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# Biology and significance of signalling pathways activated by IGF-II

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**Abstract**
Insulin-like growth factor-II (IGF-II) affects many aspects of cellular function through its ability to activate several different receptors and, consequently, numerous intracellular signalling molecules. Thus, IGF-II is a key regulator of normal foetal development and growth. However, abnormalities in IGF-II function are associated with cardiovascular disease and cancer. Here, we review the cellular mechanisms by which IGF-II's physiological and pathophysiological actions are exerted by discussing the involvement of the type 1 and type 2 IGF receptors (IGF1R and IGF2R), the insulin receptor and the downstream MAP kinase, PI-3 kinase and G-protein-coupled signalling pathways in mediating IGF-II stimulated cellular proliferation, survival, differentiation and migration.

**Keywords:** *Foetal growth, placenta, development, myogenesis, vasculogenesis, cancer*

## Introduction

Insulin-like growth factor-II (IGF-II) is a 67-amino-acid protein produced by post-translational removal of the COOH-terminal E domain from the precursor molecule, pro-IGF-II (Duguay et al. 1998). Partial cleavage of the E domain results in big IGF-II (two isoforms; 1-104 or 1-87) which, along with pro-IGF-II, are also found in the circulation (Marks et al. 2011). Mature IGF-II can itself be processed to generate des(37-40) IGF-II (also known as vesiculin; (Buchanan et al. (2010)). Little is known about the signalling properties and function of these IGF-II variants, so this review will focus on the actions of the mature protein.

IGF-II can interact with a number of cell-surface receptors (Figure 1) but it binds to the type 1 IGF receptor (IGF1R) with highest affinity and therefore it is likely that this receptor mediates much of IGF-II's effect on cellular proliferation, survival, differentiation and migration. However, IGF-I also binds to IGF1R and in general, elicits the same effects with greater potency, which has led to some speculation about the specific purpose of IGF-II. In recent years, this has been clarified through the use of receptor inhibitors and a better understanding of the pathways downstream of the type 2 IGF/mannose-6-phosphate receptor (IGF2R) and also, the A isoform of the insulin receptor (IR-A; Figure 1), both of which bind IGF-II with greater affinity than IGF-I. Here, we discuss aspects of physiology and pathophysiology that have been attributed to IGF-II.

### *Type 1 IGF receptor*

IGF1R is a heterotetramer with structural homology to the insulin receptor, thus in tissues that express both, many of the IGF binding sites are formed as hybrids of the two receptor types (Kasuya et al. 1993), though their affinity for IGF-II is similar to that of IGF1R (Kasuya et al. 1993). Activation of IGF1R results in autophosphorylation of tyrosine residues in the intracellular β-subunits and then, in general, initiation of the PI-3 kinase/AKT or MAP kinase

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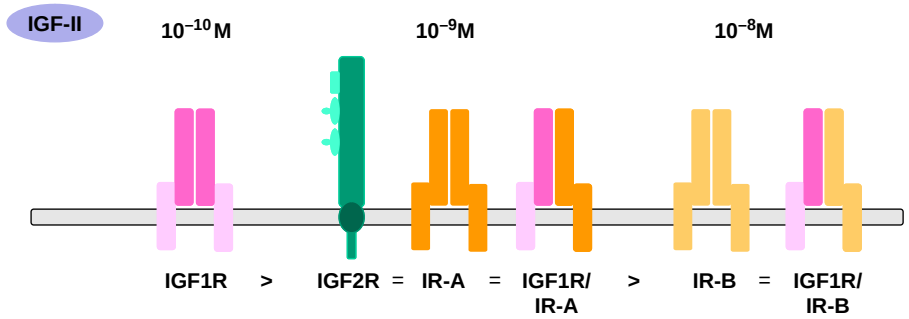


Figure 1. Affinity of IGF-II for its various receptors. IGF1R, type 1 IGF receptor; IGF2R, type 2/mannose-6-phosphate receptor; IR, insulin receptor. The IGF1R can form hybrids with either IR-A or IR-B.



signalling cascades (reviewed in Riedemann and Macaulay (2006), Laviola et al. (2007) and Tao et al. (2007)).

### *Insulin receptor*

The insulin receptor exists as two isoforms, IR-A and IR-B, depending on the absence or presence of exon 11 splicing (Belfiore et al. 2009). IR-A, but not IR-B, has a high affinity for IGF-II and in fact binds IGF-II just as well as IGF1R. IR-A is also linked to the same downstream signalling molecules as the classical IGF receptor, but IGF-II/IR-A interactions are reported to preferentially activate the MAP kinase pathway (Belfiore et al. 2009).

