Insulin-like growth factor binding proteins and angiogenesis: from cancer to cardiovascular disease

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ABSTRACT

Angiogenesis is a tightly regulated activity that is vital during embryonic development and for normal physiological repair processes and reproduction in healthy adults. Pathological angiogenesis is a driving force behind a variety of diseases including cancer and retinopathies, and inhibition of angiogenesis is a therapeutic option that has been the subject of much research, with several inhibitory agents now available for medical therapy. Conversely, therapeutic angiogenesis has been mooted as having significant potential in the treatment of ischemic conditions such as angina pectoris and peripheral arterial disease, but so far there has been less translation from lab to bedside.

The insulin-like growth factor binding proteins (IGFBP) are a family of seven proteins essential for the binding and transport of the insulin-like growth factors (IGF). It is being increasingly recognised that IGFBPs have a significant role beyond simply modulating IGF activity, with evidence of both IGF dependent and independent actions through a variety of mechanisms. Moreover, the action of the IGFBPs can be stimulatory or inhibitory depending on the cell type and environment. Specifically the IGFBPs have been heavily implicated in angiogenesis, both pathological and physiological, and they have significant promise as targeted cell therapy agents for both pathological angiogenesis inhibition and therapeutic angiogenesis following ischemic injury. In this short review we will explore the current understanding of the individual impact of each IGFBP on angiogenesis, and the pathways through which these effects occur.

1. Introduction

Angiogenesis, the formation of new blood vessels from pre-existing vasculature, is a fundamental, tightly regulated activity in many biological contexts including development, reproduction and tissue repair [1]. Angiogenesis can also become a pathological process critical to the development and progression of several diseases and may potentially be harnessed therapeutically to improve tissue perfusion in ischemia [2].

During development there are two distinct processes that form the vascular network: vasculogenesis and angiogenesis. Vasculogenesis refers to the initial differentiation of endothelial precursors in the embryonic period that occurs in order to establish a population of endothelial precursor cells. These cells can then release angiogenic factors and cytokines as well as being able to differentiate into a population of endothelial cells, which establish the rudimentary vasculature [3]. Angiogenesis is the process by which new blood vessels sprout from pre-existing vasculature. This occurs via a stepwise process that involves endothelial cell proliferation and migration to form vascular sprouts, followed by degradation and invasion of the extracellular

matrix and finally vascular tube formation [4]. Angiogenesis is a complex process, orchestrated by the local production of a range of growth factors and cytokines. These activate cellular signalling pathways and gene transcription to modulate the sprouting, proliferation and migration of endothelial cells and their interaction with the extracellular matrix [5]. Although vascular endothelial growth factors (VEGF) are the central mediators of angiogenesis, other growth factors including fibroblast growth factors (FGFs), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF) and insulin-like growth factors (IGFs) are known to play important roles. Understanding how these growth factors and their regulatory partners are involved in angiogenesis is critical to scientific advancement in the fields of development, cancer and ischemic disorders.

In pre- and post-natal development, angiogenesis is essential for organ growth but in healthy adults the vasculature is largely quiescent, with the exception of the female reproductive system. However, the need for normal physiological repair processes such as wound healing and continual remodelling of capillary beds requires angiogenesis to be a tightly regulated process that can be 'switched' on and off as needed,

depending on the balance between stimulatory and inhibitory signals [6].

'Pathological' angiogenesis is involved in the progression of several diseases including age-related macular degeneration, diabetic retinopathy and rheumatoid arthritis [7] [2]. Survival of tumour cells also relies on pathological angiogenesis: when cancer cells first develop they are initially dormant until they develop a vascular network that allows them to grow and metastasise. Tumour cells grow at an accelerated rate and in doing so cannot maintain an adequate supply of glucose and oxygen, thereby creating the perfect stressors for the release of pro-angiogenic factors and the activation of angiogenesis [8].

In ischemic states secondary to atherosclerosis or thrombosis, local release of angiogenic factors endeavours to promote perfusion of the ischemic tissues, but often the physiological process is inadequate to restore sufficient tissue perfusion [9]. A pertinent example is peripheral arterial disease (PAD), which presents clinically as intermittent claudication or critical limb ischemia, the latter of which poses a major risk of limb loss and is associated with high mortality. In some patients, revascularisation either fails or is not an appropriate option, leaving amputation as the only alternative [10].

Over the past decades, efforts have been undertaken to modulate angiogenesis as a therapeutic strategy to either promote revascularization of ischemic tissues or inhibit angiogenesis in cancer, ocular, joint or skin disorders. Angiogenesis inhibitors have been successfully developed which are now used clinically in the treatment of certain cancers and forms of macular degeneration [11]. Manipulating upregulation of angiogenesis in order to repair damaged organs and regrow blood vessels in ischemic disorders, termed 'therapeutic angiogenesis', has proved to be more challenging. Potential approaches include the systemic or local delivery of pro-angiogenic factors (as recombinant proteins or gene therapy) or cell-based strategies using autologous or ex vivo modified stem/progenitor cells [12].

Initial exploration of therapeutic angiogenesis in animal models and Phase 1 clinical trials appeared very promising. VEGF and FGF recombinant protein were used in both animal and human models to successfully induce angiogenesis and collateralisation in myocardial ischemia, with demonstrably improved perfusion of the target organ [13–15]. The delivery of autologous bone marrow derived stem cells in PAD and myocardial infarction also appeared to successfully induce angiogenesis and improve perfusion in initial trials [16,17]. The VIVA trial was the first larger scale randomised control trial (RCT) and examined the effects of intra-coronary rhVEGF administration in patients with refractory stable angina. No improvement was seen in myocardial perfusion or exercise tolerance, with only a marginal improvement in self-reported angina symptoms seen in the treated group [18]. Meta-analyses of autologous cell therapy in PAD also showed that despite promising results in several trials, in placebo controlled RCTs with a low risk of bias no benefit with cell therapy was observed [19].

Gene therapy has been mooted as a potential method to overcome the shortcomings of recombinant protein administration, with direct delivery of vectors containing VEGF-A or FGF genes to areas of ischemia. As with the other methods this also appeared to be effective in animal models and small human trials, both for PAD and myocardial ischemia [20–22]. However, larger placebo controlled studies again failed to show any significant improvement in perfusion or clinical symptoms [23,24].

Given these shortcomings when examined on a large scale, a pro-angiogenic therapy has not yet been licensed for routine clinical use. Nevertheless, the clinical need to improve tissue perfusion in ischemic disorders remains; therefore therapeutic angiogenesis remains an important research area, and new pro-angiogenic therapies need to be identified. The majority of studies so far have only examined VEGF and FGF administration, but there are several other cytokines and growth factors with significant therapeutic potential.

The insulin-like growth factor (IGF) system comprises two insulin-like growth factors (IGF-I and IGF-II), their receptors (IGF1R and IGF2R) and seven insulin-like growth factor binding proteins (IGFBPs), with 99% of all circulating IGFs bound to a member of the IGFBP family [25]. The IGFs are growth factors with molecular structural homology to proinsulin and significant overlap in signalling pathways and receptor interaction [26]. They act as circulating factors but also possess important autocrine and paracrine actions dependent on local release. Circulating IGF-I is predominantly released in response to stimulation by growth hormone (GH) and its primary role is to promote cellular growth and proliferation, although it is also involved in blood glucose regulation and plays an active role in promoting angiogenesis [25]. IGF-II expression is independent of GH and has historically been considered to have a prominent role only in pre-natal growth and development, due to negligible expression of the protein post-natally in adult rodents [27]. In humans, however, expression of IGF-II persists postnatally and it continues to play a role in cell growth, proliferation and angiogenesis [28]. Most cellular actions of IGF-I and IGF-II are mediated by the IGF1 receptor (IGF1R), a receptor tyrosine kinase which activates several signalling pathways including phosphatidylinositol 3-kinase and protein kinase B (PI3K/AKT) and Ras/Raf/ERK. The IGF2 receptor (IGF2R) is thought to be involved with IGF-II clearance but may have a limited signalling role.

