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Insulin-like growth factor axis targeting in cancer and tumour angiogenesis – the missing link

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ABSTRACT

Numerous molecular players in the process of tumour angiogenesis have been shown to offer potential for therapeutic targeting. Initially denoted to be involved in malignant transformation and tumour progression, the insulin-like growth factor (IGF) signalling axis has been subject to therapeutic interference, albeit with limited clinical success. More recently, IGFs and their receptors have received attention for their contribution to tumour angiogenesis, which offers novel therapeutic opportunities. Here we review the contribution of this signalling axis to tumour angiogenesis, the mechanisms of resistance to therapy and the interplay with other pro-angiogenic pathways, to offer insight in the renewed interest in the application of IGF axis targeting agents in anti-cancer combination therapies.

Key words: angiogenesis, IGF, insulin, IGF1R, cancer, resistance, therapy.

CONTENTS

I. INTRODUCTION

Since the initial report of specific angiogenesis inhibitors in the early 1990s (Griffioen & Molema, 2000; Kerbel & Folkman, 2002), several angiostatic therapeutics have made their entrance into the clinic (Carmeliet & Jain, 2011; Jain, 2014). While nearly all of these drugs target the vascular endothelial growth factor (VEGF)/VEGF-receptor axis, there is a great need for alternative and more specific strategies. Previous studies have identified new molecular

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targets (St Croix *et al.*, 2000; van Beijnum & Griffioen, 2005; van Beijnum *et al.*, 2006; Otsubo *et al.*, 2014). Among these are members of the insulin and insulin-like growth factor (IGF) signalling axis. The relevance of the IGF axis to cancer initiation and progression stems from the observation that mouse embryonic fibroblasts (MEFs) lacking the IGF1 receptor (IGF1R) could not be transformed, in contrast to wild-type MEFs (Sell *et al.*, 1993). Subsequently, numerous *in vitro* and *in vivo* studies demonstrated that blocking the IGF signalling axis could inhibit tumour growth and angiogenesis. However, to date clinical trials have largely failed to meet the high expectations set by preclinical mouse studies (Reidy *et al.*, 2010; Schmitz *et al.*, 2012). This can, in part, be explained by the intricate complexity of the interacting ligands, receptors and mediators of this system. Nevertheless, the relatively underappreciated importance of the IGF signalling axis in tumour angiogenesis could provide new angles for future application of (combination) therapies.

In this review, we discuss the role of insulin, IGFs and their receptors in tumour angiogenesis and the opportunities to target this signalling pathway in cancer treatment, with a focus on overcoming resistance to therapy.

II. BLOOD BROTHERS

The IGF signalling axis comprises three ligands, insulin, IGF1 and IGF2, and three receptors, insulin receptor (IR), IGF1 receptor (IGF1R) and IGF2R. Next to this, circulating IGF binding proteins (IGFBPs) have been identified that are able to modify bioavailability of IGFs.

Both IGF1R and IR are dimeric receptor tyrosine kinases, each monomer consisting of an α and a β chain generated through proteolytic cleavage of a single protein precursor (Fig. 1). The β chain has a transmembrane domain and fosters phosphorylation whereas the α chain is fully extracellular and is responsible for ligand binding (Frattali & Pessin, 1993). IR and IGF1R are highly homologous, but have subtle differences in specific regions (reviewed by Siddle, 2012). IR transcripts can be alternatively spliced to form either IR-B, the full-length sequence, or IR-A, the isoform which lacks 12 amino acids in the C-terminus of the extracellular α chain, and which is most similar to IGF1R. The general view is currently that IR and IGF1R mediate both metabolic and mitogenic effects, exerted by predominant signalling of phosphatidylinositol 3-kinase/AKT serine threonine kinase (PI3K/AKT) or RAS proto-oncogene/mitogen-activated protein kinase (Ras/MAPK) pathways [reviewed by Bach (2015); Belfiore *et al.* (2009); Bowers *et al.* (2015); Gao *et al.* (2012); Pollak (2012)]. The type of ligand binding to IGF1R or IR and their major intracellular receptor substrates, insulin receptor substrate (IRS)-1 and -2 and Src homology domain containing (SHC), influence the direction of signalling and ultimately the cellular response (Siddle, 2012). Notably, cellular context, i.e. relative expression of receptors and downstream cellular mediators, appears to be the major determinant in defining the response to insulin and IGFs (Siddle, 2012).

By lining the inside of blood vessels, endothelial cells (ECs) are continuously exposed to circulating insulin and IGFs. As such, the IGF signalling axis in endothelium has received scientific attention, although predominantly in the context of normal physiology or diabetic complications (Bach, 2015). It is now becoming clear that its role in tumour angiogenesis is of considerable importance.

