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Review

Insulin-like growth factor I and its binding proteins in the cardiovascular system

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1. Introduction

Insulin-like growth factor I (IGF I) is a ubiquitous peptide that has a fundamental role in both prenatal and postnatal development (reviewed in [1,2]). It is the major mediator of growth hormone's effects on postnatal growth. IGF II and insulin are structurally-related hormones with 40%–50% amino acid sequence similarity. This review will focus primarily on IGF I and its actions and expression in the cardiovascular system.

IGF I is the product of the IGF I gene which has been mapped to chromosome 12 in humans [3] and to chromosome 10 in mice [4]. The mammalian gene consists of at least six exons [2,5–7]. Transcription of the mammalian gene results from at least two transcription start sites located on exon 1 and exon 2. Exons 1 and 2 encode mutually exclusive 5′ untranslated regions and there are several in-frame translation initiation codons yielding signal peptides differing at their N-terminus. The mature peptide coding sequence is present in exons 3 and 4. Additional complexity results from the presence of distinct carboxyterminal E domains of the IGF I preprohormone (Ea and Eb variants). Exon 1- and Ea-containing transcripts are expressed ubiquitously whereas exon 2 and Eb transcripts are expressed more specifically in the liver. Northern blot hybridization of mammalian tissues reveals multiple IGF I transcripts varying from around 0.9 kb to 7.5 kb in length. IGF I expression is regulated both at the level of transcription, mRNA stability and post-translationally. For a more detailed discussion of the organization of the IGF I gene the reader is referred to recent reviews [2,7].

IGF I exerts all of its known physiological effects upon binding to the type 1 IGF receptor (reviewed in [8]). The related peptide IGF II binds to both the IGF I and IGF II receptors. The IGF II receptor is identical to the cation-independent mannose-6-phosphate (M-6-P) receptor [9]. It is felt, however, that the physiological effects of IGF II are mediated on binding to the IGF I receptor and that the M-6-P receptor functions essentially as a scavenging receptor mediating the degradation of IGF II. A possible exception, however, may be during early fetal development, i.e., in the pre-implantation embryo [10].

The human IGF I receptor (IGF IR) is the product of a single-copy gene located on chromosome 15 [11]. The IGF IR resembles the insulin receptor in primary and tertiary structure [12]. The mature receptor is a tetramer consisting of two extracellular α-chains and two intracellular β-chains. The putative IGF I binding-site is within the cysteine-rich domain in the extracellular α-subunit. The β-chain includes an intracellular tyrosine-kinase domain that is thought to be essential for most of the receptor's biological effects. For a detailed discussion of the molecular organization of the IGF IR gene the reader is referred to a recent review [8].

The IGF-binding proteins are proteins that are present in the circulation and in extracellular fluids and bind with high affinity to both IGF I and IGF II. Six IGF-binding proteins have been isolated (reviewed in [1]). The IGF-binding activity in rat and human serum consists predominantly of a 150 kDa complex consisting of IGFBP-3, an acid-labile subunit (ALS) and IGF I or IGF II. A smaller complex in serum (40–50 kDa) contains IGF I or II bound to IGFBP-1, IGFBP-2, or IGFBP-4. This smaller complex may also contain some IGFBP-3. IGFBP-5 and IGFBP-6 in rat and human serum are present in extremely low concentrations. It is felt that IGFBP-3 in serum is essentially saturated whereas IGFBP-1 and -2 are unsaturated [13]. The large 150 kDa ternary complex likely acts to increase the half-life of IGF I in the circulation, providing a stable serum source of bioactive peptide. This complex does not cross the endothelium [14], whereas data in the rat have demonstrated that smaller binding proteins, namely BP-1, BP-2 and BP-4, may traverse the endothelium [15,16]. It has thus been hypothesized that lower molecular weight binding proteins may increase translocation of IGF

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I into the vasculature and tissues and thus modulate IGF I action. It is of note that while the IGF-binding proteins consistently have extremely high affinity for their ligands, the N-terminally truncated des(1–3) IGF I [17] and a variety of IGF I analogs [18] have markedly reduced affinity for binding proteins but retain normal affinity for IGF receptors.

2. Expression of IGF I, the IGF I receptor and IGF-binding proteins in the cardiovascular system

2.1. In vivo studies

In situ hybridization analysis has demonstrated expression of IGF I in multiple tissues in the human fetus, as reported by Han et al. [19]. In this study, IGF I expression in the heart was localized predominantly to the epicardium and in coronary vessel walls. The presence of IGF I transcripts in conduit or resistance arteries was not reported in this study. Studies in neonatal rat have demonstrated that ventricular tissue contains both IGF I and IGF II receptors [20]. Additionally, in situ data indicate that neonatal rat cardiomyocytes express predominantly IGF II and low amounts of IGF I transcripts. In the adult rat IGF I mRNA is expressed at low levels in the left ventricle [21–23]. In situ hybridization data are lacking but immunostaining shows low level myocyte staining. IGF IR is likewise expressed at low levels in normal rat heart [22]. In conduit (elastic) arteries IGF I mRNA is expressed at low levels in the adventitia and in the media [24–26]. IGF I expression is significantly higher in resistance (muscular) arteries (P. Delafontaine, unpublished results). The IGF IR is expressed mainly in the media in normal rat aorta [27,28]. Limited data exist on expression of IGF-binding proteins in the cardiovascular system. However, in situ hybridization analysis has demonstrated that the hepatic portal venous and sinusoidal endothelium expresses abundant IGFBP-3 mRNA [29]. It is thus hypothesized that a majority of circulating BP-3 may originate from the endothelium. Recently IGFBP-3 and IGFBP-4 expression have been demonstrated in rat aorta [30].

2.2. In vitro studies

IGF I is synthesized by rat [31,32] and porcine [33–35] vascular smooth muscle cells (VSMC) in vitro. There are primarily three transcripts, sized 0.9–1.2 kb, 1.7 kb and 7.5 kb. Solution hybridization/RNase protection analysis has demonstrated that rat aortic tissue possesses only the class C 5′ untranslated IGF I mRNA transcripts [36]. Radioligand binding studies have demonstrated that VSMC in vitro express significant levels of the high-affinity type 1 IGF receptor [37–41]. Binding-affinity for IGF I is between 0.1 and 5 nM (K_d). Binding studies and cross-linking studies have indicated that VSMC in vitro possess no detectable cell surface IGF-binding proteins [42]. However, both porcine and rat aortic smooth muscle cells secrete several binding proteins, specifically IGFBP-2, IGFBP-3 and IGFBP-4 [42–46]. In rat aortic smooth muscle cells, two glycosylated forms of IGFBP-4 have been demonstrated [42]. Macrovessel and microvessel endothelial cells also express high-affinity IGF IR [47–49]. There is also low level expression of IGF I in endothelial cells [31,50], although measurements of IGF I in conditioned medium from endothelial cells may reflect to a significant extent IGF I sequestered from serum [51]. Endothelial cells in culture secrete IGF-binding proteins [52,53]. Recently porcine endothelial cells have been shown to express mRNA for IGFBP-2, 3, 4, 5 and 6 [54]. Microvessel endothelial cells secrete predominantly IGFBP-2 and IGFBP-3, while large vessel endothelial cells secrete essentially IGFBP-3 and IGFBP-4 [54]. Recently IGFBP-3 and IGFBP-5 have been shown to associate with endothelial cell surfaces through C-terminal heparin-binding domains [55]. This allows competition for binding by heparin and heparan sulfate.