A family of seven IGFBPs confers spatial and temporal regulation to IGF activity. Several IGFBPs also possess important IGF-independent effects. Three distinct structural regions are shared by all IGFBPs: an N-terminal cysteine rich region; a C-terminal cysteine rich region; and a linker region. The N-terminal and C-terminal regions contribute to IGF binding and are highly conserved across the IGFBPs; while the linker region is variable and can contain a variety of functional motifs and binding sites [29]. The linker domain is susceptible to post-translational modification and contains sites for proteolysis by a range of proteases. In the circulation, the majority of IGFs are bound to IGFBPs to form binary complexes or form large molecular mass complexes with IGFBP-3 and IGFBP-5 and an acid labile subunit (ALS) which are unable to cross to endothelial barrier. Binary complexes allow IGFBPs to localise within tissues where their action is predominantly to inhibit IGF actions, although this is context-dependent and IGFBPs may potentiate IGF actions in certain situations.

All members of the IGFBP family have been studied in the context of angiogenesis, where inhibitory and stimulatory actions have been ascribed to the different binding proteins. Local production of IGFBPs in the blood vessel wall [30], ischemic tissues or tumours [31] is of particular relevance. The predominant effects of IGFBPs on angiogenesis are mediated through modulation of IGF bioactivity, although important IGF-independent actions are emerging.

2. IGFBP-1

IGFBP-1 is a 30 kDa protein, with circulating levels produced predominantly in the liver and kidneys [32]. IGFBP-1 is expressed locally within the blood vessel, including the endothelium [33]. The predominant action of IGFBP-1 is thought to be dynamic regulation of IGF bioavailability, although an integrin binding domain (RGD) found in the C-terminus region of IGFBP-1 has been found to be an important mediator of IGF independent actions [34] [35].

IGFBP-1 levels are dynamically regulated in relation to nutritional intake: fasting levels are four to five times higher than non-fasting levels and IGFBP-1 production in the liver is inhibited directly by the increase in insulin levels in the acute post-prandial state [36]. IGFBP-1 accounts for only a small proportion of overall IGF binding capacity and so is thought to be mainly involved in acute IGF-I regulation [36] [37].

Influences of IGFBP-1 on cellular processes including migration and proliferation have been extensively researched and both IGF-dependent and IGF-independent activities have been reported. Both stimulation and inhibition of IGF actions by IGFBP-1 have been demonstrated depending on cell type and environment [35,38]. Although several studies implicate IGFBP-1 in vascular pathophysiology and endothelial

function, investigation of IGFBP-1 in the setting of angiogenesis is limited.

In the context of glioblastoma, proteomic analysis identified IGFBP-1 as the key mediator of angiogenesis secreted by microglial cells in response to macrophage colony stimulating factor (MCSF). When conditioned medium from glioma cells was added to human umbilical vein endothelial cells (HUVEC), silencing of MCSF prevented tube formation. Upregulation of IGFBP-1 was identified in the microglial cell secretome in response to MCSF, and silencing of IGFBP-1 in microglial cells blocked angiogenesis in HUVECs treated with the conditioned media [39]. The mechanism by which IGFBP-1 promoted angiogenesis was not elucidated in this study.

In human chondrocytes, lysophosphatidic acid (LPA) was shown to activate the NF-kB pathway and to enhance tube formation and cell migration of HUVECs treated with conditioned media [40]. IGFBP-1 was identified in the chondrocyte secretome along with known pro-angiogenic factors including VEGF, interleukin-8 (IL-8), matrix metalloproteinase (MMP)-9 and monocyte chemoattractant protein 1 (MCP-1). Although IGFBP-1 was found to be up-regulated, the direct contribution of IGFBP-1 to angiogenesis was not determined.

We previously reported that over-expression of IGFBP-1 in transgenic mice improved vascular endothelial function through increased basal nitric oxide (NO) production. Endothelial nitric oxide synthase (eNOS) mRNA expression was upregulated [37]. IGFBP-1 stimulated eNOS phosphorylation via the PI3K/AKT pathway – an effect independent of the IGF1 receptor. Over-expression of IGFBP-1 was also found to have an anti-atherosclerotic effect in the same transgenic mice [41]. NO is an important mediator of angiogenesis [42], and although in vitro angiogenesis was not examined in cells from these mice, it could be hypothesised that upregulation of the PI3K/AKT pathway by IGFBP-1 could stimulate angiogenesis as described elsewhere [43]. The integrin-binding RGD domain of IGFBP-1 is potentially of interest in this context, recognising that signalling downstream of integrins, for example through focal adhesion kinase, stimulates angiogenesis [44].

Amniotic membrane is developmentally avascular and has potential to inhibit neovascularisation of the cornea following ocular injury. The anti-angiogenic properties of the amniotic membrane have been shown to be mediated by a complex of hyaluronan and the heavy chain of inter-α-inhibitor. This complex significantly inhibited tube formation in HUVEC and reduced neo-vascularisation in a chorioallantoic membrane (CAM) model [45]. IGFBP-1 and the anti-angiogenic platelet factor 4 (PF4 or CXCR4) were identified in the complex by a screen of potential angiogenesis-related proteins, although the contribution of each was not specifically demonstrated.

In summary, IGFBP-1 has been shown to have both stimulatory and inhibitory effects on cells, through IGF dependent and independent pathways. Limited evidence supports a pro-angiogenic effect of IGFBP-1 but confirmatory studies are needed.

3. IGFBP-2

IGFBP-2 is a 31.4 kDa protein and the second most abundant IGFBP in the circulation [46]. It contains an RGD integrin binding domain within the C-terminus similar to IGFBP-1, and in addition contains a heparin binding domain (HBD) and nuclear localisation sequence (NLS) within the link region, both of which act as functional motifs to facilitate extracellular matrix (ECM) binding and nuclear localisation respectively [29,47]. Although IGFBP-2 levels are not as acutely regulated by insulin levels as IGFBP-1 [26], plasma IGFBP-2 levels are inversely correlated with insulin resistance [48] and over-expression of IGFBP-2 has been demonstrated to be protective against obesity and insulin resistance [49].

IGFBP-2 appears to play an important role in vascular development, as knockdown led to impaired vascular sprouting in zebrafish embryos [50]. In adult animals, IGFBP-2 expression is increased in ischemic stroke [51] and upregulation of IGFBP-2 is a signature of several types

of cancer in which it has been implicated in tumour angiogenesis. IGFBP-2 over-expression activates pro-tumorigenic gene expression in neuroblastoma cells, including significantly up-regulated VEGF mRNA transcription. Increased in vivo angiogenesis was observed when IGFBP-2 overexpressing neuroblastoma cells were studied in a quail embryo CAM assay [52]. Interestingly, upregulation of VEGF transcription was only seen in the presence of intra-cellular IGFBP-2, with nuclear translocalisation of IGFBP-2 shown to be mediated by the functional nuclear localization sequence within the link region [53]. No role was seen for IGF-I, suggesting these were IGF independent effects.

A separate study by Russo et al. also examined neuroblastoma cells and found over-expression of IGFBP-2 enhanced proliferation, migration and invasion in vitro, and through the use of IGFBP-2 mutants determined this was facilitated through ECM binding by the HBD. Within the same study IGFBP-2 inhibited exogenous IGF-I mediated proliferation, suggesting a dual role through differing, competing pathways [47]. A further study examining glioma progression linked IGFBP-2 to upregulation of NF-κB through integrin binding and activation of integrin-linked kinase (ILK) pathways. Although this study did not examine angiogenesis directly; cell migration, invasion and overall glioma progression were shown to be positively up-regulated by IGFBP-2 through IGF-independent integrin binding [54], highlighting the potential for IGFBP-2 to act through several different pathways.

IGFBP-2 has also been identified as a pro-angiogenic factor in melanoma. Das et al. investigated the role of melanoma differentiation associated gene-9 (mda-9/syntenin) in tumour angiogenesis, and found that mda-9/syntenin augmented angiogenesis in CAM assays and increased tube formation in co-cultured HUVECs. IGFBP-2 was identified as a mda-9/syntenin induced factor, and was shown to independently augment tube formation in HUVECs, with knockdown of IGFBP-2 associated with both reduced in vitro tube formation and CAM neo-vascularisation [55]. The induction of IGFBP-2 expression by mda-9/syntenin was shown to be a consequence of AKT and hypoxia-inducible factor 1 α (HIF-1α) activation. The same authors found that integrin binding by IGFBP-2 in HUVECs caused activation of the PI3K/AKT pathway and VEGF-A up-regulation, and hypothesised that endothelial recruitment and subsequent activation of this pathway by IGFBP-2 was the mechanism through which angiogenesis in melanoma occurred [55]. Interestingly, although integrin binding was seen to be key to activation of the PI3K/AKT pathway, inhibition of the IGF-1R abrogated this response, suggesting a significant role for the IGF-1R, although the mechanism of this role was not defined.