### *Type 2 IGF receptor*

IGF2R is structurally unrelated to either IGF1R or IR as it consists of just a single, primarily extracytoplasmic, polypeptide chain. This receptor binds IGF-II with greater affinity than IGF-I and whilst it does not accept insulin as a ligand (El-Shewy and Luttrell 2009), it does have high affinity for the sugar mannose-6-phosphate (M-6-P), and can therefore bind lysosomal enzymes and other growth factors and cytokines. Cloning of the type 2 IGF receptor cDNA (Morgan et al. 1987) led to the realisation that this receptor was also the cation-independent receptor for M-6-P; given the well-documented role of this receptor in the intracellular transport of lysosomal enzymes, it was suggested that rather than mediating IGF-II effects, it might be important for clearing IGF-II from the circulation. However, although IGF2R contains neither tyrosine kinase activity nor an autophosphorylation site, it does link to G-proteins which provides a mechanism for signal transduction (El-Shewy and Luttrell 2009).

IGF-II access to all of these receptors is controlled by a family of six highly specific binding proteins (IGFBPs 1–6), though their role in modulating IGF-II bioavailability and function is reviewed elsewhere (Bach et al. 2005; Duan and Xu 2005; Silha and Murphy 2005; Holly and Perks 2006; Forbes and Westwood 2008).

## Role of IGF-II and its receptors in foetal growth

It is well established that the IGF axis is essential for foetal development and growth. In humans, evidence for the importance of IGF-II comes from the observation that *Igf2* is maternally imprinted (Giannoukakis et al. 1993). Relaxation of imprinting leads to Beckwith–Wiedemann syndrome (BWS) in which excess IGF-II is associated with foetal overgrowth (Ward 1997). The genes for IGF1R, IGF2R and IR are not imprinted although all three receptors are key for normal prenatal growth (Taylor 1992; Abuzzahab et al. 2003; Kaku et al. 2007). However, most of the growth disorders associated with perturbations in the IGF axis are not due to gene defects and many studies have demonstrated that the correlation between foetal growth restriction (FGR) and decreased IGF-II levels (Bennett et al. 1983; Giudice et al. 1995; Leger et al. 1996; Bajoria et al. 2001; Westwood et al. 2001) commonly occurs in the absence of any apparent mutation. Interestingly, not all studies report a relationship between serum IGF-II concentrations and foetal weight (Osorio et al. 1996; Klauwer et al. 1997). *Igf2* and *Igf2R* are polymorphic and variants in both are associated with birth weight (Kaku et al. 2007; Adkins et al. 2010), which may account for these conflicting findings. Another possibility is the variation in the level of soluble IGF2R, which is formed by proteolytic cleavage in the transmembrane region of the expressed receptor (Nissley et al. 1993) and is known to inhibit IGF-II's actions (Scott et al. 1996; Scott and Weiss 2000). This component of the IGF axis is rarely analysed in relation to foetal growth; however, one study has shown IGF-II levels are associated with birth weight only when considered as a molar ratio to soluble IGF2R and that depending on parity, the IGF-II/IGF2R ratio accounted for up to 5% in birth weight variance (Ong et al. 2000).

In mice, the gene for IGF-II and that for IGF2R, which is thought to regulate the availability of IGF-II,

are reciprocally imprinted (DeChiara et al. 1991; Stoger et al. 1993), presenting an attractive mechanism for balancing the needs of mother and foetus during pregnancy (Haig and Graham 1991). Like humans, perturbations in ligand and receptor expression are associated with altered foetal growth in the mouse. Ablation of the IGF-II gene results in severe *in utero* growth restriction and neonatal mice which are 40% smaller than their wild-type littermates (DeChiara et al. 1990). In postnatal life, *Igf2* null animals are always remarkably smaller than their normal littermates; however, their growth velocity is unaffected, which has led to the suggestion that IGF-II's role as a growth regulator is principally relevant to foetal life. Elimination of the type 1 IGF receptor accentuates the growth-restricted phenotype (45% of normal birth weight) and results in perinatal lethality (Liu et al. 1993), whereas mice in which the *Igf2r* has been ablated have elevated levels of circulating IGF-II and are 25–30% larger than wild-type littermates (Lau et al. 1994; Ludwig et al. 1996). Unlike in humans, a null mutation in the insulin receptor gene has no effect on foetal growth (Joshi et al. 1996).

## IGF-II affects foetal growth by influencing placental development and function

IGF-II null mice and, importantly, mice lacking only the placental-specific transcript of IGF-II (Constancia et al. 2002) have small placentas (Baker et al. 1993). In contrast, animals carrying a null mutation in *Igf2r*, like the foetuses with BWS, exhibit placentomegaly and, unusually (McLaren 1965), these placentas continue to grow right uptil birth (Lau et al. 1994). Together, these studies suggest that IGF-II may influence foetal growth by promoting normal placental development and function.