3. Regulation of IGF I, the IGF IR and IGF-binding proteins in cardiac and vascular cells in vitro

3.1. IGF I

As noted previously, IGF I is secreted by VSMC in vitro [31–35]. Stiles et al. have shown that in BALB/c3T3 fibroblasts IGF I functions as a "progression" factor to stimulate passage of cells through G_1 into S phase, in contradistinction to platelet-derived growth factor (PDGF) that induces "competency," i.e., entry of cells from G_0 into the G_1 phase of the cell cycle [56]. Clemmons et al. have demonstrated that anti-IGF I antiserum inhibited PDGF-induced growth of VSMC [33]. Additionally, anti-IGF I antiserum inhibited growth hormone-induced fibroblast DNA synthesis [57]. Increased IGF I immunoreactivity in conditioned medium from PDGF-treated cells has suggested that PDGF induces synthesis of IGF I [35]. Subsequent expression studies have reported conflicting results, potentially related to differences in cell types and conditions of quiescence. Thus PDGF and serum have been reported to both increase [32] or decrease [46,58] IGF I mRNA levels in VSMC. IGF I acts additively with PDGF and insulin in the induction of the c-myc protooncogene and cellular proliferation in bovine aortic VSMC [59]. Yamamoto et al. have shown that in primary rabbit VSMC, IGF I is required for cells to enter the S phase [60]. IGF I stimulates elastin [61] and fibronectin [62] gene expression in rat aortic VSMC. Recently it has been reported that angiotensin II transcriptionally regulates the IGF I gene in rat aortic VSMC and that angiotensin II-induced growth of smooth muscle cells is completely inhibited by anti-IGF I antiserum [63]. These findings support a central role for IGF I in mediating VSMC growth. Unlike angiotensin II, thrombin downregulates IGF I mRNA and protein levels in aortic smooth muscle cells [64], as has been described in fibroblasts [65,66]. It is of note that IGF I stimulates growth of myometrial smooth muscle cells [67] and of airway [68] and pulmonary artery smooth muscle cells [69].

IGF I stimulates neutral amino acid and glucose uptake and DNA synthesis in microvessel but not macrovessel bovine endothelial cells [47]. However, IGF I stimulates DNA synthesis in human corneal endothelial cells [70]. IGF I is a potent stimulator of myogenic differentiation

[71–73], inducing expression of myogenin in L6 myoblasts [72]. Furthermore, IGF I stimulates hyperplasia and hypertrophy of skeletal myofibers [74]. A variety of recent reports have documented an important role for IGF I and the IGF IR in cardiac myocyte growth. Thus ventricular myocytes from rat ventricular tissue post myocardial infarction express higher levels of IGF I and IGF IR [75]. Ito et al. [76] have reported that IGF I induces hypertrophy of neonatal rat cardiomyocytes, with induction of expression of myosin light chain-2, troponin I, and skeletal α-actin. Kajstura et al. [77] have reported that IGF I stimulates DNA synthesis in neonatal rat cardiac myocytes and that antisense IGF IR oligonucleotides suppress cardiocyte replication. Adult rat cardiomyocytes in long-term culture upregulate their IGF IR, and respond to IGF I with enhanced myofibril development and downregulation of smooth muscle α-actin [78]. Furthermore, IGF I is a potent stimulator of adult cardiomyocyte protein synthesis [79].

3.2. IGF IR

The IGF IR is a membrane tyrosine-kinase consisting of two alpha chains and two beta chains linked through disulfide bonds (reviewed in [8]). The receptor binds IGF I and IGF II with high affinity and insulin with at least a hundred-fold lower affinity. Recently, the existence of insulin receptor–IGF IR hybrids has been demonstrated [80,81]. IGF IR signaling involves autophosphorylation and subsequent tyrosine phosphorylation of IRS-I and potentially other tyrosine-containing substrates. IRS-I serves as a docking-protein and can activate multiple signaling pathways including PI3-kinase, Syp, Nck, and the Ras–MAP kinase pathway. For a detailed discussion of IGF I/insulin signaling the reader is referred to recent reviews [8,82]. A variety of growth factors and specifically PDGF, fibroblast growth factor (FGF), angiotensin II and thrombin upregulate IGF IR on VSMC [37,83,84]. This upregulation of IGF IR may play a critical role in the growth response of smooth muscle cells. Thus antisense transcription of a rat IGF IR cDNA in VSMC markedly suppresses growth of these cells in response to 10% serum [85]. This anti-proliferative effect correlates with a reduction in receptor number of approximately 50%, without changes in binding-affinity. These findings suggest that the upregulatory effects of growth factors on IGF IR may be a important component of their ability to induce competency. This concept is supported by data from Pietrzkowski et al., indicating that in BALB/c3T3 cells overexpressing IGF I and IGF IR, IGF I mediated growth occurs independently of the EGF and PDGF receptors [86]. Furthermore, SV40 T antigen transformation of BALB/c3T3 cells markedly increases secretion of IGF I, and antisense targeting of the IGF IR inhibits the growth of these transformed cells [87]. These cells still require PDGF or 1% serum for growth; however, if the IGF IR is overexpressed in SV40 T antigen transformed cells, they will grow in serum-free medium. In mouse fibroblasts a functional IGF IR is required for the mitogenic effects of the EGF receptor [88]. These data again support the concept that IGF IR number per cell is important in cellular growth responses. Thus downregulation of IGF IR using antisense phosphorothioate oligonucleotides markedly inhibits the growth response of rat aortic VSMC to serum as well as to angiotensin II and thrombin [84,89]. A recent report has documented that a sense oligonucleotide targeting the AUG site of the rat IGF IR mRNA markedly upregulates IGF IR, leading to increased growth responses [89]. The mechanism for this effect is incompletely understood but may be related to the presence of a natural antisense transcript, a transcriptional or a translational repressor protein. It has been reported that PDGF and FGF-induced upregulation of IGF IR on VSMC is protein kinase C (PKC)-dependent, but that angiotensin II upregulation of IGF IR is PKC-independent [37]. Similarly, FGF-induced DNA synthesis has been reported to be PKC-dependent [37,90], whereas angiotensin II-induced growth responses in VSMC [37] and in cardiac fibroblasts [91] are PKC-independent. Molecular mechanisms whereby growth factors upregulate IGF IR are poorly understood. A recent report has demonstrated that the PDGF-responsive sequence of the IGF IR gene is located within ~ 100 bp proximal to the transcription start site [92].

As noted above, the ability of several growth factors to increase IGF IR density may be critical for their mitogenic effects. Thus upregulation of IGF IR could lead to stimulation of IGF IR mediated signaling events. This is consistent with cross-talk between growth factors and the IGF IR, and has been recently demonstrated in the case of thrombin stimulation of VSMC growth [84]. More direct mechanisms of cross-talk between the IGF IR and other growth factors may exist. Thus Yoshinouchi et al. [162] have suggested that FGF may transphosphorylate the IGF IR. Furthermore, angiotensin II and thrombin have been shown to increase phosphorylation of IRS-1 in VSMC (P. Delafontaine, unpublished results). Potential cross-talk between the IGF IR and other growth factors may have profound implications for understanding pathways whereby growth factors exert their effects in vivo. Thus the IGF IR could function as the final common mediator for the effects of multiple growth stimulatory peptides.

3.3. IGF-binding proteins

VSMC in culture synthesize IGFBP-2, IGFBP-3 and IGFBP-4 [42–46]. In rat aortic VSMC, FGF [42] and PDGF [46] have been reported to increase IGFBP-4 production. Porcine VSMC express BP-4 and BP-2 mRNA and secrete primarily BP-2. Cohick et al. have reported no effect of PDGF, FGF, transforming growth factor β (TGFβ), and epidermal growth factor (EGF) on BP-2 and BP-4 mRNA levels in porcine VSMC [45]. The regulation of IGF-binding protein levels in smooth muscle cells is affected by various proteases. Thus porcine VSMC secrete IGFBP-2, IGFBP-4, and IGFBP-5 proteases [93,94]. In rat aortic VSMC, biosynthesis and IGF-dependent proteolysis of IGFBP-4 are increased with the confluent state [95]. Recently angiotensin II [96] and thrombin (P. Delafontaine, unpublished results) have been shown to markedly reduce IGFBP-4 levels in rat aortic VSMC conditioned medium. It is of note that thrombin also downregulates IGF-binding-protein production by rat skeletal muscle cells and mouse myocytes [97]. The physiological significance of these binding proteins secreted by VSMC remains to be

determined. IGFBP-1 has been shown to inhibit VSMC growth in serum-free medium [98], but to increase VSMC growth in the presence of low concentrations of platelet-poor plasma [99]. BP-1 binds via its RGD sequence to the $\alpha 5\beta 1$ integrin receptor, and this may be necessary for its growth-stimulatory effect [100]. This stimulatory effect has also been ascribed to the phosphorylated isoform of BP-1 [101]. Bovine BP-2 has likewise been shown to have bifunctional effects on VSMC growth with an inhibitory effect in serum-free medium and a stimulatory effect in platelet-poor plasma [102]. The effect of BP-3 on VSMC growth has not been determined but preincubation of fibroblasts with BP-3 potentiates the IGF I response [103], possibly because of prevention of IGF IR downregulation [104]. The cell-surface association of IGFBP-3 appears to be required for this potentiating effect. Conversely, coincubation of IGF I and BP-3 results in inhibition of the IGF I response [103]. It is of note that a direct growth inhibitory effect of IGFBP-3 has been suggested [105]. BP-4 does not adhere to cell-surfaces and inhibits IGF I growth effects on VSMC [45]. It is thus possible that angiotensin II and thrombin-induced downregulation of IGFBP-4 production serves to increase availability of free IGF I. In microvessel endothelial cells BP-2 may potentiate the effect of IGF I on glucose transport and α-aminoisobutyric acid uptake [106]. Stimulation of cAMP markedly increases BP-4 mRNA levels in a clonal endothelial cell line [107]. Recently serum deprivation or contact inhibition of porcine endothelial cells has been shown to be associated with markedly increased gene expression and secretion of IGFBP-3 [108]. Because IGFBP-3 may have marked antiproliferative effects [105], it is possible that BP-3 acts as a growth-arrest gene for endothelium.

