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REVIEW

Endothelial cells and the IGF system

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Abstract

Endothelial cells line blood vessels and modulate vascular tone, thrombosis, inflammatory responses and new vessel formation. They are implicated in many disease processes including atherosclerosis and cancer. IGFs play a significant role in the physiology of endothelial cells by promoting migration, tube formation and production of the vasodilator nitric oxide. These actions are mediated by the IGF1 and IGF2/mannose 6-phosphate receptors and are modulated by a family of high-affinity IGF binding proteins. IGFs also increase the number and function of endothelial progenitor cells, which may contribute to protection from atherosclerosis. IGFs promote angiogenesis, and dysregulation of the IGF system may contribute to this process in cancer and eye diseases including retinopathy of prematurity and diabetic retinopathy. In some situations, IGF deficiency appears to contribute to endothelial dysfunction, whereas IGF may be deleterious in others. These differences may be due to tissue-specific endothelial cell phenotypes or IGFs having distinct roles in different phases of vascular disease. Further studies are therefore required to delineate the therapeutic potential of IGF system modulation in pathogenic processes.

Keywords

insulin-like growth factor · binding protein · receptor · endothelial cell · angiogenesis

Introduction

Insulin-like growth factor 1 (IGF1) and IGF2 are essential for normal pre- and postnatal growth and development (Clemmons 2007, Pollak 2008). These peptide growth factors are synthesised in most tissues and circulate at nanomolar concentrations. They have endocrine, paracrine and autocrine actions. Most IGF actions are mediated by binding to the IGF1 receptor although the insulin receptor also has a role. The IGF2/mannose 6-phosphate receptor mediates some actions of IGF2 but predominantly acts as a clearance receptor. IGF actions are regulated by a family of six high-affinity IGF binding proteins (IGFBPs). IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP5 and IGFBP6 may inhibit or potentiate IGF actions and most also have IGF-independent actions.

Endothelial cells line blood vessels and act as barriers that control the transfer of

biomolecules and cells from the circulation to tissues (Sumpio *et al.* 2002). They are metabolically active and regulate vascular tone, thrombosis, inflammatory responses and new vessel formation. Endothelial cell dysfunction is implicated in a range of diseases including atherosclerosis, inflammatory disorders and cancer. They are involved in IGF physiology and are targets of IGF actions, which is the subject of this review.

The IGF system

IGF1 and IGF2 share significant sequence homology with each other and with proinsulin (Clemmons 2007). In rodents, *Igf2* expression decreases rapidly in most tissues after birth, whereas *Igf1* expression remains widespread, which has led to the view that IGF2 is a 'prenatal growth factor' and IGF1 is a 'postnatal growth factor'. However, *IGF2* expression persists postnatally in humans and circulating IGF2 levels are higher than those of IGF1, suggesting that it also has a postnatal role. IGF1 expression is regulated by growth hormone (GH), and IGF1 mediates many of the growth-promoting effects of the latter. By contrast, IGF2 expression is largely independent of GH. IGFs promote proliferation and survival of a wide range of cell types and increase differentiation of cells including myoblasts. IGFs also have insulin-like metabolic effects, decreasing plasma glucose after injection into humans and rodents and increasing glucose uptake into cells *in vitro*.

Most actions of IGF1 and IGF2 are mediated by the IGF1 receptor, a tyrosine kinase consisting of two heterodimers that has significant homology with the insulin receptor (Garrett *et al.* 1998). IGF binding to the IGF1 receptor results in activation of a number of signalling pathways including Ras/Raf/ERK and PI3 kinase/Akt. Some metabolic IGF actions are mediated by the insulin receptor, and some proliferative actions of IGF2 but not IGF1 are mediated by the insulin receptor isoform A (Frasca *et al.* 1999). Hybrid IGF/insulin receptors consisting of one heterodimer from each receptor have also been identified in many cells (Gatenby *et al.* 2013). These receptors preferentially bind IGF1 over insulin, but their physiological significance remains uncertain. The gene for the structurally distinct IGF2/mannose 6-phosphate receptor is imprinted and this receptor is largely involved with clearance of IGF2 but not IGF1; a limited number of studies have reported IGF2 actions that are mediated by this receptor (Brown *et al.* 2009).

IGF actions are finely regulated by a family of six high-affinity IGFBPs (Firth & Baxter 2002, Bach *et al.* 2005, Baxter 2014). These proteins have three domains, with a high degree of homology within their N- and C-terminal domains, which both contain IGF-binding determinants. The linker domain between the N- and C-terminal domains is not conserved between IGFBPs and contains sites of post-translational modification (glycosylation and phosphorylation) that confer a degree of specificity to the actions of individual IGFBPs. The linker domains also contain sites for proteolysis by a number of specific proteases including matrix metalloproteases. IGFBP cleavage results in release of IGFs for binding to IGF receptors and may also modulate IGFBP interactions with the extracellular matrix. Almost all circulating IGFs are bound to IGFBPs either in binary complexes or in larger ternary complexes with IGFBP3 or IGFBP5 and an acid-labile subunit, thereby limiting their bioavailability. Ternary complexes, which incorporate ~80% of circulating IGFs, cannot cross capillary walls, but free IGFs and those in binary complexes may leave the circulation and enter tissues (Fig. 1). IGFBPs localise to different tissue compartments after leaving the circulation (Bar *et al.* 1990). In most situations, IGFBPs inhibit IGF actions, but they may also potentiate them under some conditions. Most IGFBPs also bind proteins other than IGFs, predominantly through their C-terminal and linker domains, and have actions that are independent of IGFs. IGFBPs are expressed by most tissues in a cell-type-specific manner.