In human placenta, IGF-II is expressed by the chorionic villi (cytotrophoblasts, mesodermal core and vascular endothelium) and foetal membranes (amnion and chorion laeve) from early pregnancy (Han et al. 1996), though the IGF-II present in the maternal circulation also has a role in regulating events within the placenta (Forbes and Westwood 2010). IGF1R is apparent in trophoblast and villous endothelium and stroma (Holmes et al. 1999). This finding led to the hypothesis that a reduction in the number or distribution of placental type 1 IGF receptors might be a contributing factor in pregnancies complicated by FGR. This is supported by data from a Western blot analysis of such placentas (Laviola et al. 2005); however, a study using immunohistochemistry was unable to discern any differences in receptor localisation or density (Holmes et al. 1999), and analysis by quantitative PCR detected an increase in expression (bu-Amero et al. 1998). Aberrations in the signalling molecules downstream of IGF1R (Figure 2A) could also influence placental, and consequently, foetal growth (Forbes and Westwood 2010) and decreased expression and/or activation of Akt and members of the MAP kinase pathway in placentas from FGR pregnancies have been described (Laviola et al. 2005; Street et al. 2011).

Mice containing null mutations in both the IGF-II and IGF1R genes were more severely growth restricted than those in which only the receptor had been ablated (Baker et al. 1993). This together with the fact that placental weight is reduced in IGF-II-deficient mice suggests that not all of IGF-II's effects are mediated through IGF1R; in the human placenta, candidates include IGF2R and the insulin receptor, both of which are expressed by trophoblast (Desoye et al. 1994; Rebourcet et al. 1998), though the distribution of the IR isoforms within placenta has not been documented.

## IGF-II influences placental development and function by a number of mechanisms

### *Trophoblast turnover*

The outer syncytiotrophoblast layer of the human placenta, which is bathed in maternal blood and is therefore crucial as an immune barrier and transporting epithelium, is a terminally differentiated cell that must be renewed and expanded by differentiation and fusion of cells from an underlying cytotrophoblast progenitor layer. Apoptotic elements are continuously shed into the maternal circulation. A role for IGF-II in regulating cytotrophoblast proliferation was implied by an immunohistochemical analysis of first trimester placenta which demonstrated a correlation between IGF-II expression and proliferative activity (Thomsen et al. 1997), though we have provided direct evidence using an explant model of first trimester (Forbes et al. 2008); and term (Harris et al. 2011) human placenta in which IGF-II is supplied to the syncytiotrophoblast surface, mimicking exposure to hormone in the maternal circulation. In these experiments, IGF-II stimulated the proliferation of cells in the underlying cytotrophoblast layer, which suggests the presence of pathways capable of transducing signals from the syncytiotrophoblast to the cytotrophoblast; conceivably this might be achieved either by a syncytioplasmic kinase relay activated by ligand binding at the maternal-facing microvillous membrane, or by transcytosis of ligand with exocytosis at the basal syncytial surface and rebinding to receptor on cytotrophoblast. Other data supporting a role for the maternal IGF axis come from a study on food-restricted guinea pigs in which maternal IGF-II levels were related to placental structural development (Roberts et al. 2001; Sferruzzi-Perri et al. 2006; Sferruzzi-Perri et al. 2007a, 2008; Pringle and Roberts 2007).