4. Regulation of IGF I, the IGF IR and IGF-binding proteins in cardiovascular tissues in vivo

4.1. Hemodynamic forces, hypertension, ischemia

In vitro data have indicated that stretch increases autocrine secretion of IGF I from skeletal muscle cells [109], suggesting that alterations in physical forces may regulate IGF I expression in vivo. An increase in vascular load induced by ligation of the femoral artery in the rat produces increased IGF I immunoreactivity in endothelium and smooth muscle cells in the contralateral femoral artery [110]. In the heart, supravalvular aortic stenosis in the rat results in rapid increases in IGF I mRNA levels in the left ventricle [23]. These findings are consistent with hemodynamic regulation of IGF I. A variety of studies have documented increases in cardiac IGF I mRNA and protein levels in hypertensive rats. Models have included suprarenal aortic constriction; the uninephrectomized spontaneously hypertensive rat; the uninephrectomized, deoxycorticosterone-treated, saline-fed rat (DOCA salt); and the two-kidney, one clip, hypertensive rat [21,22]. Furthermore, volume-overload induced by creation of an aortocaval fistula in the rat is associated with marked induction of IGF I expression in the right ventricle [111]. In these models conclusive demonstration of the site of IGF I synthesis remains to be determined. However, immunohistochemical analysis has suggested increases in IGF I staining in the subendocardium. In these models of cardiac hypertrophy, expression of the IGF IR appears unchanged. It is of note, however, that in right ventricular biopsies from patients with hypertrophic cardiomyopathy, there is an increase in IGF IR binding sites [112]. Consistent with hemodynamic regulation of IGF I expression are data indicating that IGF I mRNA expression is increased in the rat bladder following urethral ligation [113]. In this model IGF IR mRNA levels are unchanged, but there is a significant induction in IGFBP-2 and IGFBP-4 mRNA [114]. Recently, infusions of IGF I in the rat following myocardial infarction have been shown to enhance ventricular hypertrophy and to have potentially beneficial effects on hemodynamic function [115].

IGF I-stimulated myocardial growth could result from the effects both of systemic IGF I that crosses the endothelium [15,16,51], and of locally synthesized peptide [21–23,111]. Local synthesis of IGF I potentially derives both from myocyte and non-myocyte cells, notably endothelium, VSMC, and fibroblasts. In view of their abundance in areas of myocardial scarring, one may speculate that fibroblasts serve as a significant source of IGF I in the postischemic remodeling myocardium. Clearly myocytes from infarcted hearts have higher levels of IGF I and IGF IR [75]. In addition, monocytes in ischemic hearts may produce IGF I [128]. Although adult cardiac myocytes are terminally differentiated, evidence exists suggesting that myocytes close to the infarct zone undergo DNA replication [75], consistent with the ability of IGF I to stimulate myocyte proliferation in vitro [77]. The specific role of IGF-binding proteins in modulating IGF I effects on the heart is largely unexplored but of great potential interest.

IGF I mRNA levels have been shown to be increased in hypertensive aortae from rats following abdominal aortic coarctation [24]. In situ hybridization analysis has shown that the induction of IGF I is localized to the smooth muscle cell layer. These data are consistent with a role for IGF I as an autocrine mediator of hypertrophic/hyperplastic responses in hypertension. However, in the DOCA/salt model of hypertension in the rat, it has been reported that there is no change in IGF I mRNA [116]. The increase in aortic expression of IGF I mRNA in the abdominal coarctation model of hypertension is accompanied by a progressive decrease in IGF IR expression [117]. This is consistent with ligand-induced downregulation of the receptor, a phenomenon previously demonstrated in cultured VSMC [37]. Recently IGFBP-4 mRNA levels have been shown to be markedly elevated in the hypertensive aorta following abdominal coarctation in the rat [30]. The induction of IGFBP-4 is limited to the hypertensive blood vessel, because IGFBP-4 mRNA levels in the normotensive abdominal aorta and in the liver are transiently decreased. These data suggest that increases in vascular load directly stimulate IGFBP-4 expression. The transient decrease in hepatic IGFBP-4 and IGFBP-3 expression in this model may be related to effects of circulating angiotensin II, consistent with in vitro data [96]. Because IGFBP-4 may function as a inhibitory binding protein [45], its induction in the hypertensive vasculature may serve a counterregulatory role to blunt growth responses.

Potential regulation of IGF I and its binding proteins in human hypertension is largely unexplored. However, one group has reported higher circulating IGF I levels in patients with essential hypertension and left ventricular hypertrophy [118–120]. Larger trials to address this issue are clearly warranted.

4.2. Injury

A variety of studies are consistent with an important role for the IGF I–IGF IR autocrine system in vascular injury. An initial report by Hansson et al. demonstrated that IGF I immunoreactivity was increased in endothelial cells and in the neointima following femoral artery injury in the rat [121]. Studies from Cercek et al. [36] and Khorsandi et al. [26] showed peak induction of IGF I mRNA at 7 days in the balloon-injured rat aorta, with a reciprocal decrease in IGF IR expression. The increase in IGF I expression following balloon injury in rat aorta is localized to the smooth muscle cell layer and to the neointima. Consistent with the major role of growth hormone in regulating IGF I expression in vivo, there is a marked decrease in the intimal hyperplasia that develops following aortic balloon-injury in the hypophysectomized rat [122,123]. Interestingly, intimal proliferation is a characteristic change that occurs after subarachnoid hemorrhage [161]. It has recently been shown that exposure of rat femoral artery to periarterial blood results in a marked increase in IGF I mRNA expression and IGF IR binding sites [124]. This provides further evidence for a role of the IGF I autocrine system in vascular growth responses. Bornfeldt et al. [125] have also reported increases in IGF I mRNA in balloon-injured rat aorta; however, in this study IGF IR mRNA levels were also increased. It is of note that in this study the infusion of IGF I increased DNA synthesis in injured aorta in the diabetic rat.

4.3. Angiogenesis and wound healing

IGF I stimulates migration and tube formation by vascular endothelial cells [126] and has been shown to promote rat aortic angiogenesis in vitro [127]. Following microembolisation in the porcine heart there is increased IGF I mRNA expression in infiltrating monocytes in areas of capillary sprouting, consistent with a role for IGF I in angiogenesis in vivo [128]. It is of note that macrophage IGF I synthesis is inhibited by interferon-γ [129]. Several studies have documented that IGF I (alone or complexed to binding proteins) accelerates wound healing in vivo [130–132].

4.4. Diabetes, hyperinsulinemia

The function of IGF I as a potential mediator of vascular growth responses in insulin-dependent diabetes and in hyperinsulinemic states is unclear. Murphy et al. have reported that insulin increases IGF I expression in rat aorta [25]. In the streptozotocin diabetic rat, IGF I mRNA levels are markedly decreased in the heart, skeletal muscle and aorta, and levels are restored by insulin infusion but not by IGF I infusion [133]. In the insulin-deficient diabetic rat, DNA synthesis following balloon-injury of the aorta has been reported to be either decreased [125] or unchanged [134,135]. There are conflicting reports regarding the potential association between higher circulating IGF I levels and the incidence and progression of diabetic retinopathy [136–139]. It is of note that IGF I has been shown to significantly improve control of blood glucose in the insulin-resistant state; however, its use in humans has been associated with significant deleterious side-effects [140]. The recent demonstration that advanced glycosylation end-products (AGE) induce IGF I synthesis by human monocytes may have relevance to understanding mechanisms whereby hyperglycemia induces vascular proliferative changes [141]. Indeed, it is possible that AGE-induced IGF I synthesis by monocytes within the subendothelial space promotes VSMC growth. Furthermore, the effect of IGF I and of insulin to stimulate plasminogen activator inhibitor type 1 (PAI-1) synthesis [142] may be relevant to understanding mechanisms of accelerated atherosclerosis in diabetes.