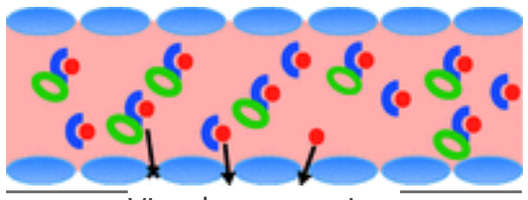

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Figure 1

Role of the endothelium in transport of circulating IGFs. Most (~80%) circulating IGFs (red) are bound in a high-molecular-weight ternary complex with IGFBP3 or IGFBP5 (blue) and an acid-labile subunit (green) that cannot leave the circulation. By contrast, IGFs in binary complexes with IGFBPs (~20%) and free IGFs (~1%) are able to traverse the endothelium into tissues.

The IGF system has been implicated in a range of diseases including atherosclerosis, diabetic complications and cancer. Endothelial cells are also implicated in these diseases and there is considerable evidence that components of the IGF system are expressed by and act upon these cells.

Endothelial cells

Endothelial cells form a monolayer that lines blood vessels (Sumpio *et al.* 2002). It has long been recognised that the endothelium functions as a barrier that regulates passage of biomolecules and cells such as leucocytes between the circulation and tissues. The endothelium is metabolically active and has an essential physiological role. Endothelial cells regulate vascular tone and blood flow via synthesis of mediators such as nitric oxide (NO), whereas thrombosis is regulated both by synthesis of key proteins and by interaction with platelets. Endothelial cells also regulate inflammatory responses by modulating interactions of inflammatory cells with blood vessel walls (Sumpio *et al.* 2002, Deanfield *et al.* 2007, Aird 2012). Endothelial cells play a key role in new vessel formation by angiogenesis and vasculogenesis. Endothelial cell responses to vascular endothelial growth factor (VEGF) via specific receptors such as VEGFR1 and VEGFR2 are an essential component of both of these processes. Many endothelial cell actions are mediated by synthesis of extracellular matrix proteins, anti-thrombotic and procoagulant factors, inflammatory mediators, proteases and vasomotor factors (Sumpio *et al.* 2002). In particular, endothelial cells synthesise and/or respond to a number of growth factors, including platelet-derived growth factor (PDGF), epidermal growth factor, transforming growth factor beta (TGFβ), and IGF1 and IGF2.

Endothelial cells show considerable heterogeneity depending on vessel type and size, and the tissue bed in which they reside (Aird 2012). For example, endothelial cells in large vessels are joined by tight junctions and form a continuous layer, whereas capillary endothelium has gaps of varying sizes in different tissues. There is also marked inter-individual variation in endothelial cell properties even if they derive from the same vessel. Endothelial cell heterogeneity is due to a combination of influences from the local microenvironment, which are changeable, and site-specific epigenetic effects, which are more stable. The phenotype of endothelial cells may therefore change as they are grown *in vitro*, hence caution is required when extrapolating from tissue culture studies to *in vivo* function.

Endothelial cells may be found in quiescent and activated states (Sumpio *et al.* 2002). Quiescent cells contribute to the maintenance of a non-thrombotic state by secreting vasodilators such as NO, which diffuses to adjacent vascular smooth muscle cells with subsequent relaxation, and prostacyclin. Cells are activated by a range of stimuli including injury and cytokines, resulting in generation of reactive oxygen species that results in a prothrombotic, proinflammatory phenotype (Deanfield *et al.* 2007).

Endothelial progenitor cells (EPCs), which are derived from bone marrow or

vessel walls, promote vessel repair and prevent endothelial dysfunction (Conti *et al.* 2011, Higashi *et al.* 2012). They may differentiate to form a new endothelium or act as paracrine sources of cytokines and growth factors to enhance endothelial repair. EPC levels decrease with ageing and low levels correlate with adverse cardiovascular outcomes.

Endothelial dysfunction has a role in a wide range of diseases including atherosclerosis, allograft vasculopathy, hypertension, congestive heart failure, primary pulmonary hypertension, sepsis and inflammatory syndromes (Sumpio *et al.* 2002, Deanfield *et al.* 2007). Endothelial dysfunction may be a consequence of damage due to prolonged activation, resulting in a loss of their ability to compensate (Deanfield *et al.* 2007, Otsuka *et al.* 2012). As mentioned earlier, endothelial cells also play a major role in angiogenesis, which is essential for tumour growth and metastasis.

