepancies between the in vivo (human and animal models) and in vitro data suggesting either tumour promoting or tumour inhibiting activity for IGFBP-2.

IGFBP-2 is overexpressed in colorectal carcinomas (Naishiro et al. 2005; Ben-Shmuel et al. 2013; Renehan et al. 2000) and its mRNA is reported to be increased in sporadic colorectal cancers (Mishra et al. 1998).

In the study by Mishra et al. (1998), it was shown by in situ hybridization and IGF binding studies that the mRNA for IGFBP-2 and its protein product were located in malignant cells in the colon cancer tissue and not in the surrounding stromal cells. Furthermore, a study by Ben-Shmuel et al. (2012) provided evidence for a role for IGFBP-2 in promotion of colon cancer progression, an event under the transcriptional control of the NF- κ B signalling pathway. In these studies (Ben-Shmuel et al. 2012), IGFBP-2 was highly expressed throughout human CRC tissue samples and co-localized with the phosphorylated p65 subunit of NF- κ B (Ben-Shmuel et al. 2012). These findings suggest a potential autocrine action for IGFBP-2 and point to a key role for IGFBP-2 in progression (Naishiro et al. 2005; Ben-Shmuel et al. 2012) and metastatic processes in CRC.

Interestingly, serum IGFBP-2 levels are elevated in CRC patients (el Atiq et al. 1994) and appears to be associated with increased risk of mortality (Liou et al. 2010). A study by Miraki-Moud et al. (2001) in patients with acromegaly, which are at increased risk of developing colorectal carcinoma, reported significantly increased IGFBP-2 serum levels, and strong immunoreactivity for IGFBP-2 in malignant colonic epithelium compared to benign epithelium.

Conversely, some in vitro work shows that IGFBP-2 inhibits proliferation of IGF-responsive colon carcinoma cell lines (Hoflich et al. 1998; Corkins et al. 1995). A study by Michell et al. (1997) in normal and tumour colonic tissue, demonstrated that IGFBP-2 mRNA is expressed in all normal and cancer cells, with IGFBP-2 only proteolysed in colon cancer tissue. Proteolysis of IGFBP-2, most likely, would result in increased local levels of IGF, thus conferring a growth advantage for the colonic tumour cells as described by (Si et al. 2007) in the human colon cancer cell line HT29.

An inhibitory role for IGFBP-2 in CRC was also demonstrated by Diehl et al. (2009), where IGFBP-2 Tg mice with high levels of IGFBP-2 both systemically and locally in the intestine, were subjected to chemically induced colorectal carcinogenesis. Although the tumour incidence in the Tg and Wt control animal was comparable, the volume of the adenomas in the IGFBP-2 Tg was dramatically reduced and IGFBP-2 serum levels negatively correlated with tumour volume (Diehl et al. 2009). In these studies it was also demonstrated that the reduced volume of the adenomas in the IGFBP-2 Tg mice was consistent with reduced number of cells positive for the proliferation marker Ki67. However, it could be argued that the persistent extreme overabundance of IGFBP-2 in these animals, might significantly account for the inhibitory effects of IGFBP-2 and reduced tumour volume.

Conclusive remarks

In this review, we have described and discussed the multifunctional roles of IGFBP-2 in health and disease, which include both IGFBP-2 IGF-dependent and IGF-independent activities. The effects of IGFBP-2 presence or absence in normal development and physiology, and the experimental evidence derived from IGFBP-2 overexpression or down regulation, suggest IGFBP-2 as a crucial molecule that could be used to enhance health and target disease.

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