4.5. IGF I and vasodilation

IGF I has been shown to induce renal arteriolar (glomerular) dilation with increases in renal plasma flow and glomerular filtration rate [143,144]. This effect is likely mediated by induction of nitric oxide (NO) production. Indeed IGF I stimulates release of NO from cultured endothelial cells [160]. A beneficial effect of IGF I in animal models of acute renal failure has been demonstrated [145,146]. Recently infusion of IGF I into the brachial artery in humans has been shown to increase forearm blood flow [147]. Consistent with those findings is the report that insulin-induced vasodilation in humans is blocked by the NO-synthase inhibitor, L-NMMA [148]. It is of note that contrary to its effect on endothelial cells, IGF I inhibits cytokine-induced production of NO in VSMC [149].

4.6. Atherosclerosis

Studies of IGF I expression in atherosclerosis are limited. A recent report has documented increased IGF I immunostaining in synthetic VSMC in human atherosclerotic plaque [150]. Furthermore, IGF IR mRNA expression has been demonstrated in smooth muscle cells in atherosclerotic lesions [151]. Inhibitors of the growth hormone–IGF I axis, namely the somatostatin analogs octreotide and angiopeptin, inhibit VSMC proliferation in vitro and in vivo [152–156]. A recent human trial has indicated that angiopeptin decreased clinical events during 12 months of follow-up after coronary balloon angioplasty by approximately 22% [157]. This clinical effect contrasted with the lack of any evident effect upon angiographic variables.

5. Summary and conclusion

A large body of evidence has conclusively shown that IGF I is an essential regulator of developmental growth.

Thus mice bearing a null mutation for the IGF IR gene invariably die shortly after birth, and mice bearing a null mutation for the IGF I gene have a high neonatal mortality rate and marked growth retardation [158,159]. The ubiquitous effects of IGF I make it likely that this autocrine/endocrine system plays an important role in cardiovascular development. Its potential role in cardiovascular pathophysiology has raised considerable interest over the last several years. There is strong evidence that IGF I is a critical determinant of vascular growth responses in vitro and in vivo. Regulation of VSMC IGF IR availability appears to be crucial for the control of VSMC growth, and as such is at a convergence point for the effects of multiple growth factors. Clinical studies relating to IGF I in hypertension are extremely limited but significant data from animal studies now suggest a role for IGF I as a mediator of hypertrophic/hyperplastic responses in hypertension. Furthermore, significant animal data now exist implicating IGF I as an important mediator of cardiac hypertrophic responses. The development of a specific pharmacologic inhibitor of the IGF IR should allow rational clinical trials to address the function of IGF I as a mediator of cardiovascular growth responses. Specifically, areas of great interest will include the potential prevention of post-angioplasty restenosis, of atherosclerotic lesion development and progression, and of the complications of hypertensive vascular disease. The use of IGF I to ameliorate myocardial growth and function post infarction, to promote angiogenesis and collateral artery formation in the setting of peripheral vascular disease, are other important directions for future research. The use of IGF I to improve wound healing, improve recovery from acute renal failure and improve glucose control is currently under investigation. Clearly ongoing studies addressing the mechanisms whereby IGF I interacts with its receptor and binding proteins to produce its effects in cardiovascular tissues, will provide a rationale for novel and pertinent clinical research.

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References

[1] Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocrine Rev 1995;16:3–34.
[2] Werner H, Adamo M, Roberts CT, Jr., LeRoith D. Molecular and cellular aspects of insulin-like growth factor action. Vitamins Horm 1994;48:1–57.
[3] Tricoli JV, Rall LB, Scott J, Bell GI, Shows TB. Localization of insulin-like growth factor genes to human chromosomes 11 and 12. Nature 1984;310:784–786.
[4] Taylor BA, Grieco D. Localization of the gene encoding insulin-like growth factor I on mouse chromosome 10. Cell 1991;56:57–58.
[5] Rotwein P. Structure, evolution, expression and regulation of insulin-like growth factors I and II. Growth Factors 1991;5:3–18.
[6] Butler AA, Ambler GR, Breier BH, LeRoith D, Roberts CT, Jr., Gluckman PD. Growth hormone (GH) and insulin-like growth factor-I (IGF-I) treatment of the GH-deficient dwarf rat: differential effects on IGF-I transcription start site expression in hepatic and extrahepatic tissues and lack of effect on type I IGF receptor mRNA expression. Mol Cell Endocrinol 1994;101:321–330.
[7] Lund PK. Insulin-like growth factor I: molecular biology and relevance to tissue-specific expression and action. Recent Prog Horm Res 1994;49:125–148.
[8] LeRoith D, Werner H, Beitner-Johnson D, Roberts CT. Molecular and cellular aspects of the insulin-like growth factor I receptor. Endocrine Rev 1995;16:143–163.
[9] Morgan DO, Edman JC, Standring DN, Fried VA, Smith MC, Roth RA, Rutter WJ. Insulin-like growth factor II receptor as a multifunctional binding protein. Nature 1987;329:301–307.
[10] Senior PV, Byrne S, Brammar WJ, Beck F. Expression of the IGF-II/mannose-6-phosphate receptor mRNA and protein in the developing rat. Development 1990;109:67–73.
[11] Abbott AM, Bueno R, Pedrini MT, Murray JM, Smith RJ. Insulin-like growth factor I receptor gene structure J Biol Chem 1992;267:10759–10763.
[12] Ullrich A, Gray A, Tam AW, Yang-Feng T, Tsubokawa M, Collins C, Henzel W, Le Bon T, Kathuria S, Chen E, Jacobs S, Francke U, Ramachandran J, Fujita-Yamaguchi T. Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. EMBO J 1986;5:2503–2512.
[13] McCusker RH, Campion DR, Clemmons DR. The ontogeny and regulation of a 31,000 Mr insulin-like growth factor/somatomedin (IGF) binding protein in fetal porcine plasma and sera. Endocrinology 1988;122:2071–2079.
[14] Binoux M, Hossenlopp P. Insulin-like growth factor (IGF) and IGF-binding proteins: comparison of human serum and lymph. J Clin Endocrinol Metab 1988;67:509–514.
[15] Bar RS, Clemmons DR, Boes M, Busby WH, Booth BA, Dake BL, Sandra A. Transcapillary permeability and subendothelial distribution of endothelial and amniotic fluid insulin-like growth factor binding proteins in the rat heart. Endocrinology 1990;127:1078–1086.
[16] Bar RS, Boes M, Clemmons DR, Busby WH, Sandra A, Dake BL, Booth BA. Insulin differentially alters transcapillary movement of intravascular IGFBP-1, IGFBP-2 and endothelial cell IGF-binding proteins in the rat heart. Endocrinology 1990;127:497–499.
[17] Szabo L, Mottershead DG, Ballard FJ, Wallace JC. The bovine insulin-like growth factor (IGF) binding protein purified from conditioned medium requires the N-terminal tripeptide in IGF I for binding. Biochem Biophys Res Commun 1988;151:207–214.
[18] Cascieri MA, Chicchi GC, Applebaum J, Hayes NS, Green BG, Bayne ML. Mutants of human insulin-like growth factor I with reduced affinity for the type I insulin-like growth factor receptor. Biochemistry 1988;27:3229–3233.
[19] Han VKM, D'Ercole AJ, Kay P. Cellular localization of somatomedin (insulin-like growth factor) messenger RNA in the human fetus. Science 1987;236:193–197.
[20] Engelmann GL, Boehm KD, Haskell JF, Khairallah PA, Ilan J. Insulin-like growth factors and neonatal cardiomyocyte development: ventricular gene expression and membrane receptor variations in normotensive and hypertensive rats. Mol Cell Endocrinol 1989;63:1–14.
[21] Wåhlander H, Isgaard J, Jennische E, Friberg P. Left ventricular insulin-like growth factor I increases in early renal hypertension. Hypertension 1992;19:25–32.
[22] Donohue TJ, Dworkin LD, Lango MN, Fliegner K, Lango RP, Benstein JA, Slater WR, Catanese VM. Induction of myocardial insulin-like growth factor-I gene expression in left ventricular hypertrophy. Circulation 1994;89:799–809.
[23] Hanson MC, Fath KA, Alexander RW, Delafontaine P. Induction of cardiac insulin-like growth factor I gene expression in pressure overload hypertrophy. Am J Med Sci 1993;306:69–74.
[24] Fath KA, Alexander RW, Delafontaine P. Abdominal coarctation increases insulin-like growth factor I mRNA levels in rat aorta. Circ Res 1993;72:271–277.
[25] Murphy LJ, Ghahary A, Chakrabarti S. Insulin regulation of IGF-I expression in rat aorta. Diabetes 1990;39:657–652.