IGF expression and regulation in endothelial cells

IGF1 and IGF2 expression levels in endothelial cells are low (Tucci *et al.* 1998, Delafontaine *et al.* 2004). In bovine aortic and pulmonary endothelial cells, hypoxia decreased IGF1 but not IGF2 levels further (Tucci *et al.* 1998). In bovine aortic endothelial cells, TGFB1 decreased *IGF1* mRNA whereas IGF1, insulin and angiotensin II had no effect (Dahlfors & Arnqvist 2000). The IGF1 receptor is expressed by endothelial cells from large and small vessels (Chisalita & Arnqvist 2004, Delafontaine *et al.* 2004), suggesting that these cells are responsive to IGFs, and IGF1 receptor levels are higher than those of insulin receptors (Chisalita & Arnqvist 2004). Endothelial cells also express hybrid IGF1/insulin receptors, and their levels appear to be regulated by the abundance of each of the holoreceptors (Gatenby *et al.* 2013). The IGF2/mannose 6-phosphate receptor is also expressed in endothelial cells (Volpert *et al.* 1996).

Endothelial cells from large vessels and microvessels express IGFBP2, IGFBP3, IGFBP4, IGFBP5 and IGFBP6, with IGFBP2 and IGFBP3 predominating in microvessel cells, and IGFBP3 and IGFBP4 predominating in large vessel cells (Moser *et al.* 1992). By contrast, another study showed that IGFBP4 was the predominant IGFBP in two microvessel endothelial cell lines, and levels were increased by cAMP (Yang *et al.* 1993). In bovine aortic endothelial cells, VEGF decreased IGFBP3 and increased IGFBP5 mRNA and protein levels, whereas TGFB1 decreased *IGFBP3* and *IGFBP4* mRNAs, but only IGFBP3 protein (Dahlfors & Arnqvist 2000). IGF1 increased IGFBP5 protein in these cells, but insulin and angiotensin II had no effect on *IGFBP* mRNA (Dahlfors & Arnqvist 2000). Hypoxia increased low levels of IGFBP6 but had no effect on IGFBP4 levels in bovine aortic and pulmonary endothelial cells (Tucci *et al.* 1998). IGFBP3 was increased by hypoxia in pulmonary but not aortic cells, whereas IGFBP5 was decreased by hypoxia in aortic cells.

IGF actions in endothelial cells

Endothelial cells are metabolically active, and IGF1 increased amino acid and glucose uptake in microvessel endothelial cells (Boes *et al.* 1991). MAP kinase (MAPK), protein kinase C and PI3 kinase pathways were involved in IGF1-mediated glucose uptake in retinal endothelial cells (DeBosch *et al.* 2002). IGF1 stimulated migration of a number of microvessel and large vessel endothelial cell lines (Grant *et al.* 1987, Nakao-Hayashi *et al.* 1992), but had no effect on proliferation of bovine carotid endothelial cells (Nakao-Hayashi *et al.* 1992). Endothelial cells form vessel-like tubes when grown on extracellular matrix and IGF1 enhanced this effect. IGF1 activated ERK and PI3 kinase pathways in bovine aortic endothelial cells, but only PI3 kinase activation was required for IGF1-induced tube formation (Liu *et al.* 2001).

There is evidence that IGF1 acts as a vasodilator *in vivo*. GH-deficient patients with low IGF1 levels had impaired flow-mediated arterial dilation, which was endothelium dependent and NO mediated (Higashi *et al.* 2010). In untreated hypertensive adults, low plasma IGF1 levels were associated with reduced endothelial function as measured by acetylcholine (ACh)-stimulated forearm blood flow; by contrast, there was no correlation with endothelium-independent, sodium nitroprusside-induced vasodilation (Perticone *et al.* 2008). Mice with decreased circulating IGF1 levels due to liver-specific knockout of the *Igf1* gene had higher blood pressure than control mice, and this was attributed to impaired endothelium-dependent, NO-mediated vasorelaxation and enhanced expression of the vasoconstrictor endothelin (Tivesten *et al.* 2002). In another study, ACh-induced vasodilation of aortic rings from mice with liver-specific knockdown of *Igf1* was not significantly different from that of control mice; however, the response in *Igf1*-deficient mice was significantly impaired by oxidative stress induced by high glucose or oxidised LDL. The authors attributed this to an impaired antioxidant response that is dependent on the transcription factor *Nrf2* (*Nfe2l2*) (Bailey-Downs *et al.* 2012). IGF1-mediated vasorelaxation was impaired in spontaneously hypertensive rats, and a single session of aerobic exercise reversed this in an endothelium-dependent manner via increased PI3 kinase and NO synthase activity (Yang *et al.* 2010). *In vitro*, IGF1 stimulated NO production via PI3 kinase activation and endothelial NO synthase in human umbilical vein endothelial cells (Zeng & Quon 1996).

IGF1 enhanced TNF-induced inflammatory responses in bovine aortic endothelial cells via Gab1, c-Jun and NFκB (Che *et al.* 2002). By contrast, IGF1 had no effect on TNF-induced inflammatory responses in human cardiac microvascular endothelial cells (Back *et al.* 2012). Another study showed that IGF1 inhibited proinflammatory human coronary artery endothelial cell activation by C-reactive protein through activating the PI3K/Akt pathway and suppressing the JNK and p38 pathways (Liu *et al.* 2014). The apparent contradiction between these results may reflect phenotypic differences between cells from different vascular beds.