Png et al. also described an IGF-I dependent pro-angiogenic role for IGFBP-2. They had previously identified the micro-RNA miR-126 as a suppressor of breast cancer metastasis [56]. They went on to find that this was achieved through the suppression of IGFBP-2 induced metastatic angiogenesis in endothelial cells and that IGFBP-2 secreted by breast cancer cells positively modulated IGF-I mediated activation of IGF-1R on endothelial cells, promoting their recruitment for metastatic angiogenesis [57]. The involvement of the IGF-1R in the pro-angiogenic role of IGFBP-2 was also seen by Shen et al., who found binding of IGFBP-2 to receptor protein tyrosine phosphatase β (RPTPβ) caused inactivation of RPTPβ and subsequently inhibited transcription of the tumour suppressor gene PTEN. Inhibition of PTEN allowed increased activation of the PI3K/AKT pathway by IGF-I, promoting vascular smooth muscle proliferation, and raising the potential for a pro-angiogenic action as well. Inhibiting IGF-1R expression however prevented the inactivation of RPTPβ, suggesting the effects of IGFBP-2 on PTEN phosphorylation required coordination of IGFBP-2, IGF-I and IGF-1R [58].

In summary, although IGFBP-2 has been shown in some environments to inhibit the mitogenic actions of IGF-I [59], the overwhelming evidence suggests a pro-angiogenic role for IGFBP-2. The effects of IGFBP-2 have been proven to occur through a variety of both IGF dependent and independent pathways, creating the possibility of dual, competing roles. Further understanding of these pro-angiogenic

pathways could allow the use of IGFBP-2 as an agent for therapeutic angiogenesis after ischemic insult, and conversely the inhibition of IGFBP-2 may be a novel avenue in the treatment of various cancers.

4. IGFBP-3

IGFBP-3 is a 28.7 kDa protein primarily produced in the liver and is the most abundant IGFBP, responsible for over 80% of IGF-I and IGF-II binding in the circulation. It binds IGF-I in a ternary complex with an acid-labile subunit, forming a stable 150 kDa complex and preventing transport of IGF-I into the tissues [25]. Both pro-angiogenic and anti-angiogenic roles of IGFBP-3 have been reported.

Granata et al. demonstrated a pro-angiogenic role for IGFBP-3 in HUVECs both *in vitro* and *in vivo*. IGFBP-3 induced the expression of several angiogenesis-related genes including VEGF, MMP-2, and MMP-9. This pro-angiogenic action was mediated by IGFBP-3 induction of the sphingosine kinase (SphK1) pathway and was dependent on IGF-I signalling [60].

IGFBP-3 was released with other angiogenic factors, such as hypoxia inducible factor alpha (HIF-α) and VEGF when human retinal pigmented epithelial cells were stimulated with IGF-I, suggesting that IGFBP-3 may have a pro-angiogenic effect in choroidal neovascularisation [61]. Several *in vivo* studies have provided further evidence of this. Lofqvist et al. showed that IGFBP-3 deficient mice had a decrease in retinal vessel regrowth following hypoxia and wild type mice treated with IGFBP-3 had a significant increase in vessel regrowth, independent of IGF-I levels. This study also incorporated a prospective clinical study of premature human infants showing an association between IGFBP-3 levels and a decrease in retinal neovascularisation [62]. These findings were corroborated by Chang et al., who injected an IGFBP-3 expressing plasmid into the vitreous of mice and showed that this IGFBP-3 over-expression promoted angiogenic repair of the eye [63].

Fanton et al. demonstrated that cardiac atrial appendage stem cells (CASCs) secrete high levels of IGFBP-3, VEGF and endothelin-1 (ET-1), and promoted microvascular endothelial cell proliferation, tube formation and migration *in vitro* and stimulated angiogenesis *in vivo* in a CAM assay. Combined inhibition of the three growth factors was required to inhibit tube formation, with individual inhibition having no effect. The authors postulated that these growth factors acted synergistically to enhance angiogenesis, although a pathway for this was not elucidated, and the authors did not explore the effect of inhibition of VEGF and ET-1 only [64].

In contrast to the previous reports, there is also significant evidence for IGFBP-3 as an anti-angiogenic agent. IGFBP-3 was found to block tumour angiogenesis in both non-small cell lung cancer and squamous cell carcinoma of the head and neck. This was demonstrated to occur via an IGF-independent pathway involving the inactivation of ERK1/2 and Elk-1, leading to inhibition of basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) transcription: growth factors known to have significant roles in angiogenesis and cancer cell survival [65]. IGFBP-3 has also been shown to inhibit endothelial cell adhesion to the extracellular matrix [66], which is important in angiogenesis and endothelial repair. Han et al. demonstrated that a peroxisome proliferator-activated receptor (PPAR-δ) agonist caused endothelial progenitor cells to produce MMP-9, which degraded IGFBP-3, leading to a higher capillary-to-myocyte ratio and faster recovery of blood flow in an ischemic hind-limb mouse model. Muscle architecture was also more intact and no fibrosis was observed, both of which were reversed with the introduction of IGFBP-3. IGFBP-3 degradation has also been shown to induce IGF-1R phosphorylation, resulting in augmented angiogenesis in an *in vivo* mouse hindlimb ischemia model [67].

It has been shown that in head and neck cancers, IGFBP-3 expression reduces the tumours' angiogenic capacity, thereby limiting lymph node and wider body metastasis, and treatment with IGFBP-3 above the physiological concentration of 2-7 μg/ml will inhibit VEGF expression [68]. An *in vivo* study conducted in mouse endothelial progenitor cells

showed that the transcription factor Runx1 promoted angiogenesis by down-regulating mRNA expression of IGFBP-3 through directly binding to the mouse IGFBP-3 gene and preventing its transcription [69]. Increasing expression of IGFBP-3 has already been exploited therapeutically by way of SCH6633, a farnesyl transferase inhibitor, which is an anti-angiogenic oral agent designed to block tumour growth [70].

To summarise, a pro-angiogenic role for IGFBP-3, especially in the context of hypoxia, has been revealed, with potential for its utilisation as a therapeutic agent in the context of ischemic insult. However, this needs to be balanced against its anti-angiogenic function that has also been demonstrated to occur through a variety of pathways, which could potentially be exploited as a novel cancer therapeutic inhibitor.

5. IGFBP-4

IGFBP-4 is a 24 kDa protein produced in the liver and is the smallest IGFBP [71]. Unlike the other IGFBPs, IGFBP-4 inhibits the action of IGF-I and IGF-II in almost all cellular environments [25]. Several studies have investigated how the inhibitory effect of IGFBP-4 on IGF-I can be manipulated to restrict tumour size through a reduction in angiogenesis. Colon cancer cells implanted into mice to create subcutaneous tumours had significantly fewer micro-vessels per area when treated with IGFBP-4 compared to controls. This increased IGFBP-4 exposure led to a concomitant rise in IGF-1R levels, potentially indicating the anti-angiogenic effects of IGFBP-4 were secondary to inhibition of IGF-I and IGF-1R interaction [72].

Contois et al. also showed that IGFBP-4 inhibited IGF-I induced angiogenesis *in vivo*, but interestingly did not have an effect on VEGF-induced angiogenesis [73]. The continued stimulatory effects of VEGF were dependent on sustained p38/MAPK activity, indicating IGFBP-4 may have pathway specific anti-angiogenic properties. An *in vivo* model of murine breast cancer also demonstrated that a protease-resistant IGFBP-4 mutant restricted the actions of IGF-I by preventing binding to its receptor, subsequently decreasing tumour growth through reduced angiogenesis [74].