[26] Khorsandi MJ, Fagin JA, Giannella-Neto D, Forrester JS, Cercek B. Regulation of insulin-like growth factor-I and its receptor in rat aorta after balloon denudation: evidence for local bioactivity. J Clin Invest 1992;90:1926–1931.

[27] Delafontaine P, Ku L, Ververis JJ, Cohen C, Runge MS, Alexander RW. Epitope mapping of the α-chain of the insulin-like growth factor I receptor using antipeptide antibodies. J Mol Cell Cardiol 1994;26:1659–1673.

[28] Sidawy AN, Termanini B, Nardi RV, Harmon JW, Korman LY. Insulin-like growth factor I receptors in the arteries of the rabbit: autoradiographic mapping and receptor characterization. Surgery 1990;108:165–171.

[29] Chin E, Zhou J, Dai J, Baxter RC, Bondy CA. Cellular localization and regulation of gene expression for components of the insulin-like growth factor ternary binding protein complex. Endocrinology 1994;134:2498–2504.

[30] Anwar A, Delafontaine P. Hypertension increases insulin-like growth factor binding protein-4 mRNA levels in rat aorta. Hypertension 1994;24:679–685.

[31] Delafontaine P, Bernstein KE, Alexander RW. Insulin-like growth factor I gene expression in vascular cells. Hypertension 1991;17:693–699.

[32] Delafontaine P, Lou H, Alexander RW. Regulation of insulin-like growth factor I messenger RNA levels in vascular smooth muscle cells. Hypertension 1991;18:742–747.

[33] Clemmons DR, Van Wyk JJ. Evidence for a functional role of endogenously produced somatomedin-like peptides in the regulation of DNA synthesis in cultured human fibroblasts and porcine smooth muscle cells. J Clin Invest 1985;75:1914–1918.

[34] Clemmons DR. Exposure to platelet-derived growth factor modulates the porcine aortic smooth muscle cell response to somatomedin-C. Endocrinology 1985;117:77–83.

[35] Clemmons DR. Variables controlling the secretion of a somatomedin-like peptide by cultured porcine smooth muscle cells. Circ Res 1985;56:418–426.

[36] Cercek B, Fishbein MC, Forrester JS, Helfant RH, Fagin JA. Induction of insulin-like growth factor I messenger RNA in rat aorta after balloon denudation. Circ Res 1990;66:1755–1760.

[37] Ververis JJ, Ku L, Delafontaine P. Regulation of insulin-like growth factor I receptors on vascular smooth muscle cells by growth factors and phorbol esters. Circ Res 1993;72:1285–1292.

[38] Pfeifle B, Ditschuneit HH, Ditschuneit H. Binding and biological actions of insulin-like growth factors on human arterial smooth muscle cells. Horm Metab Res 1982;14:409–414.

[39] Bornfeldt KE, Arnqvist HJ, Dahlkvist HH, Skottner A, Wikberg JES. Receptors for insulin-like growth factor-I in plasma membranes isolated from bovine mesenteric arteries. Acta Endocrinol 1988;117:428–434.

[40] King GL, Goodman AD, Buzney S, Moses A, Kahn CR. Receptors and growth-promoting effects of insulin and insulin-like growth factors on cells from bovine retinal capillaries and aorta. J Clin Invest 1985;75:1028–1036.

[41] Bornfeldt KE, Gidlof RA, Wasteson A, Lake M, Skottner A, Arnqvist HJ. Binding and biological effects of insulin, insulin analogues and insulin-like growth factors in rat aortic smooth muscle cells. Comparison of maximal growth promoting activities. Diabetologia 1991;34:307–313.

[42] Ververis J, Ku L, Delafontaine P. Fibroblast growth factor regulates insulin-like growth factor-binding protein production by vascular smooth muscle cells. Am J Med Sci 1994;307:77–81.

[43] McCusker RH, Clemmons DR. Insulin-like growth factor binding protein secretion by muscle cells: effect of cellular differentiation and proliferation. J Cell Physiol 1988;137:505–512.

[44] McCusker RH, Camacho-Hübner C, Clemmons DR. Identification of the types of insulin-like growth factor-binding proteins that are secreted by muscle cells in vitro. J Biol Chem 1989;264:7795–7800.

[45] Cohick WS, Gockerman A, Clemmons DR. Vascular smooth muscle cells synthesize two forms of insulin-like growth factor binding proteins which are regulated differently by the insulin-like growth factors. J Cell Physiol 1993;157:52–60.

[46] Giannella-Neto D, Kamyar A, Sharifi B, Pirola CJ, Kupfer J, Rosenfeld RG, Forrester JS, Fagin JA. Platelet-derived growth factor isoforms decrease insulin-like growth factor I gene expression in rat vascular smooth muscle cells and selectively stimulate the biosynthesis of insulin-like growth factor binding protein 4. Circ Res 1992;71:646–656.

[47] Boes M, Dake BL, Bar RS. Interactions of cultured endothelial cells with TGF-β, bFGF, PDGF and IGF-I. Life Sci 1991;48:811–821.

[48] Bar RS, Boes M, Booth BA, Dake BL, Henley SA, Hart MN. The effects of platelet-derived growth factor in cultured microvessel endothelial cells. Endocrinology 1989;124:1841–1848.

[49] Bar RS, Boes M. Distinct receptors for IGF-I, IGF-II and insulin are present on bovine capillary endothelial cells and large vessel endothelial cells. Biochem Biophys Res Commun 1984;124:203–209.

[50] Kern PA, Svoboda ME, Eckel RH, Van Wyk JJ. Insulin-like growth factor action and production in adipocytes and endothelial cells from human adipose tissue. Diabetes 1989;38:710–717.

[51] Gajdusek CM, Luo Z, Mayberg MR. Sequestration and secretion of insulin-like growth factor-I by bovine aortic endothelial cells. J Cell Physiol 1993;154:192–198.

[52] Bar RS, Harrison LC, Baxter RC, Boes M, Dake BL, Booth B, Cox A. Production of IGF-binding proteins by vascular endothelial cells. Biochem Biophys. Res Commun 1987;148:734–739.

[53] Booth BA, Bar RS, Boes M, Dake BL, Bayne M, Cascieri M. Intrinsic bioactivity of insulin-like growth factor-binding proteins from vascular endothelial cells. Endocrinology 1990;127:2630–2638.

[54] Moser DR, Lowe WL, Dake BL, Booth BA, Boes M, Clemmons DR, Bar RS. Endothelial cells express insulin-like growth factor-binding proteins 2 to 6. Mol Endocrinol 1992;6:1805–1814.

[55] Booth BA, Boes M, Andress DL, Dake BL, Kiefer MC, Maack C, Linhardt RJ, Bar K, Caldwell EEO, Weiler J, Bar RS. IGFBP-3 and IGFBP-5 association with endothelial cells: role of C-terminal heparin binding domain. Growth Regul 1995;5:1–17.

[56] Stiles CD, Capone GT, Scher CD, Antoniades HN, Van Wyk JJ, Pledger WJ. Dual control of cell growth by somatomedins and platelet-derived growth factor. Proc Natl Acad Sci USA 1979;76:1279–1283.

[57] Cook JJ, Haynes KM, Werther GA. Mitogenic effects of growth hormone in cultured human fibroblasts. Evidence for action via local insulin-like factor I production. J Clin Invest 1988;81:206–212.