Senescence is a cellular state of growth arrest and unresponsiveness to growth stimuli while remaining metabolically active. Endothelial cell senescence is associated with a proinflammatory phenotype and atherosclerosis. IGF1 receptor activation by IGF1 and IGF2 has been implicated in radiation-induced senescence of human pulmonary artery endothelial cells (Panganiban & Day 2013). By contrast, IGF1 inhibited oxidative stress-induced senescence of human aortic endothelial cells (Higashi *et al.* 2013). IGFBP3 and IGFBP5 were upregulated in endothelial cells made senescent by serial culture *in vitro* (Shelton *et al.* 1999, Grillari *et al.* 2000). IGFBP3 overexpression increased aspects of the senescent phenotype in human umbilical vein endothelial cells, whereas *IGFBP3* knockdown impaired this phenotype (Kim *et al.* 2007*a*). Some effects of IGFBP3 on senescence varied in cells from different donors (Muck *et al.* 2008). IGFBP5 induced senescence in human umbilical vein endothelial cells by a p53-dependent mechanism (Kim *et al.* 2007*b*). In these cells, IGFBP5 was identified as a potential candidate for senescence induced by radiation (Kim *et al.* 2014, Rombouts *et al.* 2014).

Similar to IGF1, IGF2 promoted migration and tube formation of human umbilical vascular endothelial cells, but had no effect on proliferation (Lee *et al.* 2000). Additionally, IGF2 increased p38 MAPK and p125 FAK phosphorylation. New vessel formation requires degradation of basement membranes by endothelial cells and this process involves proteases such as matrix metalloprotease 2 (MMP2); IGF2 promoted invasion of endothelial cells through extracellular matrix and increased MMP2 expression (Lee *et al.* 2000).

Depletion of the IGF1 receptor may contribute to endothelial cell apoptosis induced by advanced glycation end products (AGEs) (Pan *et al.* 2014), which increase with diabetes and ageing and are thought to be pathogenic.

Microvesicles containing miRNA 223 were taken up by human umbilical vein endothelial cells resulting in decreased levels of the IGF1 receptor, and AGE-induced apoptosis was potentiated by this microRNA or by siRNA to the IGF1 receptor.

The IGF2/mannose 6-phosphate receptor was reported to mediate IGF2-induced migration of bovine adrenal capillary endothelial cells (Volpert *et al.* 1996). Similar to other endothelial cells, IGF2 increased migration and tube formation but had no effect on proliferation of human uterine endothelial cells (Herr *et al.* 2003). The IGF2/mannose 6-phosphate receptor mediated IGF2-induced tube formation whereas the IGF1 and/or insulin receptor mediated tube formation induced by IGF1 or insulin. Protein kinase C and G_i proteins were implicated in the actions of IGF2. IGF2 also stimulated homing of EPCs, which may be important for vasculogenesis following hypoxia, via the IGF2/mannose 6-phosphate receptor, G_i proteins and phospholipase C (Maeng *et al.* 2009). The IGF2/mannose 6-phosphate receptor binds many ligands other than IGF2, and interaction of mini-plasminogen with this receptor mediated TGFB-induced apoptosis of endothelial cells (Leksa *et al.* 2005).

IGFs and atherosclerosis

There is substantial interest in the role of the IGF system in cardiovascular disease. Many studies suggested that IGF1 may be proatherogenic, because it promotes vascular smooth muscle cell proliferation and migration, and its expression is increased in atherosclerotic plaques (Clemmons 2007). In particular, it is postulated that locally synthesised IGF1 from activated macrophages may directly stimulate atherogenic processes including smooth muscle cell proliferation.

However, IGF1 is also potentially protective both by enhancing endothelial cell function and promoting plaque stability through increased vascular smooth muscle cell survival (Conti *et al.* 2004, Ezzat *et al.* 2008). Many but not all studies have suggested that low-normal serum IGF1 levels correlate with an increased risk of adverse cardiovascular outcomes, including myocardial infarction and heart failure (Ezzat *et al.* 2008, Higashi *et al.* 2012). One potential explanation is that low IGF1 levels result in insulin resistance and accelerated atherosclerosis (Clemmons 2007). Obesity is associated with insulin resistance and atherosclerosis. Obesity induced by high-fat feeding in mice resulted in impaired IGF1-mediated endothelial NO synthase activity and vasorelaxation (Imrie *et al.* 2009).

Another potential explanation is the effect of IGF1 on the number and function of EPCs, which decrease with age. These changes were reversed by increasing IGF1 levels with GH treatment in humans and mice (Thum *et al.* 2007*a*). IGF1 stimulated differentiation, migratory capacity and vascular network formation of EPCs from elderly individuals via the IGF1 receptor *in vitro*. IGF1 increased endothelial NO synthase expression, phosphorylation and activity via PI3 kinase/Akt in these cells. Additionally, GH-induced augmentation of IGF1 increased systemic NO bioavailability and the number of EPCs in healthy humans (Thum *et al.* 2007*b*). This is supported in part by a population study demonstrating that flow-mediated dilation, a measure of endothelial function, correlated with serum IGF1 levels in adult men but not women (Empen *et al.* 2010). IGF1 infusion increased the number of circulating EPCs and reduced inflammation and oxidative stress in the *Apoe*-null mouse model of atherosclerosis (Sukhanov *et al.* 2007). Additionally, IGF1 secreted by EPCs mediated myocardial repair after myocardial infarction in a porcine model (Hynes *et al.* 2013). *In vivo*, IGF1 infusion accelerated endothelial repair via an NO-dependent mechanism and increased EPC mobilisation after carotid artery injury in the rat (Cittadini *et al.* 2009).