A recent study by Smith et al. highlighted a potential mechanism through which the interaction between IGFBP-4, IGF-I and the IGF-1R could be exploited therapeutically. IGF-I is cleaved from IGFBP-4 by the protease pregnancy-associated plasma protein-A (PAPP-A), allowing the free IGF-I molecule to bind to IGF-1R. A PAPP-A resistant IGFBP-4 mutant was shown to prevent binding of IGF-I to IGF-1R through competitive antagonism. This in turn reduced IGF-I mediated activation of the PI3K/AKT pathway; inhibiting angiogenesis, migration and invasion both *in vitro* and *in vivo* [75].

An IGF-independent role for IGFBP-4 in the inhibition of angiogenesis has also been seen. Human glioblastoma cells exposed to Dibutyryl cAMP (dB-cAMP) have been found to be less aggressive, invasive and have a reduced ability to stimulate angiogenesis in human brain endothelial cells. A study by Moreno et al. found that this response appeared to be mediated by high levels of IGFBP-4 secreted by the glioblastoma cells after they had by exposed to dB-cAMP. An IGFBP-4 neutralising antibody reversed the anti-angiogenic effect seen. It was speculated that this inhibitory effect of IGFBP-4 on angiogenesis was independent of IGF-I, as the levels of IGF-I secreted by the cells were undetectable [76]. This IGF-independent effect appeared to be mediated by the C-terminal protein fragment of IGFBP-4 (CIBP-4). This fragment contains a thyroglobulin type 1 domain and a recombinant fragment was found to inhibit tubulogenesis, cathepsin activity and glioblastoma tumour growth to the same extent as the complete IGFBP-4 protein [77].

In summary, although the molecular mechanisms underlying the anti-angiogenic effect of IGFBP-4 are yet to be fully determined, it is clear that IGFBP-4 is an anti-angiogenic molecule with significant potential as an inhibitor in novel cancer therapies.

6. IGFBP-5

IGFBP-5 is a 28.6 kDa protein and binds the IGFs in a ternary complex with ALS, in a similar manner to IGFBP-3 [46]. Association with extracellular matrix proteins results in a reduced affinity to the IGFs, releasing them from the ternary complex that IGFBP-5 forms with IGF and the acid labile subunit [78]. Through this mediation IGFBP-5 modulates IGF action, but IGF-independent actions have also been seen.

In vitro, VEGF-induced proliferation, tube formation and migration in HUVEC were directly inhibited by over-expression of IGFBP-5. This inhibitory effect was reversed using siRNA to silence IGFBP-5 over-expression. IGFBP-5 has also been shown to significantly inhibit blood vessel formation *in vivo* in the CAM assay and in an *in vivo* tumour growth model in which SKOV-3 ovarian cancer cells were xenografted into mice. Subcutaneous injection of IGFBP-5 markedly inhibited tumour growth and decreased the number of blood vessels in IGFBP-5 treated mice compared to controls. Reduced expression of phosphorylated AKT and phosphorylated eNOS was observed, indicating this anti-angiogenic activity may be mediated through inhibition of the PI3K/AKT pathway [79]. These findings have been echoed recently, with a truncated IGFBP-5 peptide derived from the c-terminus inhibiting angiogenesis and ovarian tumour growth *in vivo* and *ex vivo*. Both the AKT/ERK and NF-kB-VEGF/MMP pathways were down-regulated by this c-terminal region in an IGF-independent manner, as no inhibition was seen with a peptide containing the IGF-binding site only [80].

In contrast to the inhibitory actions described above, IGFBP-5 has been found to stimulate proliferation in intestinal smooth muscle cells [81] and prostate cancer cells [82]. In summary, although these findings support a primarily anti-angiogenic role, any future therapeutic option will need to be assessed with caution.

7. IGFBP-6

IGFBP-6 is a 22.8 kDa protein produced in the liver, and uniquely has a 50-fold increased preference to IGF-II compared to IGF-I [83]. The primary role of IGFBP-6 is to inhibit the actions of IGF-II, such as cell proliferation, migration and differentiation, but IGF independent actions have also been reported [84].

HUVECs treated with IGFBP-6 showed impaired tube formation *in vitro* and addition of IGFBP-6 negated VEGF-induced HUVEC tube formation. Angiogenesis inhibition was also examined *in vivo* by injection of human IGFBP-6 mRNA into *flk1*:GFP zebrafish, resulting in impaired embryonic angiogenesis. Furthermore, an IGFBP-6 mutant with a 10,000 times lower affinity for IGF-II exerted the same anti-angiogenic effect, suggesting an IGF-independent mechanism [85]. Exposing HUVECs to hypoxic conditions over a prolonged time period induced IGFBP-6 mRNA expression, likely through HIF-1α mediated activation. The authors therefore hypothesised that IGFBP-6 could be a negative regulator of hypoxia induced angiogenesis [85].

A recent study by Qiu et al. also showed an inhibitory role for IGFBP-6 with dose-dependent IGFBP-6 inhibition of proliferation, invasion and migration of colorectal cancer cells seen *in vitro* [86]. However, Fu et al. demonstrated IGFBP-6 promoted migration in rhabdomyosarcoma cell lines. This was demonstrated to be IGF independent, as a non-IGF-binding IGFBP-6 mutant also stimulated cell migration, with p38/MAPK activation implicated as the causative pathway [87]. Furthermore, opposing actions of IGFBP-6 have been seen in two different ovarian cancer cell lines, with promotion of SKOV3 cell migration and inhibition of HEY cell migration, despite upregulation of MAPK pathways in both [88].

In summary, evidence for the involvement of IGFBP-6 in angiogenesis is limited, and both inhibitory and stimulatory roles have been observed in different cellular environments. Further research is required before we have a definitive understanding of IGFBP-6 and its place in angiogenesis regulation.

8. IGFBP-7

IGFBP-7, also known as insulin like growth factor binding protein related protein [89], has an amino acid sequence with high similarity to the other human IGFBPs [90]. IGFBP-7 (formally known as mac25) meets structural criteria as a member of the IGFBP family and affinity cross-linking data has shown that IGFBP-7 specifically binds IGF-I and IGF-II, indicating that it is a bona fide IGFBP [90]. IGFBP-7 also binds to unoccupied IGF-1R and suppresses downstream signalling, thereby inhibiting protein synthesis, cell growth, and survival [91].

In endothelial cells, IGFBP-7 is stored in Weibel-Palade Bodies, suggesting that on its release it is involved in vascular homeostasis [92]. Evidence to support a role for IGFBP-7 in angiogenesis is conflicting, although the majority of data suggest IGFBP-7 is anti-angiogenic. A study in glioblastoma indicated that IGFBP-7 was strongly expressed in tumour endothelial cells, was pro-angiogenic and enhanced tube formation in brain endothelial cells [93]. Subsequent reports, however, have shown that IGFBP-7 treatment reduced angiogenesis in hepatocellular carcinoma, when nude mice received xenografts from human hepatocellular carcinoma cells [94,95]. In human endothelial cells and vascular endothelial cells from rat corpus luteum, IGFBP-7 reduced VEGF tube formation, proliferation and the phosphorylation of MEK/ERK 1/2 [96,97]. Not only did IGFBP-7 down regulate VEGF signalling, it also down regulated VEGF expression [96,97]. Recent data from a murine angiogenesis model show that IGFBP-7 inhibited retinal angiogenesis by blocking ERK signalling pathway and down-regulating VEGF expression [98]. This is supported *in vitro* using a retinal endothelial cell line which showed that IGFBP-7 can inhibit the stimulatory effect of VEGF on retinal angiogenesis in vitro by inhibiting expression of B-Raf to induce apoptosis [99]. In epithelial cells, knock down of IGFBP-7 upregulates the MAPK signalling pathway further strengthening studies from endothelial cells that suggest IGFBP-7 down regulates MAPK signalling [100].

In summary, although IGFBP-7 has been ascribed pro-angiogenic effects in glioblastoma, other reports collectively suggest a robust anti-angiogenic effect of IGFBP-7.