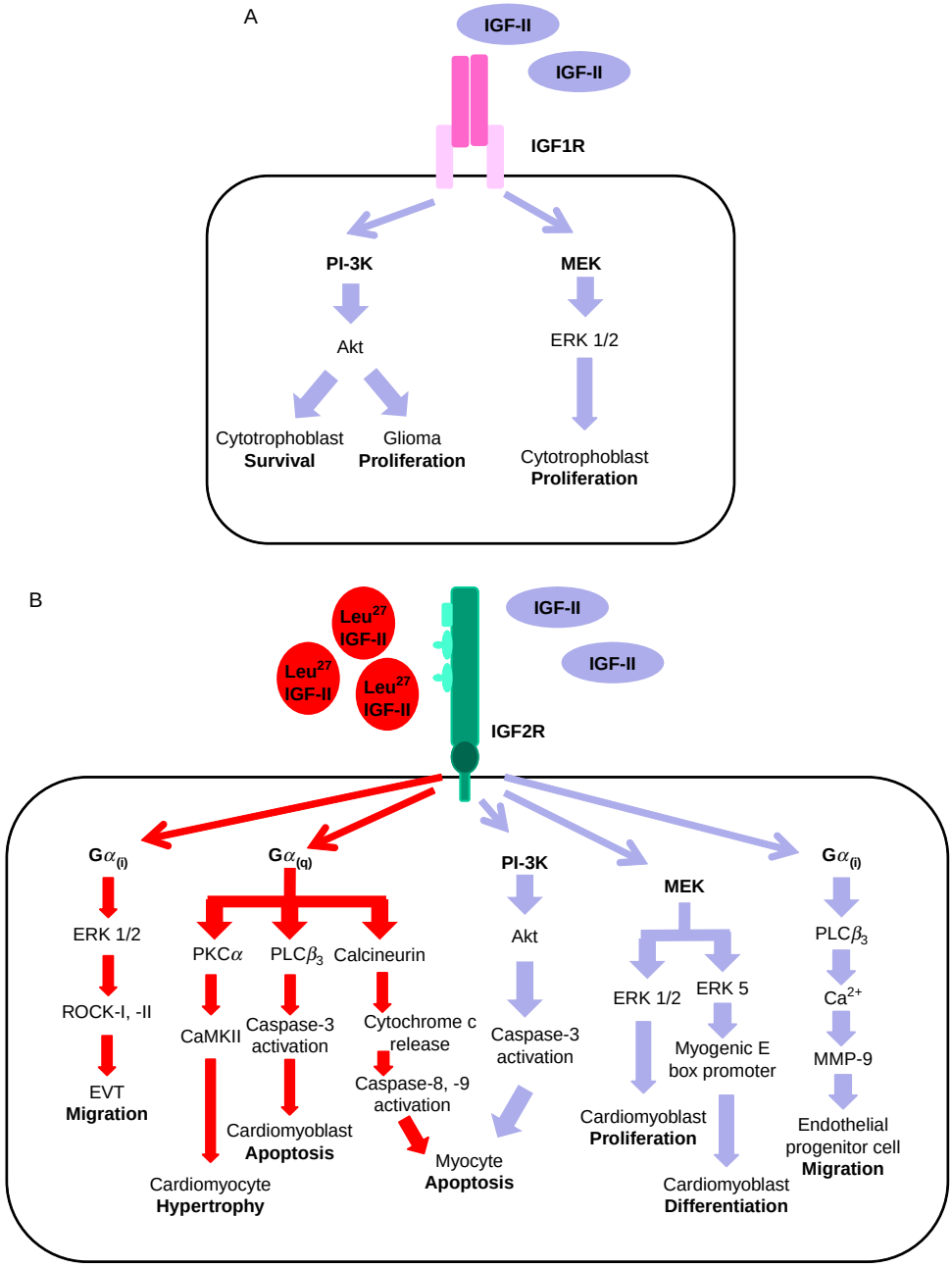


Figure 2. Signalling pathways activated following IGF-II binding to the type 1 or type 2 IGF receptor (IGF1R and IGF2R; Panel A and B, respectively). PKC, protein kinase C; PLC, phospholipase C; CaMKII, calcium/calmodulin-dependent protein kinase II; MMP, matrix metalloproteinase. Leu$^{27}$IGF-II, IGF-II analogue that binds primarily to IGF2R; pathways identified through the use of this analogue are shown in red.

The majority of IGF-II's mitogenic actions are thought to be mediated through IGF1R (Figure 2A) and this is certainly supported by the studies on mice with a null mutation in this gene, as these animals have a much more severe phenotype than those lacking either of the ligands. We found that IGF-II-stimulated cytotrophoblast proliferation was reduced in the presence of a specific IGF1R inhibitor (Forbes et al. 2008); however, IGF2R may also have a role in mediating IGF-II's mitogenic effects as proliferation was enhanced in term placental explants exposed to an IGF-II analogue, Leu$^{27}$IGF-II (Harris et al. 2011). Leu$^{27}$IGF-II primarily binds to IGF2R (Forbes et al. 2002) and is commonly used to distinguish between cellular signalling and function initiated by IGF-II/IGF1R versus IGF-II/IGF2R interactions. We have shown, again by using pharmacological inhibitors, that the MAP kinase pathway is responsible for mediating the proliferative effects of IGF-II (Forbes et al. 2008), thus it is interesting to note that through activation of sphingosine kinase and the production of sphingosine-1-phosphate, the ligand for G-protein-coupled S1P receptors, IGF2R can also link into this signalling cascade (El-Shewy et al. 2007).

Flux through the MAP kinase, and other signalling pathways, is regulated by protein tyrosine phosphatases. Although expressed by placenta (Norris et al. 1997), relatively little is known about their importance in this tissue, though we have recently found that one of the enzymes, SHP-2, is required for IGF-II stimulation of cytotrophoblast proliferation (Forbes et al. 2009).

IGF-II is known to provide a survival signal in many cell systems, and recent work suggests that at the maternal–foetal interface also, it may play a role in this context since it can protect both first trimester (Forbes et al. 2008) and term (Harris et al. 2011) cytotrophoblast from apoptosis. IGF1R is clearly involved in mediating this effect, though downstream, the PI-3 kinase/Akt rather than the MAP kinase pathway seems to be key (Figure 2A) (Forbes et al. 2008). Again, the contribution of IGF2R must be considered; $Leu^{27}$IGF-II promoted cytotrophoblast survival; however, our data indicate that IGF2R also functions as a clearance receptor, since in tissue with reduced IGF2R, IGF-II activity was enhanced thereby suggesting that IGF-II signalling can be redirected through IGF1R (Harris et al. 2011).