[58] Bornfeldt KE, Arnqvist HJ, Norstedt G. Regulation of insulin-like growth factor-I gene expression by growth factors in cultured vascular smooth muscle cells. J Endocrinol 1990;125:381–386.

[59] Banskota NK, Taub R, Zellner K, King GL. Insulin, insulin-like growth factor I and platelet-derived growth factor interact additively in the induction of the protooncogene c-*myc* and cellular proliferation in cultured bovine aortic smooth muscle cells. Mol Endocrinol 1989;3:1183–1190.

[60] Yamamoto M, Yamamoto K. Growth regulation in primary culture of rabbit arterial smooth muscle cells by platelet-derived growth factor, insulin-like growth factor-I, and epidermal growth factor. Exp Cell Res 1994;212:62–68.

[61] Jensen DE, Rich CB, Terpstra AJ, Farmer SR, Foster JA. Transcriptional regulation of the elastin gene by insulin-like growth factor-I involves disruption of Sp1 binding: evidence for the role of Rb in mediating Sp1 binding in aortic smooth muscle cells. J Biol Chem 1995;270:6555–6563.

[62] Tamaroglio TA, Lo CS. Regulation of fibronectin by insulin-like growth factor-I in cultured rat thoracic aortic smooth muscle cells and glomerular mesangial cells. Exp Cell Res 1994;215:338–346.

[63] Delafontaine P, Lou H. Angiotensin II regulates insulin-like growth factor I gene expression in vascular smooth muscle cells. J Biol Chem 1993;268:16866–16870.

[64] Anwar A, Runge M, Delafontaine P. Thrombin downregulates insulin-like growth factor I mRNA levels in vascular smooth muscle cells. Circulation 1994;90:I–305.

[65] Lowe Jr., WL, Yorek MA, Teasdale RM. Ligands that activate protein kinase-C differ in their ability to regulate basic fibroblast growth factor and insulin-like growth factor-I messenger ribonucleic acid levels. Endocrinology 1993;132:1593–1602.

[66] Lowe Jr., WL, Yorek MA, Karpen CW, Teasdale RM, Hovis JG, Albrecht B, Prokopiou C. Activation of protein kinase-C differen-

tially regulates insulin-like growth factor-I and basic fibroblast growth factor messenger RNA levels. Mol Endocrinol 1992;6:741–752.

[67] Tang X-M, Rossi MJ, Masterson BJ, Chegini N. Insulin-like growth factor I (IGF-I), IGF-I receptors, and IGF-binding proteins 1–4 in human uterine tissue: tissue localization and IGF-I action in endometrial stromal and myometrial smooth muscle cells in vitro. Biol Reprod 1994;50:1113–1125.

[68] Noveral JP, Bhala A, Hintz RL, Grunstein MM, Cohen P. Insulin-like growth factor axis in airway smooth muscle cells. Am J Physiol 1994;267:L761–L765.

[69] Dempsey EC, Badesch DB, Dobyns EL, Stenmark KR. Enhanced growth capacity of neonatal pulmonary artery smooth muscle cells in vitro: Dependence on cell size, time from birth, insulin-like growth factor I, and auto-activation of protein kinase C. J Cell Physiol 1994;160:469–481.

[70] Hyldahl L, Engström W, Schofield PN. Stimulatory effects of insulin-like growth factors on DNA synthesis in the human embryonic cornea. J Embryol Exp Morphol 1986;98:71–83.

[71] Tollefsen SE, Lajara R, McCusker RH, Clemmons DR, Rotwein P. Insulin-like growth factors (IGF) in muscle development: expression of IGF-I, the IGF-I receptor, and an IGF-binding protein during myoblast differentiation. J Biol Chem 1989;264:13810–13817.

[72] Florini JR, Ewton DZ. Induction of gene expression in muscle by the IGFs. Growth Regul 1992;2:23–29.

[73] James PL, Jones SB, Busby Jr., WH, Clemmons DR, Rotwein P. A highly conserved insulin-like growth factor-binding protein (IGFBP-5) is expressed during myoblast differentiation. J Biol Chem 1993;268:22305–22312.

[74] Vandenburgh HH, Karlisch P, Shansky J, Feldstein R. Insulin and IGF-I induce pronounced hypertrophy of skeletal myofibers in tissue culture. Am J Physiol 1991;260:C475–C484.

[75] Reiss K, Meggs LG, Li P, Olivetti G, Capasso JM, Anversa P. Upregulation of IGF_1, IGF_1-receptor, and late growth related genes in ventricular myocytes acutely after infarction in rats. J Cell Physiol 1994;158:160–168.

[76] Ito H, Hiroe M, Hirata Y, Tsujino M, Adachi S, Shichiri M, Koike A, Nogami A, Marumo F. Insulin-like growth factor-I induces hypertrophy with enhanced expression of muscle specific genes in cultured rat cardiomyocytes. Circulation 1993;87:1715–1721.

[77] Kajstura J, Cheng W, Reiss K, Anversa P. The IGF-I–IGF-I receptor system modulates myocyte proliferation but not myocyte cellular hypertrophy in vitro. Exp Cell Res 1994;215:273–283.

[78] Donath MY, Zapf J, Eppenberger-Eberhardt M, Froesch ER, Eppenberger HM. Insulin-like growth factor I stimulates myofibril development and decreases smooth muscle α-actin of adult cardiomyocytes. Proc Natl Acad Sci USA 1994;91:1686–1690.

[79] Fuller SJ, Mynett JR, Sugden PH. Stimulation of cardiac protein synthesis by insulin-like growth factors. Biochem J 1992;282:85–90.

[80] Moxham CP, Duronio V, Jacobs S. Insulin-like growth factor I receptor β subunit heterogeneity. Evidence for hybrid tetramers composed of insulin-like growth factor I and insulin receptor heterodimers. J Biol Chem 1989;264:13238–13244.

[81] Soos MA, Siddle K. Immunological relationships between receptors for insulin and insulin-like growth factor I. Evidence for structural heterogeneity of insulin-like growth factor I receptors involving hybrids with insulin receptors. Biochem J 1989;263:553–563.

[82] Cheatham B, Kahn CR. Insulin action and the insulin signaling network. Endocrine Rev 1995;16:117–142.

[83] Pfeifle B, Boeder H, Ditschuneit H. Interaction of receptors for insulin-like growth factor I, platelet-derived growth factor, and fibroblast growth factor in rat aortic cells. Endocrinology 1987;120:2251–2258.

[84] Delafontaine P, Anwar A, Ku L. Thrombin-induced mitogenesis of vascular smooth muscle cells is dependent on activation of the insulin-like growth factor I receptor. Circulation 1994;90:I–626.

[85] Du J, Delafontaine P. Inhibition of vascular smooth muscle cell growth through antisense transcription of a rat insulin-like growth factor I receptor cDNA. Circ Res 1995;76:963–972.

[86] Pietrzkowski Z, Lammers R, Carpenter G, Soderquist AM, Limardo M, Phillips PD, Ullrich A, Baserga R. Constitutive expression of insulin-like growth factor I and insulin-like growth factor I receptor abrogates all requirements for exogenous growth factors. Cell Growth Differ 1992;3:199–205.

[87] Porcu P, Ferber A, Pietrzkowski Z, Roberts CT, Adamo M, LeRoith D, Baserga R. The growth-stimulatory effect of simian virus 40 T antigen requires the interaction of insulin-like growth factor I with its receptor. Mol Cell Biol 1992;12:5069–5077.

[88] Coppola D, Ferber A, Miura M, Sell C, D'Ambrosio C, Rubin R, Baserga R. A functional insulin-like growth factor I receptor is required for the mitogenic and transforming activities of the epidermal growth factor receptor. Mol Cell Biol 1994;14:4588–4595.

[89] Delafontaine P, Meng XP, Ku L, Du J. Regulation of vascular smooth muscle cell insulin-like growth factor I receptors by phosphorothioate oligonucleotides: effects on cell growth and evidence that sense targeting at the ATG site increases receptor expression. J Biol Chem 1995;270:14383–14388.

[90] Ali S, Becker MW, Davis MG, Dorn II, GW. Dissociation of vasoconstrictor-stimulated basic fibroblast growth factor expression from hypertrophic growth in cultured vascular smooth muscle cells: relevant roles of protein kinase C. Circ Res 1994;75:836–843.