9. Conclusion

The actions of the different members of the IGFBP family are widely varied and their role in angiogenesis is summarised in Table 1. Furthermore, the IGFBP family have been shown to have both IGF dependent and independent actions, with their role in angiogenesis especially appearing to have both IGF-independent and IGF-dependent mechanisms.Fig. 1

Recombinant proteins are one of the fasting growing classes of therapeutic compounds and several of the IGFBPs have pro- or anti-angiogenic actions that could potentially be explored clinically. Amongst IGFBP family members, IGFBP-1 and IGFBP-2 have consistent pro-angiogenic effects that could potentially be exploited in the future for therapeutic angiogenesis during times of ischemia. However, more information is required regarding the specific pathways through which these effects occur, and there is need for a greater volume of *in vivo* data

Table 1
Summary of Insulin-like growth factor binding proteins' (IGFBPs) role in angiogenesis.

Binding Protein	Pro- angiogenic	Anti-angiogenic
IGFBP-1	✓	✓
IGFBP-2	✓	×
IGFBP-3	✓	✓
IGFBP-4	×	✓
IGFBP-5	×	✓
IGFBP-6	×	✓
IGFBP-7	✓	✓
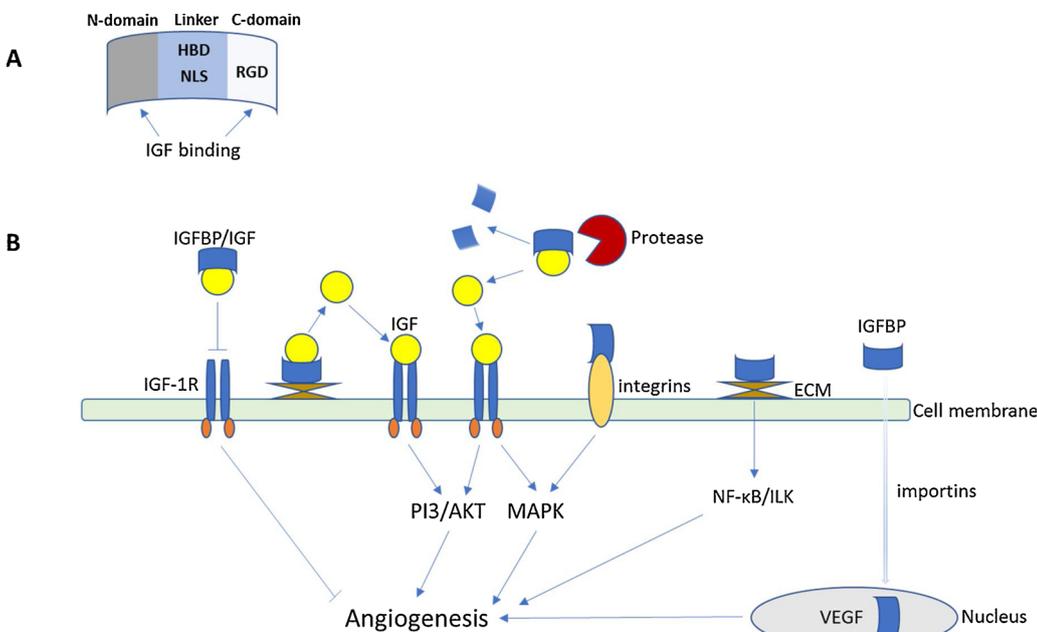
Fig. 1. (A) Demonstration of potential domains and functional motif locations within the Insulin-like growth factor binding proteins (IGFBP). IGF binding domains are demonstrated to be present in the N-terminal and C-terminal domains, and are highly conserved across the IGFBPs. The linker domain is variable between the IGFBPs, and can contain a range of functional motifs. The heparin binding domain (HBD) and nuclear localisation sequence (NLS) demonstrated here are most well described in IGFBP-2, but both are also present in IGFBP-3 and 5, with IGFBP-6 possessing an NLS but not an HBD. The RGD motif demonstrated within the C-terminal domain is present in both IGFBP-1 and IGFBP-2. (B) Schematic of the different potential modes of action of the IGFBPs and how they can influence angiogenesis. Both stimulatory and inhibitory interactions with the IGF-1R are evident, as well as IGF-independent interaction with various cell surface receptors, upregulation of pro-angiogenic signalling pathways and direct upregulation of VEGF mRNA transcription via nuclear localisation.

demonstrating a beneficial effect. IGFBP-3 and IGFBP-7 appear to have both pro and anti-angiogenic properties, whilst IGFBP-4 and IGFBP-5 have predominantly anti-angiogenic properties, and could provide potential new targets and avenues for research in developing novel cancer therapeutics.

Over the last twenty years we have developed a much greater understanding of the individual structure and actions of the IGFBPs and the influence they can exert within distinct cellular environments. However, a great deal more investigation is required to fully elucidate the mechanisms through which they exert their effects, and to understand their tissue distribution and the proteases which regulate their local activity before their promise can be translated into therapeutic agents.

Declarations of interest

References

[1] A. Hoeben, B. Landuyt, M.S. Highley, et al., Vascular endothelial growth factor and angiogenesis, Pharmacol Rev 56 (2004) 549–580, https://doi.org/10.1124/pr.56.4.3.

[2] M. Simons, J.A. Ware, Therapeutic angiogenesis in cardiovascular disease, Nat Rev Drug Discov 2 (2003) 863–871, https://doi.org/10.1038/nrd1226.

[3] A. Czirok, C.D. Little, Pattern formation during vasculogenesis, Birth Defects Res C Embryo Today 96 (2012) 153–162, https://doi.org/10.1002/bdrc.21010.

[4] O.H. Lee, S.K. Bae, M.H. Bae, et al., Identification of angiogenic properties of insulin-like growth factor II in in vitro angiogenesis models, Br J Cancer 82 (2000) 385–391, https://doi.org/10.1054/bjoc.1999.0931.

[5] M. Potente, H. Gerhardt, P. Carmeliet, Basic and therapeutic aspects of angiogenesis, Cell 146 (2011) 873–887, https://doi.org/10.1016/j.cell.2011.08.039.

[6] D. Hanahan, J. Folkman, Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis, Cell 86 (1996) 353–364, https://doi.org/10.1016/S0092-8674(00)80108-7.

[7] P.J. Rosenfeld, D.M. Brown, J.S. Heier, et al., Ranibizumab for neovascular age-related macular degeneration, N Engl J Med 355 (2006) 1419–1431, https://doi.org/10.1056/NEJMoa054481.

[8] A. Wicki, G. Christofori, The Angiogenic Switch in Tumorigenesis, in: D. Marmé, N. Fusenig (Eds.), Tumor Angiogenesis: Basic Mechanisms and Cancer Therapy, Springer Berlin Heidelberg, Berlin, Heidelberg, 2008, pp. 67–88, , https://doi.org/10.1007/978-3-540-33177-3_4.

[9] S. Banai, D. Shweiki, A. Pinson, et al., Upregulation of vascular endothelial growth factor expression induced by myocardial ischaemia: implications for coronary angiogenesis, Cardiovasc Res 28 (1994) 1176–1179, https://doi.org/10.1093/cvr/28.8.1176.

[10] G. Peach, M. Griffin, K.G. Jones, et al., Diagnosis and management of peripheral arterial disease, BMJ 345 (2012) e5208, https://doi.org/10.1136/bmj.e5208.

[11] N. Ferrara, R.D. Mass, C. Campa, et al., Targeting VEGF-A to treat cancer and age-related macular degeneration, Annu Rev Med 58 (2007) 491–504, https://doi.org/10.1146/annurev.med.58.061705.145635.

[12] H. Al Sabti, Therapeutic angiogenesis in cardiovascular disease, J Cardiothorac Surg 2 (2007) 49, https://doi.org/10.1186/1749-8090-2-49.

[13] K. Sato, R.J. Laham, J.D. Pearlman, et al., Efficacy of intracoronary versus intravenous FGF-2 in a pig model of chronic myocardial ischemia, Ann Thorac Surg 70 (2000) 2113–2118, https://doi.org/10.1016/S0003-4975(99)01383-1.

[14] B. Schumacher, P. Pecher, B.U. von Specht, et al., Induction of neoangiogenesis in ischemic myocardium by human growth factors: first clinical results of a new treatment of coronary heart disease, Circulation 97 (1998) 645–650 http://www.ncbi.nlm.nih.gov/pubmed/9495299.

[15] J.D. Pearlman, M.G. Hibberd, M.L. Chuang, et al., Magnetic resonance mapping demonstrates benefits of VEGF-induced myocardial angiogenesis, Nat Med 1 (1995) 1085–1089 http://www.ncbi.nlm.nih.gov/pubmed/7489368.