### *Trophoblast migration*

Successful implantation and placental development depends on adequate extravillous trophoblast invasion (EVT) of the maternal endometrium and there are several lines of evidence to implicate IGF-II as a mediator of this process. mRNA localisation studies have demonstrated abundant IGF-II expression in the trophoblastic columns of the anchoring villi, particularly in those cells at the leading edge of the column (Han et al. 1996). Moreover, *in vitro* studies have shown that in monolayer wounding (Irving and Lala 1995) or trans-Matrigel barrier assays (Hamilton et al. 1998), the migration of these cells is increased in response to IGF-II. Several reports suggest that IGF-II's ability to promote migration is dependent on IGF2R (Minniti et al. 1992; Volpert et al. 1996), and in trophoblast also, $Leu^{27}$IGF-II and QAYL-$Leu^{27}$IGF-II, another analogue that is selective for IGF2R, enhanced migration whereas function-blocking IGF2R antibodies were inhibitory (McKinnon et al. 2001). The authors also report that IGF-II signalling through this receptor involves $G_i$ proteins and activation of the MAP kinase pathway (McKinnon et al. 2001) as well as the Rho kinases, ROCK-I and -II (Figure 2B) (Shields et al. 2007). Members of the Rho GTPase family (RhoA and RhoC) are also required for IGF-II stimulation of EVT through IGF1R (Shields et al. 2007). There is some controversy about the role of IR-A in mediating IGF-II-directed EVT migration, as Shields and colleagues suggest from their work using an IR tyrosine kinase inhibitor that this receptor is not involved (Shields et al. 2007), whereas a study using a choriocarcinoma cell model of EVT found that the actions of IGF-II were reduced in the presence of a different IR inhibitor (Diaz et al. 2007).

### *Nutrient transport*

IGF-II is also a potent metabolic factor and could therefore modulate foetal growth by influencing nutrient transfer across the placenta. IGF-II is known to stimulate both glucose and amino acid uptake by cultured human trophoblast (Kniss et al. 1994; Karl 1995; Yu et al. 1998; Fang et al. 2006), and in the guinea pig, maternal administration of IGF–II has been shown to increase placental transport of nutrients to the foetus resulting in enhanced foetal growth (Sferruzzi-Perri et al. 2006, 2007a,b). Similar effects were observed when animals were treated with $Leu^{27}$IGF-II, suggesting that maternal IGF-II promotes, at least in part, nutrient delivery to the foetus via IGF2R (Sferruzzi-Perri et al. 2008). Indeed, rather than a direct effect of IGF-II, enhanced nutrient transfer across an enlarged placenta has been proposed as an explanation for the increased embryo weights noted in the mice null for IGF2R (Lau et al. 1994). Correspondingly, deletion of placental IGF-II leads to a reduction in the surface area of the nutrient exchange barrier (Constancia et al. 2002; Sibley et al. 2004), decreased amino acid transfer (Sferruzzi-Perri et al. 2011) and, consequently, FGR.

## Role of IGF-II in early cardiac development, myogenesis and vasculogenesis

IGF-II gene expression has been reported as early as embryonic day 5.5 (E5.5) in the pre-implantation mouse blastocyst, where it is localised to the extraembryonic ectoderm and the ectoplacental cone, but not the epiblast (Lee et al. 1990). At E6.5, *Igf2* transcripts are also expressed in the columnar visceral endoderm, extraembryonic mesoderm and in trophoblast giant cells. At E7.5, expression is observed in all extraembryonic structures, including the allantois, the amnion, the chorion and the visceral yolk sac. At this time, *Igf2* expression is noted in a restricted region of embryonic mesoderm which at E8.0 extends to include the developing heart, the lateral mesoderm, the head mesenchyme and the lining of the foregut. The functional importance of IGF-II signalling during cardiac development was highlighted when the differentiation potential of murine embryonic stem cells expressing reduced levels of IGF-II was investigated: the absence of *Igf2* severely impaired the expression of mesoderm markers, and the subsequent formation of mesoderm derivatives including cardiomyocytes and muscle fibres (Morali et al. 2000). In addition, IGF-II synthesised by the epicardium is required to activate MAP kinase signalling pathway and induce

cardiomyocyte proliferation in the developing mouse heart from E10.5-E14.5. *Igf2* null mice exhibit significantly decreased rates of cardiomyocyte proliferation in the ventricular wall at E11.5, resulting in ventricular wall hypoplasia (Li et al. 2011). IGF-II signalling also regulates differentiation of adult myoblasts, inducing exit from the cell cycle, expression of muscle-specific genes and formation of multinucleated myotubes. Initiation of myogenesis is achieved by an IGF-II-mediated increase in ERK5 phosphorylation and kinase activity, translocation of ERK5 to the nucleus and myogenic E box promoter activity (Figure 2B) (Carter et al. 2009).

The IGF2R signalling axis also regulates post-natal vasculogenesis by controlling homing of endothelial progenitor cells (EPC). EPC isolated from human placental cord blood express high levels IGF2R, and IGF-II signalling through IGF2R, but not IGF1R, increased EPC migration, invasion, adhesion to fibrinogen and MMP-9 secretion *in vitro (*Maeng et al. *2009)*. Signalling was mediated via the G-protein subunit G(i) and phospholipase-C$\beta_2$ (PLC$\beta_2$), leading to an increase in intracellular $Ca^{2+}$ (Figure 2B) (Maeng et al. 2009). IGF-II promoted recruitment of murine bone marrow mononuclear cells (MBMMC) and neo-vascularisation in a mouse Matrigel plug assay, and increased the number of MBMMC incorporated into the capillaries in a mouse model of hindlimb ischaemia (Maeng et al. 2009).