[91] Booz GW, Dostal DE, Singer HA, Baker KM. Involvement of protein kinase C and Ca^{2+} in angiotensin II-induced mitogenesis of cardiac fibroblasts. Am J Physiol 1994;267:C1308–C1318.

[92] Rubini M, Werner H, Gandini E, Roberts Jr., CT, LeRoith D, Baserga R. Platelet-derived growth factor increases the activity of the promoter of the IGF I receptor gene. Exp Cell Res 1994;211:374–379.

[93] Gockerman A, Clemmons DR. Porcine aortic smooth muscle cells secrete a serine protease for insulin-like growth factor binding protein-2. Circ Res 1995;76:514–521.

[94] Parker A, Gockerman A, Busby WH, Clemmons DR. Properties of an insulin-like growth factor-binding protein-4 protease that is secreted by smooth muscle cells. Endocrinology 1995;136:2470–2476.

[95] Kamyar A, Pirola CJ, Wang H-M, Sharifi B, Mohan S, Forrester JS, Fagin JA. Expression and insulin-like growth factor-dependent proteolysis of insulin-like growth factor-binding protein-4 are regulated by cell confluence in vascular smooth muscle cells. Circ Res 1994;74:576–585.

[96] Anwar A, Ku L, Delafontaine P. Angiotensin II regulates expression of insulin-like growth factor binding proteins in vascular smooth muscle cells J Am Coll Cardiol 1994:294A.

[97] McCusker RH, Clemmons DR. Effects of cytokines on insulin-like growth factor-binding protein secretion by muscle cells in vitro. Endocrinology 1994;134:2095–2102.

[98] Busby WH, Klapper DG, Clemmons DR. Purification of a 31000 dalton insulin like growth factor binding protein from human amniotic fluid. J Biol Chem 1988;263:14203–14210.

[99] Elgin RG, Busby WH, Clemmons DR. An insulin-like growth factor binding protein enhances the biologic response to IGF-I. Proc Natl Acad Sci USA 1987;84:3254–3258.

[100] Jones JI, Gockerman A, Busby WH, Wright G, Clemmons DR. Insulin-like growth factor binding protein 1 stimulates cell migration and binds to the $\alpha 5\beta 1$ integrin by means of its Arg-Gly-Asp sequence. Proc Natl Acad Sci USA 1993;90:10553–10557.

[101] Jones JI, D'Ercole AJ, Camacho-Hubner C, Clemmons DR. Phosphorylation of insulin-like growth factor binding protein in cell culture and in vivo: effects on affinity for IGF-I. Proc Natl Acad Sci USA 1991;88:7481–7485.

[102] Bourner MJ, Busby WH, Seigel NR, Krivi GG, McCusker RH, Clemmons DR. Cloning and sequence determination of bovine insulin-like growth factor binding protein-2 (IGFBP-2): comparison of its structural and functional properties with IGFBP-1. J Cell Biochem 1992;48:215–226.

[103] DeMellow JSM Baxter RC. Growth hormone dependent insulin-like growth factor binding protein both inhibits and potentiates IGF-I stimulated DNA synthesis in skin fibroblasts. Biochem Biophys Res Commun 1988;156:199–204.

[104] Conover CA, Powell DR. Insulin-like growth factor (IGF) binding protein-3 blocks IGF-I-induced receptor downregulation and cell densitization in cultured bovine fibroblasts. Endocrinology 1991;129:710–716.

[105] Valentinis B, Bhala A, DeAngelis T, Baserga R, Cohen P. The human insulin-like growth factor (IGF) binding protein-3 inhibits the growth of fibroblasts with a targeted disruption of the IGF-I receptor gene. Mol Endocrinol 1995;9:361–367.

[106] Bar RS, Booth BA, Bowes M, Drake BL. Insulin-like growth factor binding proteins from cultured endothelial cells: purification, characterization, and intrinsic biologic activities. Endocrinology 1989;125:1910–1920.

[107] Yang YW-H, Pioli P, Fiorelli G, Brandi ML, Rechler MM. Cyclic adenosine monophosphate stimulates insulin-like growth factor binding protein-4 and its messenger ribonucleic acid in a clonal endothelial cell line. Endocrinology 1993;133:343–351.

[108] Hayzer D, Anwar A, Bernstein K, Delafontaine P. Insulin-like growth factor binding protein-3 synthesis by bovine aortic endothelial cells is induced by cell quiescence and contact inhibition. FASEB J 1995;9:A872.

[109] Perrone CE, Fenwick-Smith D, Vandenburgh HH. Collagen and stretch modulate autocrine secretion of insulin-like growth factor-I and insulin-like growth factor binding proteins from differentiated skeletal muscle cells. J Biol Chem 1995;270:2099–2106.

[110] Hanson H-A, Jennische E, Skottner A. IGF-I expression in blood vessels varies with vascular load. Acta Physiol Scand 1987;129:165–169.

[111] Isgaard J, Wåhlander H, Adams MA, Friberg P. Increased expression of growth hormone receptor mRNA and insulin-like growth factor-I mRNA in volume-overloaded hearts. Hypertension 1994;23:884–888.

[112] Toyozaki T, Hiroe M, Hasumi M, Horie T, Hosoda S, Tsushima T, Sekiguchi M. Insulin-like growth factor I receptors in human cardiac myocytes and their relation to myocardial hypertrophy. Jpn Circ J 1993;57:1120–1127.

[113] Chen Y, Bornfeldt KE, Arner A, Jennische E, Malmqvist U, Uvelius B, Arnqvist HJ. Increase in insulin-like growth factor I in hypertrophying smooth muscle. Am J Physiol 1994;266:E224–E229.

[114] Chen Y, Arner A, Bornfeldt KE, Uvelius B, Arnqvist HJ. Development of smooth muscle hypertrophy is closely associated with increased gene expression of insulin-like growth factor binding protein-2 and -4. Growth Regul 1995;5:45–52.

[115] Duerr RL, Huang S, Miraliakbar HR, Clark R, Chien KR, Ross J. Insulin-like growth factor-I enhances ventricular hypertrophy and function during the onset of experimental cardiac failure. J Clin Invest 1995;95:619–627.

[116] Sarzani R, Brecher P, Chobanian AV. Growth factor expression in aorta of normotensive and hypertensive rats. J Clin Invest 1989;83:1404–1408.

[117] Fath KA, Alexander RW, Delafontaine P. Hypertension modulates insulin-like growth factor I receptor mRNA levels. Circulation 1991;84:II–397.

[118] Diez J, Ruilope LM, Rodicio JL. Insulin response to oral glucose in essential hypertensives with increased circulating levels of insulin growth factor I. J Hypertens 1991;9:S174–S175.

[119] Laviades C, Mayor G, Diez J. Elevated circulating levels of insulin-like growth factor I in essential hypertensive patients with left ventricular hypertrophy. Arch Mal Coeur 1991;84:1039–1041.

[120] Díez J, Laviades C. Insulin-like growth factor-I and cardiac mass in essential hypertension: comparative effects of captopril, lisinopril and quinapril. J Hypertens 1994;12:S31–S36.

[121] Hansson H-A, Jennische E, Skottner A. Regenerating endothelial cells express insulin-like growth factor-I immunoreactivity after arterial injury. Cell Tissue Res 1987;250:499–505.

[122] Tiell ML, Stemerman MB, Spaet TH. The influence of the pituitary on arterial intimal proliferation in the rat. Circ Res 1978;42:644–649.

[123] Khorsandi M, Fagin JA, Fishbein MC, Forrester JS, Cercek B. Effects of hypophysectomy on vascular insulin-like growth factor-I gene expression after balloon denudation in rats. Atherosclerosis 1992;93:115–122.

[124] Kasuya H, Weir BKA, Shen YJ, Tredget EE, Ghahary A. Insulin-like growth factor-I in the arterial wall after exposure to periarterial blood. Neurosurgery 1994;35:99–105.

[125] Bornfeldt KE, Arnqvist HJ, Capron L. In vivo proliferation of rat vascular smooth muscle in relation to diabetes mellitus, insulin-like growth factor I and insulin. Diabetologia 1992;35:104–108.