A provocative hypothesis implicates hybrid IGF1/insulin receptor formation in the development of insulin resistance and atherosclerosis. Hybrid receptors predominantly bind IGF1 rather than insulin, and, according to this hypothesis, formation of hybrids results in depletion of insulin receptors, thereby reducing insulin sensitivity (Gatenby *et al.* 2013). Several studies support this contention. At physiological concentrations, insulin activated the insulin receptor but not hybrid receptors in bovine aortic endothelial cells, whereas higher concentrations activated both (Li *et al.* 2005). Furthermore, endothelial cells have more IGF1 receptors than insulin receptors. Haploinsufficient *Igf1r*$^{+/-}$ mice had decreased IGF1 receptors, enhanced insulin-mediated glucose disposal, restored insulin-mediated aortic vasorelaxation, and increased insulin-stimulated endothelial cell NO release (Abbas *et al.* 2011). Haploinsufficient *Igf1r*$^{+/-}$ mice also had fewer angiogenic progenitor cells than WT mice, but these cells had increased adhesion to fibronectin, increased IGF1 secretion and enhanced tube formation (Yuldasheva *et al.* 2014). Furthermore, infusion of these cells into WT mice increased endothelial repair without altering atherosclerotic lesion formation. Overexpression of IGF1 receptors on endothelial cells increased hybrid formation and reduced NO bioavailability (Imrie *et al.* 2012). However, perhaps surprisingly, endothelial regeneration was enhanced by IGF1 receptor overexpression after arterial denudation. The interplay between IGF1 and insulin, and the relative roles of their receptors in endothelial cells, clearly requires further study.

IGFBPs may also play a role in atherosclerosis via modulation of insulin sensitivity. IGFBP1 is acutely regulated by insulin, whereas IGFBP2 is also regulated by this hormone (Wheatcroft & Kearney 2009). There is evidence that both of these IGFBPs may improve insulin sensitivity, especially in the context of overfeeding and obesity (Wheatcroft & Kearney 2009, Hedbacker *et al.* 2010). Although most studies have not shown IGFBP1 expression in endothelial cells and direct regulation of IGFBP2 by insulin has also not been demonstrated in these cells, they would be exposed to circulating IGFBPs that could act by modulating IGF actions or through IGF-independent mechanisms.

IGFs and new vessel formation

Vasculogenesis refers to new blood vessel formation from mesodermal EPCs during embryogenesis. By contrast, angiogenesis refers to the sprouting of new vessels from pre-existing vessels (Potente *et al.* 2011). Angiogenesis proceeds through a number of stages commencing with mobilisation of endothelial cells by proangiogenic signals. Endothelial cells then proliferate to extend the new vessel and a lumen is formed. Anastomoses between vessels result in loop formation, and vessels remodel and mature once blood flow commences.

Hypoxia is a major angiogenic stimulus, and hypoxia-inducible factors (HIFs) regulate transcription of key angiogenic genes, including *VEGF* (Semenza 2012). HIF1 consists of HIF1A and ARNT subunits, and HIF1A, which is increased by hypoxia, is the main determinant of transcriptional activity. Both HIF1 and HIF2 play important roles in blood vessel development in vertebrates.

Angiogenesis is involved in a number of disease processes. Inadequate vessel formation contributes to ischaemic damage in diseases such as myocardial infarction and stroke. Therapeutic enhancers of angiogenesis are under development and have not reached clinical use. By contrast, increased angiogenesis is implicated in diseases including cancer and some forms of retinal damage. Indeed, it is integral to cancer progression at all stages, as it is required for oxygen and nutrient delivery to enlarging solid tumours beyond 1–2 mm^3 (Potente *et al.* 2011), and it enhances metastasis by facilitating cancer cell entry into abnormal, tumour-induced vessels (Valastyan & Weinberg 2011). Angiogenesis inhibitors are in clinical use for the treatment of a number of advanced cancers and eye diseases.

Insulin-like growth factors

Although VEGF pathways are central mediators of angiogenesis, IGFs also play a role (Carmeliet & Jain 2000). As discussed in detail above, IGFs promote endothelial cell migration and tube formation *in vitro*. IGF1 and IGF2 stimulate HIF1A expression (Hoeben *et al.* 2004), and IGF1 induces VEGF synthesis (Warren *et al.* 1996, Stearns *et al.* 2005) via HIF1-dependent and -independent pathways (Slomiany & Rosenzweig 2006). IGF2 also plays a role in angiogenesis (Wang *et al.* 1998, Kim *et al.* 2012), at least in part by inducing VEGF synthesis (Hoeben *et al.* 2004). Although most IGF actions, including proangiogenic actions, are mediated by the IGF1 receptor, IGF2 may also promote angiogenesis via the insulin receptor (Bid *et al.* 2012). There has been considerable interest in IGF inhibition as treatment for a range of cancers, and some of the *in vivo* efficacy of these inhibitors is mediated by impaired angiogenesis (Dransfield *et al.* 2010).