[16] K. Hamano, M. Nishida, K. Hirata, et al., Local implantation of autologous bone marrow cells for therapeutic angiogenesis in patients with ischemic heart disease: clinical trial and preliminary results, Jpn Circ J 65 (2001) 845–847 http://www.ncbi.nlm.nih.gov/pubmed/11548889.

[17] E. Tateishi-Yuyama, H. Matsubara, T. Murohara, et al., Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial, Lancet (London, England) 360 (2002) 427–435, https://doi.org/10.1016/S0140-6736(02)09670-8.

[18] T.D. Henry, B.H. Annex, G.R. McKendall, et al., The VIVA trial: Vascular endothelial growth factor in Ischemia for Vascular Angiogenesis, Circulation 107 (2003) 1359–1365, https://doi.org/10.1161/01.CIR.0000061911.47710.8A.

[19] M. Rigato, M. Monami, G.P. Fadini, Autologous Cell Therapy for Peripheral Arterial Disease: Systematic Review and Meta-Analysis of Randomized, Nonrandomized, and Noncontrolled Studies, Circ Res 120 (2017) 1326–1340, https://doi.org/10.1161/CIRCRESAHA.116.309045.

[20] Y. Tsurumi, S. Takeshita, D. Chen, et al., Direct intramuscular gene transfer of naked DNA encoding vascular endothelial growth factor augments collateral development and tissue perfusion, Circulation 94 (1996) 3281–3290.

[21] I. Baumgartner, A. Pieczek, O. Manor, et al., Constitutive expression of phVEGF165 after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia, Circulation 97 (1998) 1114–1123.

[22] D.W. Losordo, P.R. Vale, J.M. Isner, Gene therapy for myocardial angiogenesis, Am Heart J 138 (1999) S132–41.

[23] M. Giacca, S. Zacchigna, VEGF gene therapy: Therapeutic angiogenesis in the clinic and beyond, Gene Ther 19 (2012) 622–629, https://doi.org/10.1038/gt.2012.17.

[24] J. Belch, W.R. Hiatt, I. Baumgartner, et al., Effect of fibroblast growth factor NV1FGF on amputation and death: A randomised placebo-controlled trial of gene therapy in critical limb ischaemia, Lancet 377 (2011) 1929–1937, https://doi.org/10.1016/S0140-6736(11)60394-2.

[25] N.J. Haywood, T.A. Slater, C.J. Matthews, et al., The insulin like growth factor and binding protein family: Novel therapeutic targets in obesity & diabetes, Mol Metab (2018) 1–11, https://doi.org/10.1016/j.molmet.2018.10.008.

[26] R. Hjortebjerg, A. Flyvbjerg, J. Frystyk, Insulin growth factor binding proteins as therapeutic targets in type 2 diabetes, Expert Opin Ther Targets 18 (2014) 209–224, https://doi.org/10.1517/14728222.2014.858698.

[27] L.A. Bach, Endothelial cells and the IGF system, J Mol Endocrinol 54 (2015), https://doi.org/10.1530/JME-14-0215 R1–13.

[28] Q. Yang, P. Wang, X. Du, et al., Direct repression of IGF2 is implicated in the anti-angiogenic function of microRNA-210 in human retinal endothelial cells, Angiogenesis 21 (2018) 313–323, https://doi.org/10.1007/s10456-018-9597-6.

[29] J.B. Allard, Duan C. IGF-Binding Proteins: Why Do They Exist and Why Are There So Many? Front Endocrinol (Lausanne) 9 (2018) 117, https://doi.org/10.3389/fendo.2018.00117.

[30] P. Delafontaine, Y.H. Song, Y. Li, Expression, regulation, and function of IGF-1, IGF-1R, and IGF-1 binding proteins in blood vessels, Arterioscler Thromb Vasc Biol 24 (2004) 435–444, https://doi.org/10.1161/01.ATV.0000105902.89459.09.

[31] H. Yu, T. Rohan, Role of the insulin-like growth factor family in cancer development and progression, J Natl Cancer Inst 92 (2000) 1472–1489 http://www.ncbi.nlm.nih.gov/pubmed/10995803.

[32] S.B. Wheatcroft, M.T. Kearney, IGF-dependent and IGF-independent actions of IGF-binding protein-1 and -2: implications for metabolic homeostasis, Trends Endocrinol Metab 20 (2009) 153–162, https://doi.org/10.1016/j.tem.2009.01.002.

[33] A.F. Messmer-Blust, M.J. Philbrick, S. Guo, et al., RTEF-1 attenuates blood glucose levels by regulating insulin-like growth factor binding protein-1 in the endothelium, Circ Res 111 (2012) 991–1001, https://doi.org/10.1161/CIRCRESAHA.112.268110.

[34] N.J. Haywood, P.A. Cordell, K.Y. Tang, et al., Insulin-Like Growth Factor Binding Protein 1 Could Improve Glucose Regulation and Insulin Sensitivity Through Its RGD Domain, Diabetes 66 (2017) 287–299, https://doi.org/10.2337/db16-0997.

[35] J. Jones, A. Gockerman, W. Busby, et al., Insulin-Like Growth Factor Binding Protein 1 Stimulates Cell Migration and Binds to the alpha5beta1 Integrin by Means of its Arg-Gly-Asp Sequence, EMBO J 8 (1993) 2497–2502, https://doi.org/10.1073/pnas.90.22.10553.

[36] D.R. Clemmons, Modifying IGF1 activity: an approach to treat endocrine disorders, atherosclerosis and cancer, Nat Rev Drug Discov 6 (2007) 821–833, https://doi.org/10.1038/nrd2359.

[37] S.B. Wheatcroft, M.T. Kearney, A.M. Shah, et al., Vascular endothelial function and blood pressure homeostasis in mice overexpressing IGF binding protein-1, Diabetes 52 (2003) 2075–2082 http://www.ncbi.nlm.nih.gov/pubmed/12882925.

[38] B. Dai, B. Ruan, J. Wu, et al., Insulin-like growth factor binding protein-1 inhibits cancer cell invasion and is associated with poor prognosis in hepatocellular carcinoma, Int J Clin Exp Pathol 7 (2014) 5645–5654, https://doi.org/10.1101/gad.228825.113.

[39] M.B. Nijaguna, V. Patil, S. Urbach, et al., Glioblastoma-derived macrophage colony-stimulating factor (MCSF) induces microglial release of insulin-like growth factor-binding protein 1 (IGFBP1) to promote angiogenesis, J Biol Chem 290 (2015) 23401–23415, https://doi.org/10.1074/jbc.M115.664037.

[40] Y.W. Chuang, W.M. Chang, K.H. Chen, et al., Lysophosphatidic acid enhanced the angiogenic capability of human chondrocytes by regulating Gi/NF-kB-dependent angiogenic factor expression, PLoS One 9 (2014) e95180, , https://doi.org/10.1371/journal.pone.0095180.

[41] A. Rajwani, V. Ezzat, J. Smith, et al., Increasing circulating IGFBP1 levels improves insulin sensitivity, promotes nitric oxide production, lowers blood pressure, and protects against atherosclerosis, Diabetes 61 (2012) 915–924, https://doi.org/10.2337/db11-0963.

[42] J.P. Cooke, NO and angiogenesis, Atheroscler 4 (Suppl) (2003) 53–60, https://doi.org/10.1016/S1567-5688(03)00034-5.

[43] J. Karar, A. Maity, PI3K/AKT/mTOR Pathway in Angiogenesis, Front Mol Neurosci 4 (2011) 1–8, https://doi.org/10.3389/fnmol.2011.00051.

[44] X. Peng, H. Ueda, H. Zhou, et al., Overexpression of focal adhesion kinase in vascular endothelial cells promotes angiogenesis in transgenic mice, Cardiovasc Res 64 (2004) 421–430, https://doi.org/10.1016/j.cardiores.2004.07.012.

[45] E. Shay, H. He, S. Sakurai, et al., Inhibition of angiogenesis by HC·HA, a complex of hyaluronan and the heavy chain of inter-α-Inhibitor, purified from human amniotic membrane, Investig Ophthalmol Vis Sci 52 (2011) 2669–2678, https://doi.org/10.1167/iovs.10-5888.