## IGF-II signalling in cardiovascular development and disease

Cardiomyocyte apoptosis is one of the primary causes of cardiovascular pathology following myocardial infarction. The adult cardiomyocyte cannot proliferate, thus the signalling pathways that regulate cell survival have been extensively studied. IGF-I signalling through IGF1R promotes physiological cardiac growth and function, and improves cardiac output after myocardial infarction by stimulating contractility and tissue remodelling (Ren et al. 1999). In contrast, evidence from cell culture studies and animal models is mixed, suggesting that IGF-II signalling can induce hypertrophy, extracellular matrix remodelling and apoptosis, but also that IGF-II overexpression can enhance cardiomyocyte survival.

Treatment of the cardiomyoblast cell line H9c2 with angiotensin-II upregulated IGF-II and IGF2R expression, induced activation of caspase-8 and -9 and increased cardiomyoblast apoptosis via an IGF2R-dependent mechanism (Lee et al. 2006). Similarly, Leu$^{27}$IGF-II has been shown to enhance angiotensin-II-induced H9c2 cell apoptosis, mediated by the interaction of IGF2R with the G-protein subunit G$\alpha$q and phosphorylation of PLC$\beta_3$, leading to increased caspase activation and DNA fragmentation (Figure 2B) (Chen et al. 2009). These findings are mirrored in neonatal rat ventricular myocytes following knock-down of IGF1R expression, where IGF-II treatment induced phosphorylation of Akt, increased caspase-3 activation and induced apoptosis (Chu et al. 2009). Interestingly, Leu$^{27}$IGF-II treatment also increased apoptosis in cells lacking IGF1R, but did so in the absence of Akt phosphorylation. Instead, activation of G$\alpha$q and calcineurin leads to translocation of the pro-apoptotic protein Bad to the mitochondria, cytochrome c release and activation of caspase-3 and caspase-9 (Figure 2B) (Chu et al. 2009).

Signalling through IGF2R induces H9c2 cell hypertrophy, via its interaction with G$\alpha$q, and phosphorylation of protein kinase C-$\alpha$ and calcium/calmodulin-dependent protein kinase II, leading to increased expression of the cardiac hypertrophy markers atrial natriuretic peptide and brain natriuretic peptide (Figure 2B) (Chu et al. 2008). IGF-II signalling through IGF2R also promoted extracellular matrix catabolism in these cells, by increasing the expression of matrix metalloproteinase-9, urokinase plasminogen activator and tissue plasminogen activator, and by reducing expression of tissue inhibitor of metalloproteinases-2 (Chang et al. 2008).

*In vivo*, rats subjected to ligation of the abdominal aorta exhibit increased expression of IGF-II and IGF2R in the left ventricle, and display hypertension and enhanced myocyte apoptosis (Lee et al. 2006). As expression of IGF2R is increased in areas of infarcted human myocardium (Chu et al. 2008), and elevated expression is maintained in the resulting scar tissue (Chang et al. 2008), signalling through the IGF2R may enhance pathological myocardial apoptosis and hypertrophy, exacerbating the existing damage.

In contrast to the studies cited above, transduction of cardiomyocytes with an adenoviral vector encoding IGF-II significantly reduced apoptosis induced by heat shock or ischaemia-reoxygenation (Su et al. 2003). Moreover, downregulation of the IGF-II clearance receptor IGF2R in neonatal rat cardiac myocytes reduced cell susceptibility to hypoxia- and tumour necrosis factor-induced apoptosis (Chen et al. 2004). These findings suggest that IGF-II signalling through IGF1R can enhance cardiomyocyte survival.

## IGF-II signalling in cancer

Aberrant autocrine and paracrine IGF-II signalling, leading to the enhancement of cell proliferation and resistance to apoptosis, has long been implicated in the initiation and progression of tumour growth (Toretsky and Helman 1996). Epigenetic alterations, such as the loss of DNA imprinting, occur in cancer at least as commonly as genetic mutations. The majority of imprinted genes exist in clusters, and their expression is regulated by the methylation status of CpG-rich *cis*-elements, known as differently

methylated regions (DMRs) (Mann et al. 2000). The DMRs are differentially methylated on CpG sites by DNA methyltransferases, depending on the parental origin of the allele (Mann et al. 2000). *Igf2* is an example of an imprinted gene; the loss of imprinting (LOI) of the normally silent maternal allele of *Igf2* leads to overexpression of IGF-II protein and an increased risk of malignancy.