IGFs are involved in early vascular development. Human embryonic and fetal lung explants expressed IGF1, IGF2, and IGF1 receptor as early as 4 weeks, and inhibiting the IGF1 receptor decreased the number of endothelial cells, due at least in part to apoptosis (Han *et al.* 2003). IGF2 increased vessel formation in a chick chorioallantoic membrane assay, which, together with its effects on human uterine endothelial cells, may be relevant to the vascular adaptation to pregnancy (Herr *et al.* 2003).

Angiogenesis is impaired in the diabetic heart, and a recent study has demonstrated that cardiomyocytes from rats with diabetes secreted exosomes containing miR320, which were subsequently taken up by endothelial cells (Wang *et al.* 2014). This microRNA decreased expression of a number of protein targets including IGF1, and inhibited endothelial cell migration and tube formation. However, the specific role of IGF1 as opposed to other protein targets was not studied.

Insulin-like growth factor-binding proteins

The roles of IGFBP2, IGFBP3, IGFBP4, IGFBP5 and IGFBP6 in angiogenesis have been studied. In general, IGFBP4, IGFBP5 and IGFBP6 inhibit angiogenesis and IGFBP2 enhances it, whereas there is evidence for both enhancement and inhibition by IGFBP3.

IGFBP2

Knockdown of *IGFBP2* resulted in impaired vessel sprouting in zebrafish embryos (Wood *et al.* 2005). IGFBP2 increased angiogenesis via transcriptional regulation of VEGF (Azar *et al.* 2011). miR126 suppressed metastatic endothelial cell recruitment and angiogenesis by targeting a number of genes including *IGFBP2* (Png *et al.* 2012). In this study, IGFBP2 increased endothelial cell chemotaxis and migration *in vitro* through an IGF1-dependent pathway.

IGFBP3

IGFBP3 inhibited vessel formation in prostate cancer xenografts, and a mutant of IGFBP3 that does not bind IGFs inhibited vessel formation in zebrafish embryos, indicating an IGF-independent effect (Liu *et al.* 2007). IGF1- and VEGF-induced proliferation and survival of human umbilical vein endothelial cells were both inhibited by IGFBP3 (Franklin *et al.* 2003). IGFBP3 alone increased apoptosis, and IGFBP3 antagonised VEGF actions by an IGF-independent mechanism involving inhibition of PI3 kinase/Akt. IGFBP3 was also implicated in the antiangiogenic actions of a farnesyl transferase inhibitor that has anti-tumorigenic effects on head and neck squamous cell carcinomas (Oh *et al.* 2006). Furthermore, RUNX1, a transcription factor, promoted angiogenesis by suppressing *Igfbp3* expression (Iwatsuki *et al.* 2005).

In contrast to the above, comparison of doxorubicin and serum starvation-induced apoptosis of human umbilical vein endothelial cells suggests a more

complex role for IGFBP3 (Granata *et al.* 2004). IGFBP3 enhanced the former but inhibited the latter, and these effects were mediated in an IGF1-dependent manner via sphingosine kinase and altered ceramide levels. IGFBP3 increased IGF1 secretion and phosphorylation of the IGF1 receptor, AKT and ERK according to this study. A subsequent study showed that IGFBP3 increased tube formation and angiogenesis-related gene expression including VEGF, MT1-MMP and MMP2, as well as increasing MMP2, and MMP9 activation (Granata *et al.* 2007). Incubation in high glucose increased death of primary human retinal microvascular endothelial cells and IGFBP3 inhibited this effect via the LRP1/TGFB receptor (Zhang *et al.* 2013*a*), which has been reported to mediate IGF-independent actions of this IGFBP (Huang *et al.* 2003). The anti-apoptotic effect of IGFBP3 was associated with increased AKT phosphorylation and Bcl-xL levels while cytochrome *c*, BAX and cleaved caspase 3 were decreased in these cells. Clearly, further studies are required to delineate the role of IGFBP3 in endothelial survival, function and angiogenesis in the face of these apparently disparate findings.

IGFBP4

Media conditioned by glioblastoma multiforme cells increased tube formation by human brain endothelial cells, and this effect was inhibited by IGFBP4. IGFBP4 also inhibited tube formation induced by IGF1, VEGF, FGF2 and PIGF, suggesting that its effects are IGF-independent (Moreno *et al.* 2006). IGFBP4 expression decreased upon exposure of human umbilical vein endothelial cells to denatured type IV collagen, which is found in angiogenic vessels (Contois *et al.* 2011). IGFBP4 inhibited angiogenesis induced by IGF1 and FGF2 but not VEGF in a chick chorioallantoic membrane assay. Adipose tissue expansion in response to high-fat feeding requires an expanded vasculostromal network. The angiogenic response was increased by insulin, which decreased IGFBP4 as well as altering expression of other genes (Gealekman *et al.* 2014). Insulin increased IGF1 levels and both peptides stimulated sprout formation; IGFBP4 inhibited these effects.