[46] V. Hwa, Y. Oh, R.G. Rosenfeld, The insulin-like growth factor-binding protein (IGFBP) superfamily, Endocr Rev 20 (1999) 761–787, https://doi.org/10.1210/edrv.20.6.0382.

[47] V.C. Russo, B.S. Schütt, E. Andaloro, et al., Insulin-like growth factor binding protein-2 binding to extracellular matrix plays a critical role in neuroblastoma cell proliferation, migration, and invasion, Endocrinology 146 (2005) 4445–4455, https://doi.org/10.1210/en.2005-0467.

[48] J.M. Ko, H.K. Park, S. Yang, et al., Influence of catch-up growth on IGFBP-2 levels and association between IGFBP-2 and cardiovascular risk factors in Korean children born SGA, Endocr J 59 (2012) 725–733, https://doi.org/10.1507/endocrj.EJ12-0080.

[49] S.B. Wheatcroft, M.T. Kearney, A.M. Shah, et al., IGF-binding protein-2 protects against the development of obesity and insulin resistance, Diabetes 56 (2007) 285–294, https://doi.org/10.2337/db06-0436.

[50] A.W. Wood, P.J. Schlueter, C. Duan, Targeted knockdown of insulin-like growth

[51] L. Fletcher, E. Isgor, S. Sprague, et al., Spatial distribution of insulin-like growth factor binding protein-2 following hypoxic-ischemic injury, BMC Neurosci 14 (2013) 158, https://doi.org/10.1186/1471-2202-14-158.

[52] W.J. Azar, S.H.X. Azar, S. Higgins, et al., IGFBP-2 enhances VEGF gene promoter activity and consequent promotion of angiogenesis by neuroblastoma cells, Endocrinology 152 (2011) 3332–3342, https://doi.org/10.1210/en.2011-1121.

[53] W.J. Azar, S. Zivkovic, G.A. Werther, et al., IGFBP-2 nuclear translocation is mediated by a functional NLS sequence and is essential for its pro-tumorigenic actions in cancer cells, Oncogene 33 (2014) 578–588, https://doi.org/10.1038/onc.2012.630.

[54] K.M. Holmes, M. Annala, C.Y.X. Chua, et al., Insulin-like growth factor-binding protein 2-driven glioma progression is prevented by blocking a clinically significant integrin, integrin-linked kinase, and NF-κB network, Proc Natl Acad Sci 109 (2012) 3475–3480, https://doi.org/10.1073/pnas.1120375109.

[55] S.K. Das, S.K. Bhutia, B. Azab, et al., MDA-9/syntenin and IGFBP-2 promote angiogenesis in human melanoma, Cancer Res 73 (2013) 844–854, https://doi.org/10.1158/0008-5472.CAN-12-1681.

[56] S.F. Tavazoie, C. Alarcon, T. Oskarsson, et al., Endogenous human microRNAs that suppress breast cancer metastasis, Nature 451 (2008) 147–152, https://doi.org/10.1038/nature06487.

[57] K. Png, N. Halberg, S. Yoshida, et al., A microRNA regulon that mediates endothelial recruitment and metastasis by cancer cells, Nature 481 (2012) 190–194.

[58] X. Shen, G. Xi, L.A. Maile, et al., Insulin-Like Growth Factor (IGF) Binding Protein 2 Functions Coordinately with Receptor Protein Tyrosine Phosphatase and the IGF-I Receptor To Regulate IGF-I-Stimulated Signaling, Mol Cell Biol 32 (2012) 4116–4130, https://doi.org/10.1128/MCB.01011-12.

[59] A. Grimberg, C. Coleman, Z. Shi, et al., Insulin-Like Growth Factor Factor Binding Protein-2 is a Novel Mediator of p53 Inhibition of Insulin-Like Growth Factor Signaling, Cancer Biol Ther 5 (2006) 1408–1414.

[60] R. Granata, L. Trovato, E. Lupia, et al., Insulin-like growth factor binding protein-3 induces angiogenesis through IGF-I- and SphK1-dependent mechanisms, J Thromb Haemost 5 (2007) 835–845, https://doi.org/10.1111/j.1538-7836.2007.02431.x.

[61] M.G. Slomiany, S.A. Rosenzweig, IGF-1-induced VEGF and IGFBP-3 secretion correlates with increased HIF-1 alpha expression and activity in retinal pigment epithelial cell line D407, Invest Ophthalmol Vis Sci 45 (2004) 2838–2847, https://doi.org/10.1167/iovs.03-0565.

[62] C. Lofqvist, J. Chen, K.M. Connor, et al., IGFBP3 suppresses retinopathy through suppression of oxygen-induced vessel loss and promotion of vascular regrowth, Proc Natl Acad Sci U S A 104 (2007) 10589–10594, https://doi.org/10.1073/pnas.0702031104.

[63] K.H. Chang, T. Chan-Ling, E.L. McFarland, et al., IGF binding protein-3 regulates hematopoietic stem cell and endothelial precursor cell function during vascular development, Proc Natl Acad Sci 104 (2007) 10595–10600, https://doi.org/10.1073/pnas.0702072104.

[64] Y. Fanton, C. Houbrechts, L. Willems, et al., Cardiac atrial appendage stem cells promote angiogenesis in vitro and in vivo, J Mol Cell Cardiol 97 (2016) 235–244, https://doi.org/10.1016/j.yjmcc.2016.06.005.

[65] J. Kim, D.S. Choi, O.H. Lee, et al., Antiangiogenic antitumor activities of IGFBP-3 are mediated by IGF-independent suppression of Erk1/2 activation and Egr-1-mediated transcriptional events, Blood 118 (2011) 2622–2631, https://doi.org/10.1182/blood-2010-08-299784.

[66] H.J. Lee, J.S. Lee, S.J. Hwang, et al., Insulin-like growth factor binding protein-3 inhibits cell adhesion via suppression of integrin β4 expression, Oncotarget 6 (2015) 15150–15163, https://doi.org/10.18632/oncotarget.3825.

[67] J. Han, H.L. Kim, K. Jeon, et al., Peroxisome proliferator-activated receptor-δ activates endothelial progenitor cells to induce angio-myogenesis through matrix metallo-proteinase-9-mediated insulin-like growth factor-1 paracrine networks, Eur Heart J 34 (2013) 1755–1765, https://doi.org/10.1093/eurheartj/ehr365.

[68] S.H. Oh, W.Y. Kim, O.H. Lee, et al., Insulin-like growth factor binding protein-3 suppresses vascular endothelial growth factor expression and tumor angiogenesis in head and neck squamous cell carcinoma, Cancer Sci 103 (2012) 1259–1266, https://doi.org/10.1111/j.1349-7006.2012.02301.x.

[69] K. Iwatsuki, K. Tanaka, T. Kaneko, et al., Runx1 promotes angiogenesis by down-regulation of insulin-like growth factor-binding protein-3, Oncogene 24 (2005) 1129–1137, https://doi.org/10.1038/sj.onc.1208287.

[70] S.H. Oh, W.Y. Kim, J.H. Kim, et al., Identification of insulin-like growth factor binding protein-3 as a farnesyl transferase inhibitor SCH66336-induced negative regulator of angiogenesis in head and neck squamous cell carcinoma, Clin Cancer Res 12 (2006) 653–661, https://doi.org/10.1158/1078-0432.CCR-05-1725.

[71] L.A. Wetterau, M.G. Moore, K.W. Lee, et al., Novel aspects of the insulin-like growth factor binding proteins, Mol Genet Metab 68 (1999) 161–181, https://doi.org/10.1006/mgme.1999.2920.

[72] R. Durai, S.Y. Yang, K.M. Sales, et al., Insulin-like growth factor binding protein-4 gene therapy increases apoptosis by altering Bcl-2 and Bax proteins and decreases angiogenesis in colorectal cancer, Int J Oncol 30 (2007) 883–888.

[73] L.W. Contois, D.P. Nugent, J.M. Caron, et al., Insulin-like Growth Factor Binding Protein-4 Differentially Inhibits Growth Factor-induced Angiogenesis, J Biol Chem 287 (2012) 1779–1789, https://doi.org/10.1074/jbc.M111.267732.