### *Colon cancer*

Biallelic expression of *Igf2* in a mouse model of intestinal neoplasia induced intestinal adenoma formation, elongation of intestinal crypts and an increased population of epithelial progenitor cells in the mucosa (Sakatani et al. 2005). This increase in mucosal epithelial progenitor cells is also observed in the normal gut mucosa of humans presenting with colon-specific LOI of *Igf2* (Sakatani et al. 2005), leading to an elevated risk of colorectal cancer for affected individuals (Kaneda and Feinberg 2005). Similar findings were observed when azoxymethane was used to induce the formation of pre-malignant aberrant crypt foci in mice with *Igf2* LOI: expression of proliferation-related genes in the intestinal crypts was increased, leading to enhanced tumour formation (Kaneda et al. 2007). Blockade of the IGF1R signalling pathway using the competitive inhibitor NVP-AEW541 decreased expression of proliferation-related genes and significantly reduced pre-malignant aberrant crypt foci formation (Kaneda et al. 2007). Using mouse embryo fibroblast cell lines from *Igf2* LOI and wild-type embryos, the authors also demonstrated that LOI cells showed an enhanced sensitivity to IGF-II signalling. Low doses of IGF-II induced sustained Akt activation in LOI cells, whereas cells from wild-type embryos exhibited only a transient increase in Akt activation (Kaneda et al. 2007). IGF2R and insulin receptor expression were also increased in these cells.

### *Breast cancer*

Female transgenic mice engineered to exhibit enhanced IGF-II expression in the mammary gland displayed an increased incidence of aggressive, metastatic, mammary tumours (Pravtcheva and Wise 1998), implicating chronic IGF-II signalling as a tumorigenic stimulus. As predicted, when these animals were crossed with transgenic mice overexpressing IGF2R, their offspring exhibited a significant delay in the onset of mammary tumour formation and reduced tumour burden (Wise and Pravtcheva 2006). Biallelic IGF-II expression has been observed in human breast cancer samples: one study reported LOI in 67% of benign lesions and 60% of malignant lesions, whereas all control samples displayed normal IGF-II imprinting (McCann et al. 1996). However, only three benign and five malignant tissue samples were analysed, so these data must be interpreted with caution. ProIGF-II has been shown to promote the survival of the MCF7 breast cancer cell line by activating PI3K/Akt signalling and upregulating the expression of the anti-apoptotic proteins Bcl-2 and Bcl-$X_L$ (Singh et al. 2008). Mature IGF-II can also promote breast cancer progression by activating oestrogen receptor-α (ER-α) and ER-β in the absence of oestrogen. In breast cancer cells, IGF-II binding to IGF1R and the insulin receptor induced translocation of ER-α and ER-β to the mitochondria, facilitating activation of cell survival pathways (Richardson et al. 2011).

### *Pancreatic cancer*

Transgenic mice expressing the SV40 large T-antigen (Tag) under the control of the insulin gene regulatory region develop hyperplasia within the islets of Langerhans, followed by the occurrence of pancreatic tumours. Increased IGF-II expression, due to LOI of the *Igf2* gene, is observed in this population of hyperproliferative β cells (Christofori et al. 1994). When crossed with *Igf2* null mice, Tag mice displayed a dramatically reduced tumour burden and had a fivefold higher incidence of tumour cell apoptosis (Christofori et al. 1994), again highlighting both the proliferative and pro-survival effects of IGF-II signalling. Interestingly, Tag mice that carried a disruption in either the paternal or maternal *Igf2* allele developed tumours of a similar size and histology to wild-type Tag mice, indicating that both the developmentally expressed paternal allele or the inactive maternal allele could contribute to tumour development (Christofori et al. 1995). In humans, evidence of a role for IGF-II in pancreatic cancer is mixed: a nested case–control study has shown no correlation between increased serum concentration of IGF-II and increased risk of pancreatic cancer (Douglas et al. 2010), and no change in IGF-II mRNA expression was observed in human pancreatic cancer samples, despite biallelic *Igf2* expression (Micha et al. 1999). However, a recent study has reported hypermethylation of the *Igf2* DMR2 in insulinomas, which was associated with LOI and overexpression of IGF-II at the mRNA and protein level (Dejeux et al. 2009).

### *Lung cancer*

Immortalised mouse embryonic fibroblasts from wild type, and but not *integrin* $\alpha_{11}$ null mice, significantly enhanced the growth of A549 human lung adenocarcinoma cells, when co-implanted into immune-deficient mice (Zhu et al. 2007). Gene profiling of the resulting tumours revealed a 100-fold reduction in IGF-II mRNA expression in tumours formed in mice injected with the integrin α11 null fibroblasts

(Zhu et al. 2007). siRNA-mediated knockdown of fibroblast IGF-II expression reduced the growth of A549 tumours to a similar extent, suggesting that in this system, the growth promoting effects of stromal fibroblasts were (i) mediated by the paracrine actions of IGF-II and (ii) dependent on fibroblast integrin α11 expression (Zhu et al. 2007).

*Brain tumours*

IGF-II is overexpressed in a subset of high-grade glioblastomas that lack amplification or overexpression of the EGF receptor, and are characterised by poor survival (Soroceanu et al. 2007). Tumours overexpressing IGF-II were highly proliferative, exhibited enhanced Akt phosphorylation and displayed PTEN loss. IGF-II signalling through IGF1R and PI3-kinase regulatory subunit 3 recapitulated the tumorigenic effects of EGF and promoted the growth of glioblastoma-derived neurospheres *in vitro* (Figure 2B) (Soroceanu et al. 2007).