IGFBP5

In human umbilical vein endothelial cells, IGFBP5 inhibited VEGF-induced proliferation, invasion, tube formation, and AKT and endothelial NO synthase phosphorylation (Rho *et al.* 2008). In the same study, IGFBP5 also inhibited vessel formation in a chick chorioallantoic membrane assay and angiogenesis in ovarian cancer xenografts.

IGFBP6

Overexpression of IGFBP6 inhibited angiogenesis in rhabdomyosarcoma xenografts and zebrafish embryos, as well as inhibiting tube formation in human umbilical vein endothelial cells through an IGF-independent mechanism (Zhang *et al.* 2012). Prolonged hypoxia increased IGFBP6 expression in human umbilical vein endothelial cells through a HIF1A-dependent mechanism. These findings suggest that IGFBP6 may be part of a negative feedback mechanism limiting hypoxia-induced angiogenesis (Messmer-Blust *et al.* 2009). Consistent with this, IGFBP6 was identified as a substrate for MMP2 in a proteomic screen of human umbilical vein endothelial cells for candidate angiogenic inhibitors that may be neutralised by this protease (Dean *et al.* 2007).

Retinopathy

Endothelial dysfunction and neovascularisation are implicated in a number of retinal diseases, including retinopathy of prematurity (ROP) and diabetic retinopathy.

Retinopathy of prematurity

ROP is a potentially sight-threatening disorder that arises in children born prematurely (<31 weeks, birth weight <1250g) before the retinal vasculature has fully developed. It is thought to arise from inadequately vascularised, hypoxic retinal tissue generating signals that promote neovascularisation. These new vessels are abnormal and prone to bleeding and cause retinal detachment. ROP was more prevalent in the past when high concentrations of oxygen were used to treat premature babies in intensive care units.

In oxygen-induced retinopathy, a mouse model of ROP, IGF1 was required for maximal VEGF-dependent neovascularisation via the IGF1 receptor and MAPK activation (Smith *et al.* 1999). VEGF and IGF1 additively increased AKT phosphorylation in retinal endothelial cells, which promoted cell survival (Hellstrom *et al.* 2001). *Igf1* knockout mice had impaired retinal vascular growth despite normal VEGF levels. ROP is associated with impaired vascular growth, and serum IGF1 levels were significantly lower in infants with ROP than in those without ROP. The authors postulated that the phase of neovascularisation occurs when IGF1 levels increased to reach a threshold after prolonged low levels. Knockout of IGF1 receptors in endothelial cells also decreased neovascularisation in oxygen-induced retinopathy (Kondo *et al.* 2003).

IGFBP3 has also been studied in oxygen-induced retinopathy (Chang *et al.* 2007, Lofqvist *et al.* 2007). IGFBP3 expression was regulated by hypoxia and it promoted differentiation of EPCs to endothelial cells. IGFBP3 also increased EPC migration, VEGFR1 and VEGFR 2 expression, and tube formation *in vitro* (Chang *et al.* 2007), as well as NO synthesis, which is required for homing into ischaemic tissue (Kielczewski *et al.* 2009). *Igfbp3*-deficient mice have more vessel loss after high oxygen exposure and less vessel regrowth after restoration of normoxia in a model of oxygen-induced retinopathy (Lofqvist *et al.* 2007). Exogenous IGFBP3 increased vessel regrowth in wild-type mice, possibly by promoting progenitor cell chemoattraction. Premature babies with ROP had lower IGFBP3 levels than those without ROP. The authors postulated that IGFBP3 might prevent retinal neovascularisation by inhibiting oxygen-induced vascular loss and promoting regrowth after vascular destruction. In support of this, plasmid-mediated IGFBP3 overexpression protected the retina from hyperoxia-induced vascular regression and subsequent neovascularisation (Chang *et al.* 2007).

Diabetic retinopathy

Diabetes mellitus is associated with a range of microvascular complications including retinopathy. Background retinopathy, which includes microaneurysm formation and vessel leakage resulting in protein exudates, small haemorrhages and infarcts, occurs in the vast majority of patients with an increasing duration of diabetes. A minority of patients then progress to sight-threatening proliferative retinopathy with neovascularisation, vitreous haemorrhage and retinal detachment. A significant proportion of patients also develop macular oedema. Diabetic retinopathy is the commonest cause of blindness in adults of working age in the western world.

The GH/IGF1 axis is implicated in diabetic retinopathy (Wilkinson-Berka *et al.* 2006). Early studies suggested that GH-deficient patients with diabetes were protected from retinopathy, and pituitary ablation, which decreases GH levels, was a treatment for severe retinopathy before the advent of laser photocoagulation. Overexpression of IGF1 in the retina resulted in changes similar to those of diabetic retinopathy (Ruberte *et al.* 2004). Subsequently, intraocular overexpression of IGF1 has been shown to result in breakdown of the blood–retinal barrier and increased retinal vascular permeability (Haurigot *et al.* 2009).

Expression of components of the IGF system was compared in human retinal endothelial cells from subjects with and without diabetes (Spoerri *et al.* 1998).

Immunoreactive IGF1 receptor and IGFBP1, IGFBP3 and IGFBP5 were increased with diabetes, whereas IGF1 and IGFBP4 were decreased. *IGFBP1*, *IGFBP2* and *IGFBP5* mRNA levels were increased with diabetes, whereas *IGF1* mRNA decreased and mRNA levels of *IGFBP3, IGFBP4* and *IGF1R* were unchanged. These findings suggest that diabetes alters IGF system components at both transcriptional and post-transcriptional levels.