[74] A.J. Ryan, S. Napoletano, P.A. Fitzpatrick, et al., Expression of a protease-resistant insulin-like growth factor-binding protein-4 inhibits tumour growth in a murine model of breast cancer, Br J Cancer 101 (2009) 278–286, https://doi.org/10.1038/sj.bjc.6605141.

[75] Y.E. Smith, S. Toomey, S. Napoletano, et al., Recombinant PAPP-A resistant insulin-like growth factor binding protein 4 (dBP4) inhibits angiogenesis and metastasis in

a murine model of breast cancer, BMC Cancer 18 (2018) 1016, https://doi.org/10.1186/s12885-018-4950-0.

[76] M. Moreno, M. Ball, M. Andrade, et al., Insulin-like growth factor binding protein-4 (IGFBP-4) is a novel anti-angiogenic and anti-tumorigenic mediator secreted by dibutyryl cyclic AMP (dB-cAMP)-differentiated glioblastoma cells, Glia 53 (2006) 845–857, https://doi.org/10.1002/glia.20345.

[77] M.J. Moreno, M. Ball, M. Rukhlova, et al., IGFBP-4 Anti-Angiogenic and Anti-Tumorigenic Effects Are Associated with Anti-Cathepsin B Activity, Neoplasia 15 (2013) 554–567, https://doi.org/10.1593/neo.13212.

[78] S.M. Twigg, R.C. Baxter, Insulin-like growth factor (IGF)-binding protein 5 forms an alternative ternary complex with IGFs and the acid-labile subunit, J Biol Chem 273 (1998) 6074–6079 http://www.ncbi.nlm.nih.gov/pubmed/9497324.

[79] S.B. Rho, S.M. Dong, S. Kang, et al., Insulin-like growth factor-binding protein-5 (IGFBP-5) acts as a tumor suppressor by inhibiting angiogenesis, Carcinogenesis 29 (2008) 2106–2111, https://doi.org/10.1093/carcin/bgn206.

[80] J.R. Hwang, Y.J. Cho, Y. Lee, et al., The C-terminus of IGFBP-5 suppresses tumor growth by inhibiting angiogenesis, Sci Rep 6 (2016) 1–12, https://doi.org/10.1038/srep39334.

[81] J.F. Kuemmerle, H. Zhou, Insulin-like growth factor-binding protein-5 (IGFBP-5) stimulates growth and IGF-I secretion in human intestinal smooth muscle by Ras-dependent activation of p38 MAP kinase and Erk1/2 pathways, J Biol Chem 277 (2002) 20563–20571, https://doi.org/10.1074/jbc.M200885200.

[82] H. Miyake, C. Nelson, P.S. Rennie, et al., Overexpression of insulin-like growth factor binding protein-5 helps accelerate progression to androgen-independence in the human prostate LNCaP tumor model through activation of phosphatidylinositol 3′-kinase pathway, Endocrinology 141 (2000) 2257–2265, https://doi.org/10.1210/endo.141.6.7520.

[83] W. Zhu, Y. Wu, C. Cui, et al., Expression of IGFBP-6 in proliferative vitreoretino-pathy rat models and its effects on retinal pigment epithelial-J cells, Mol Med Rep 9 (2014) 33–38, https://doi.org/10.3892/mmr.2013.1794.

[84] L.A. Bach, Recent insights into the actions of IGFBP-6, J Cell Commun Signal 9 (2015) 189–200, https://doi.org/10.1007/s12079-015-0288-4.

[85] C. Zhang, L. Lu, Y. Li, et al., IGF binding protein-6 expression in vascular en-dothelial cells is induced by hypoxia and plays a negative role in tumor angio-genesis, Int J cancer 130 (2012) 2003–2012, https://doi.org/10.1002/ijc.26201.

[86] F. Qiu, W. Gao, B. Wang, Correlation of IGFBP-6 expression with apoptosis and migration of colorectal carcinoma cells, Cancer Biomark 21 (2018) 893–898, https://doi.org/10.3233/CBM-170947.

[87] P. Fu, J.A. Thompson, L.A. Bach, Promotion of cancer cell migration: An insulin-like growth factor (IGF)-independent action of IGF-binding protein-6, J Biol Chem 282 (2007) 22298–22306, https://doi.org/10.1074/jbc.M703066200.

[88] Z. Yang, L.A. Bach, Differential Effects of Insulin-Like Growth Factor Binding Protein-6 (IGFBP-6) on Migration of Two Ovarian Cancer Cell Lines, Front Endocrinol (Lausanne) 5 (2014) 231, https://doi.org/10.3389/fendo.2014.00231.

[89] S. Ahmed, K. Yamamoto, Y. Sato, et al., Proteolytic processing of IGFBP-related protein-1 (TAF/angiomodulin/mac25) modulates its biological activity, Biochem Biophys Res Commun 310 (2003) 612–618.

[90] Y. Oh, S.R. Nagalla, Y. Yamanaka, et al., Synthesis and characterization of insulin-like growth factor-binding protein (IGFBP)-7. Recombinant human mac25 protein specifically binds IGF-I and -II, J Biol Chem 271 (1996) 30322–30325, https://doi.org/10.1109/AEECT.2013.6716443.

[91] V. Evdokimova, C.E. Tognon, T. Benatar, et al., IGFBP7 Binds to the IGF-1 Receptor and Blocks Its Activation by Insulin-Like Growth Factors, Sciecne Signal 5 (2012) 1–12.

[92] D. van Breevoort, E.L. van Agtmaal, B.S. Dragt, et al., Proteomic screen identifies IGFBP7 as a novel component of endothelial cell-specific Weibel-Palade bodies, J Proteome Res 11 (2012) 2925–2936, https://doi.org/10.1021/pr300010r.

[93] A. Pen, M.J. Moreno, Y. Durocher, et al., Glioblastoma-secreted factors induce IGFBP7 and angiogenesis by modulating Smad-2-dependent TGF-beta signaling, Oncogene 27 (2008) 6834–6844, https://doi.org/10.1038/onc.2008.287.

[94] D. Chen, A. Siddiq, L. Emdad, et al., Insulin-like Growth Factor-binding Protein-7 (IGFBP7): A Promising Gene Therapeutic for Hepatocellular Carcinoma (HCC), Mol Ther 21 (2013) 758–766, https://doi.org/10.1038/mt.2012.282.

[95] D. Chen, B.K. Yoo, P.K. Santhekadur, et al., Insulin-like growth factor-binding protein-7 functions as a potential tumor suppressor in hepatocellular carcinoma, Clin Cancer Res 17 (2011) 6693–6701, https://doi.org/10.1158/1078-0432.CCR-10-2774.

[96] K. Tamura, K. Hashimoto, K. Suzuki, et al., Insulin-like growth factor binding protein-7 (IGFBP7) blocks vascular endothelial cell growth factor (VEGF)-induced angiogenesis in human vascular endothelial cells, Eur J Pharmacol 610 (2009) 61–67, https://doi.org/10.1016/j.ejphar.2009.01.045.

[97] K. Tamura, M. Yoshie, K. Hashimoto, et al., Inhibitory effect of insulin-like growth factor-binding protein-7 (IGFBP7) on in vitro angiogenesis of vascular endothelial cells in the rat corpus luteum, J Reprod Dev 60 (2014) 447–453, https://doi.org/10.1262/jrd.2014-069.

[98] R. Chen, H. Chen, P. Jian, et al., Intratumoral injection of pEGFC1-IGFBP7 inhibits malignant melanoma growth in C57BL / 6J mice by inducing apoptosis and down-regulating VEGF expression, Oncol Rep 23 (2010) 981–988, https://doi.org/10.3892/or.

[99] T. Sun, H. Cao, L. Xu, et al., Insulin-like growth factor binding protein-related protein 1 mediates VEGF-induced proliferation, migration and tube formation of retinal endothelial cells, Curr Eye Res 36 (2011) 341–349, https://doi.org/10.3109/02713683.2010.545498.

[100] M. Kutsukake, K. Tamura, M. Yoshie, et al., Knockdown of IGF-Binding Protein 7 Inhibits Transformation of the Endometrial Gland in an In Vitro Model, Mol Reprod Dev 272 (2010) 265–272, https://doi.org/10.1002/mrd.21143.

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