*Other cancers*

As mentioned above, individuals with BWS exhibit biallelic *Igf2* expression, along with aberrant expression of p57, CDKN1C, H19 and LIT1, and have an increased risk of developing childhood cancers (Ward 1997). Approximately 5–10% of BWS patients develop embryonal tumours, including Wilms' tumour of the kidney, but they are also at increased risk of adrenocortical carcinoma, hepatoblastoma and rhabdomyosarcoma (Ward 1997). Wilms' tumour is associated with defects in the *Wt1* gene, which encodes a transcriptional repressor of *Igf2*, and with mutations in the 11p15.5 region which alter *Igf2* imprinting. As such, biallelic *Igf2* expression is observed in the majority of pathological cases (Ravenel et al. 2001). Biallelic IGF-II expression has also been reported in human cases of testicular germ cell tumours (van Gurp et al. 1994), choriocarcinoma (Hashimoto et al. 1995), primary lung cancers including adenocarcinoma, squamous cell carcinoma, large and small cell carcinoma (Suzuki et al. 1994), and cervical carcinomas (Douc-Rasy et al. 1996), uterine leiomyosarcoma (Vu et al. 1995) and endometrial cancer (Roy et al. 2000).

## IGF2R signalling in cancer

The tumour suppressor function of the IGF2R was first demonstrated by O'Gorman et al. who showed that down-regulation of IGF2R expression in JEG-3 choriocarcinoma cells enhanced proliferation *in vitro,* and increased tumour growth rate *in vivo (*O'Gorman et al. *1999)*. Conversely, IGF2R overexpression reduced JEG-3 cell proliferation *in vitro,* and decreased tumour growth in nude mice (O'Gorman et al. 2002). IGF2R overexpression did not alter endogenous IGF-II production, or secretion of the IGF2R ligands procathepsin D and L, but did promote secretion and activation of latent TGF-β1. Overexpression of a soluble form of the receptor dramatically reduced tumour cell growth *in vitro* and *in vivo,* but did not alter the level of TGF-β1 (O'Gorman et al. 2002). These data suggest that increased levels of soluble IGF2R inhibit cell proliferation.

Unlike its murine homologue, human *Igf2r* exhibits biallelic expression, (Kalscheuer et al. 1993; Ogawa et al. 1993), although a few individuals exclusively express the maternal allele (Xu et al. 1993). Mutations in *Igf2r*, or loss of heterozygosity at the 6q26–27 locus where *Igf2r* resides, lead to reduced IGF2R expression and increased circulating concentrations of IGF-II. Loss of biallelic *Igf2r* expression has been reported in cancers of the breast, liver, prostate, lung, adrenal gland, head, neck and endometrium (Martin-Kleiner and Gall 2010) and in the kidneys of Wilms' tumour patients (Xu et al. 1997). Loss of heterozygosity proximal to the *Igf2r* locus is also predictive of the presence of disseminated tumour cells in the bone marrow of ovarian cancer patients, before and after chemotherapy (Kuhlmann et al. 2011).

## IR-A signalling in cancer

Ligation of IR-A by IGF-II initiates a proliferative response (Frasca et al. 1999), and aberrant IR-A signalling has been implicated in a number of diseases, including cancer (Reviewed in Belfiore et al. (2009)). IGF-II signalling through IR-A has also been shown to induce differential expression of genes involved in signal transduction, cell cycle, metabolism, angiogenesis and adhesion, when compared with insulin signalling (Pandini et al. 2004).

IR-A is the predominant IR isoform expressed by carcinomas of the breast, colon and lung (Frasca et al. 1999), and the relative abundance of IR-A is increased in thyroid cancer, compared with normal thyroid tissue (Vella et al. 2002). Activation of IR by IGF-II in human breast cancer cell lines stimulated proliferation, with IGF-II exhibiting 63% of the potency of insulin. In contrast, IGF-II signalling through IR in non-malignant human breast cells was less than 1% as potent as insulin (Sciacca et al. 1999). IGF-II is also reported to be a more potent stimulator of SKUT-1 leiomyosarcoma cell migration than insulin, a cell line that expresses IR-A but not IGF1R (Sciacca et al. 2002). However, siRNA-mediated knockdown of IR-A in SW480 human colon adenocarcinoma cells increased viability and enhanced IGF1R activation by IGF-II (Brierley et al. 2010), suggesting that IGF-II bioactivity is mediated most effectively by IGF1R.

## Summary

In summary, IGF-II is a critical mediator of cell fate, regulating normal embryonic development and placental function, but also inducing aberrant proliferation and cell survival in cancer and cardiovascular disease. By understanding in more detail how the actions of IGF-II are regulated, either through genetic, epigenetic or post-translational modifications, downstream signalling cascades, or via its interactions with IGF binding proteins or the IGF-2R, we will be better placed to combat its pathophysiological effects.

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