Another study demonstrated that human retinal endothelial cells secrete IGFBP2, IGFBP3, IGFBP4 and IGFBP5 (Giannini *et al.* 2001). GH, VEGF, FGF2 and PDGF had no effect on IGFBP levels, whereas IGF1 decreased IGFBP4 and increased IGFBP5 levels. IGF1 increased thymidine incorporation in these cells, while IGFBP5 alone also had a minor effect. High glucose (22 mM) incubation decreased basal thymidine incorporation after 14–21 days and also decreased IGFBP2, IGFBP3 and IGFBP5 protein but not mRNA levels. These results differ from the above comparison of retinal endothelial cells from patients with and without diabetes (Spoerri *et al.* 1998), reflecting the need for caution in interpreting *in vitro* results.

IGFBP3 reduced monocyte–endothelial cell adhesion in an IGF-independent manner by decreasing ICAM1 levels in human retinal endothelial cells cultured in a high glucose medium (Zhang *et al.* 2013*b*). It was postulated that this anti-inflammatory effect may be protective in the setting of diabetic nephropathy, which is supported by a study showing that TNFA, which is proinflammatory, reduced IGFBP3 expression in retinal endothelial cells via activation of p38α MAPK and casein kinase 2 under high glucose conditions (Zhang *et al.* 2014). Furthermore, IGFBP3 inhibited whereas TNFA increased retinal endothelial cell apoptosis (Zhang *et al.* 2013*c*).

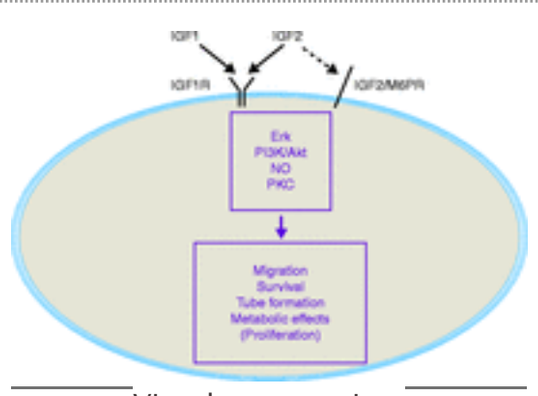
There appears to be a paradox whereby IGF1 deficiency is thought to be deleterious in ROP, whereas IGF1 excess is implicated in diabetic retinopathy. However, this may be an oversimplistic interpretation, as these disease processes are dynamic and the role of the IGF system may differ depending on the stage of the disease. Thus, increasing IGF1 may promote normal retinal vascular growth and prevent tissue hypoxia in the early phases of ROP, whereas IGF inhibition may limit neovascularisation later in the course of ROP and in diabetic retinopathy.

Conclusions

Endothelial cells play an important role in maintaining homoeostasis, and they are constantly exposed to circulating IGFs. They express IGF and insulin receptors and are responsive to IGFs at concentrations sufficient to activate these receptors (Fig. 2). This raises the question as to how IGF responses are limited. One mechanism is binding of the vast majority of circulating IGFs to IGFBPs in binary and ternary complexes, within which they are not accessible to receptors. Another question is how circulating IGFs traverse the endothelium to access tissues. Capillary endothelium contains gaps between cells, and there is evidence that IGFs utilise this paracellular pathway (Bastian *et al.* 1997). Insulin traverses the endothelium by a transcytotic pathway that involves insulin and IGF1 receptors (Wang *et al.* 2006), so it remains possible that IGFs also utilise this pathway. Binary complexes containing IGFs and IGFBPs can traverse the endothelium and the latter have a role in regulating this process (Boes *et al.* 2003).

Figure 2

Actions of IGFs on endothelial cells. Most actions of IGF1 and IGF2 are mediated by the IGF1 receptor (IGF1R), although there is evidence that some IGF2 actions are mediated by IGF2/mannose 6-phosphate receptor (IGF2/M6PR). A number of signalling pathways, including Erk, PI3K/Akt, NO



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and PKC are activated by IGFs resulting in endothelial cell migration, survival, nutrient uptake and tube formation. In some studies, IGFs also stimulate proliferation.

The evidence suggests that the effects of IGFs on endothelial cells can be both protective and pathogenic in a range of diseases. For example, ROP is biphasic, and IGF deficiency appears to have a role in the first ischaemic phase, whereas IGFs may contribute to the subsequent phase of neovascularisation. At a more general level, angiogenesis, to which IGFs contribute, is important for tissue repair in situations such as myocardial ischaemia but deleterious in malignancy. The interplay between insulin and IGFs and their receptors, including hybrid receptors, also appears to contribute to insulin sensitivity and endothelial responses that may contribute to atherosclerosis. There are means of enhancing IGF action such as recombinant GH or IGF1 itself, and means of inhibition such as IGF1 receptor antibodies and small molecule inhibitors. It is important to develop approaches to target these potential treatments to specific sites, but it is equally important for further studies to delineate potential positive and negative effects and define possible switches between them.

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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