[126] Nakao-Hayashi J, Ito H, Kanayasu T, Morita I, Murota S. Stimulatory effects of insulin and insulin-like growth factor I on migration and tube formation by vascular endothelial cells. Atherosclerosis 1992;92:141–149.

[127] Nicosia RF, Nicosia SV, Smith M. Vascular endothelial growth factor, platelet-derived growth factor, and insulin-like growth factor-I promote rat aortic angiogenesis in vitro. Am J Pathol 1994;145:1023–1029.

[128] Kluge A, Zimmermann R, Münkel B, Mohri M, Sack S, Schaper J, Schaper W. Insulin-like growth factor I is involved in inflammation linked angiogenic processes after microembolisation in porcine heart. Cardiovasc Res 1995;29:407–415.

[129] Arkins S, Rebeiz N, Brunke-Reese DL, Biragyn A, Kelley KW. Interferon-γ inhibits macrophage insulin-like growth factor-I synthesis at the transcriptional level. Mol Endocrinol 1995;9:350–360.

[130] Suh DY, Hunt TK, Spencer EM. Insulin-like growth factor-I reverses the impairment of wound healing induced by corticosteroids in rats. Endocrinology 1992;131:2399–2403.

[131] Jyung RW, Mustoe TA, Busby WH, Clemmons DR. Increased wound-breaking strength induced by insulin-like growth factor I in combination with insulin-like growth factor binding protein-1. Surgery 1994;115:233–239.

[132] Tsuboi R, Shi C-M, Sato C, Cox GN, Ogawa H. Co-administration of insulin-like growth factor (IGF)-I and IGF-binding protein-1 stimulates wound healing in animal models. J Invest Dermatol 1995;104:199–203.

[133] Bornfeldt KE, Skottner A, Arnqvist HJ. In-vivo regulation of messenger RNA encoding insulin-like growth factor-I (IGF-I) and its receptor by diabetes, insulin and IGF-I in rat muscle. J Endocrinol 1992;135:203–211.

[134] Capron L, Jarnet J, Kazandjian S, Housset E. Growth-promoting effects of diabetes and insulin on arteries. An in vivo study of rat aorta. Diabetes 1986;35:973–978.

[135] Haudenschild CC, Van Sickle W, Chobanian AV. Response of the aorta of the obese Zucker rat to injury. Arteriosclerosis 1981;1:186–191.

[136] Wang Q, Dills DG, Klein R, Klein BEK, Moss SE. Does insulin-like growth factor I predict incidence and progression of diabetic retinopathy? Diabetes 1995;44:161–164.

[137] Dills DG, Moss SE, Klein R, Klein BEK, Davis M. Is insulin-like growth factor I associated with diabetic retinopathy? Diabetes 1990;39:191–195.

[138] Merimee TJ, Zapf J, Froesch ER. Insulin-like growth factors: studies in diabetics with and without retinopathy. N Engl J Med 1983;309:527–530.

[139] Dills DG, Moss SE, Klein R, Klein BEK. Association of elevated IGF-I levels with increased retinopathy in late-onset diabetes. Diabetes 1991;40:1725–1730.

[140] Jabri N, Schalch DS, Schwartz SL, Fischer JS, Kipnes MS, Radnik BJ, Turman NJ, Marcsisin VS, Guler H-P. Adverse effects of recombinant human insulin-like growth factor I in obese insulin-resistant type II diabetic patients. Diabetes 1994;43:369–374.

[141] Kirstein M, Aston C, Hintz R, Vlassara H. Receptor-specific induction of insulin-like growth factor I in human monocytes by advanced glycosylation end product-modified proteins. J Clin Invest 1992;90:439–446.

[142] Schneider DJ, Sobel BE. Augmentation of synthesis of plasminogen activator inhibitor type I by insulin and insulin-like growth factor type I: implications for vascular disease in hyperinsulinemic states. Proc Natl Acad Sci USA 1991;88:9959–9963.

[143] Hirschberg R, Kopple JD. Effects of growth hormone and IGF I on renal function. Kidney Int 1989;36:S20–S26.

[144] Haylor J, Singh I, El Nahas AM. Nitric oxide synthesis inhibitor prevents vasodilation by insulin-like growth factor I. Kidney Int 1991;39:333–335.

[145] Ding H, Kopple JD, Cohen A, Hirschberg R. Recombinant human insulin-like growth factor-I accelerates recovery and reduces catabolism in rats with ischemic acute renal failure. J Clin Invest 1993;91:2281–2287.

[146] Miller SB, Martin DR, Kissane J, Hammerman MR. Insulin-like

growth factor I accelerates recovery from ischemic acute tubular necrosis in the rat. Proc Natl Acad Sci USA 1992;89:11876–11880.
[147] Copeland KC, Nair KS. Recombinant human insulin-like growth factor I increases forearm blood flow. J Clin Endocrinol Metab 1994;79:230–232.
[148] Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P. Nitric oxide release accounts for insulin's vascular effects in humans. J Clin Invest 1994;94:2511–2515.
[149] Schini VB, Catovsky S, Schray-Utz B, Busse R, Vanhoutte PM. Insulin-like growth factor I inhibits induction of nitric oxide synthase in vascular smooth muscle cells. Circ Res 1994;74:24–32.
[150] Grant MB, Wargovich TJ, Ellis EA, Caballero S, Mansour M, Pepine CJ. Localization of insulin-like growth factor I and inhibition of coronary smooth muscle cell growth by somatostatin analogues in human coronary smooth muscle cells: a potential treatment for restenosis. Circulation 1994;89:1511–1517.
[151] Polanco JI, Berciano MT, Lafarga M, León J, Pocoví M, Rodríguez-Rey JC. Expression of insulin-like growth factor receptor mRNA in rabbit atherosclerotic lesions. Biochem Biophys Res Commun 1995;209:182–190.
[152] Asotra S, Foegh M, Conte JV, Cai BR, Ramwell PW. Inhibition of ^{3}H-thymidine incorporation by angiopeptin in the aorta of rabbits after balloon angioplasty. Transplant Proc 1989;21:3695–3696.
[153] Conte JV, Foegh ML, Calcagno D, Wallace RB, Ramwell PW. Peptide inhibition of myointimal proliferation following angioplasty in rabbits. Transplant Proc 1989;21:3686–3688.
[154] Lundergan C, Foegh ML, Vargas R, Eufemio M, Bormes GW, Kot PA, Ramwell PW. Inhibition of myointimal proliferation of the rat carotid artery by the peptides, angiopeptin and BIM 23034. Atherosclerosis 1989;80:49–55.
[155] Foegh ML. Angiopeptin: a treatment for accelerated myointimal hyperplasia? J Heart Lung Transplant 1992;11:S28–S31.
[156] Howell M, Ørskov H, Frystyk J, Flyvbjerg A, Gronbæk H Foegh ML. Lanreotide, a somatostatin analog, reduces IGF I accumulation in proliferating aortic tissue in rabbits in vivo: a preliminary study. Eur J Endocrinol 1994;130:422–425.
[157] Emanuelsson H, Beatt KJ, Bagger J-P, Balcon R, Heikkilä J, Piessens J, Schaeffer M, Suryapranata H, Foegh M. Long-term effects of angiopeptin treatment in coronary angioplasty: reduction of clinical events but not angiographic restenosis. Circulation 1995;91:1689–1696.
[158] Liu J-P, Baker J, Perkins AS, Robertson EJ, Efstratiadis A. Mice carrying null mutations of the genes encoding insulin-like growth factor I (IGF-I) and type 1 IGF receptor (IGF-IR). Cell 1993;75:59–72.
[159] Baker J, Liu J-P, Robertson EJ, Efstratiadis A. Role of insulin-like growth factors in embryonic and postnatal growth. Cell 1993;75:73–82.
[160] Tsukahara H, Gordienko D, Tonshoff B, Gelator M, Goligorsky M. Direct demonstration of insulin-like growth factor-I-induced nitric oxide production by endothelial cells. Kidney Int 1994;45:598–604.
[161] Conway LW, MacDonald WM. Structural changes of the intradural arteries following subarachnoid hemorrhage. J Neurosurg 1972;37:715–723.
[162] Yoshinouchi M, Miura M, Gaozza E, Li SW, Baserga R. Basic fibroblast growth factor stimulates DNA synthesis in cells overexpressing the insulin-like growth factor I receptor. Mol Endocrinol 1993;7:1161–1168.