(1) Insulin-like growth factor 1 (IGF1) receptor and angiogenesis

Insulin-like growth factor 1 receptor (IGF1R) has a predominant affinity for IGF1 (Fig. 1), although IGF2 and insulin are also able to activate IGF1R. IGF1 expression is mainly regulated by growth hormone and originates predominantly in the liver, but can also be induced in an autocrine or paracrine fashion in target tissues, including malignancies (Bowers *et al.*, 2015). Increased IGF–IGF1R signalling is frequently observed in cancer, and in malignant transformation. Concordantly, mutations in IGF1R in cancer are rarely reported (Gao *et al.*, 2012). Tumour cells can also produce IGF1 making it available for endocrine, paracrine and autocrine interactions in the tumour microenvironment. IGFs predominantly act on ECs to promote angiogenesis through stimulation of VEGF, hypoxia-inducible factor 1α (HIF1α) and endothelial nitric oxide (NO) synthase (eNOS) expression (Bach, 2015).

Although ECs themselves express negligible levels of IGF1, IGF1R is expressed by both large and small vessels (Delafontaine, Song & Li, 2004). Human umbilical vein ECs (HUVECs) express several-fold more IGF1R than IR, although this ratio may change over time in culture (Nitert *et al.*, 2005). Direct effects of IGF1 on ECs have been shown, however, not all studies controlled for the relative contributions of IR and IGF1R in the response to IGF1. Nevertheless, IGF1–IGF1R signalling is involved in EC migration and tube formation, vasorelaxation as a consequence of NO production and mediation of inflammatory responses. However, effects differed in ECs derived from various vascular beds (Nakao-Hayashi *et al.*, 1992; Shigematsu *et al.*, 1999; Che *et al.*, 2002; Bid *et al.*, 2013; Bach, 2015). *In vivo*, high levels of IGF1 were found to be associated with the development of diabetic retinopathy, a condition characterized by neovascularization in the eye (Merimee, Zapf & Froesch, 1983). These pro-angiogenic properties of IGF1 were later confirmed in both corneal and retinal neovascularization (Grant, Caballero & Millard, 1993). Of note, overexpression of IRS-1 has also been reported in corneal neovascularization (Cursiefen *et al.*, 2014). In a tumour setting, IGF1 was shown to stimulate VEGF expression from tumour cells in both HIF1α-dependent and -independent mechanisms, contributing to sustained angiogenesis (Slomiany & Rosenzweig, 2006; Kurmasheva *et al.*, 2009; Li *et al.*, 2011). In addition, IGF1R activation may protect against apoptosis of ECs (Pan *et al.*, 2014).

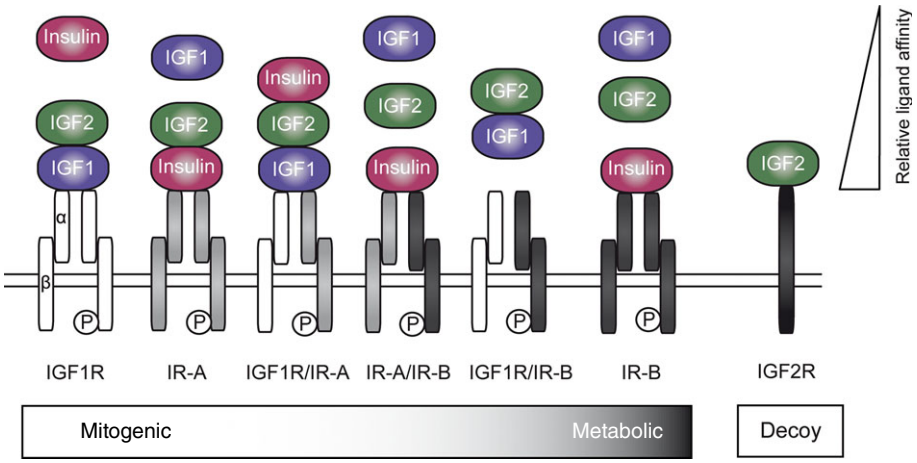

Fig. 1. Receptors and ligands of the insulin-like growth factor (IGF) signalling axis. IGF1 receptor (IGF1R), insulin receptor A (IR-A) and IR-B consist of an extracellular ligand-binding α-subunit and a catalytic transmembrane β-subunit derived from a single protein precursor, and are present in the membrane as dimers. Homo- and heterodimeric as well as hybrid receptor combinations are possible and driven by the relative expression of each protein. Depending on their composition, downstream signalling is predominantly mitogenic (IGF1R and IR-A) or metabolic (IR-B). IGF2R is a single-pass transmembrane monomer without tyrosine kinase activity which acts predominantly as a decoy receptor for circulating IGF2. The relative affinity of the different receptor combinations for the ligands IGF1, IGF2 and insulin is depicted in distance from the receptor. Adapted from Belfiore *et al.* (2009) and Chao & D'Amore (2008).

In vivo experiments demonstrated that a vascular-specific knockout of IGF1R reduced hypoxia-mediated retinal neovascularization in mice, albeit to a lesser extent than knockout of IR (Kondo *et al.*, 2003). In another study, knockdown of IRS-1 downstream of IGF1R, using an antisense oligonucleotide, inhibited corneal neovascularization in a rat model (Andrieu-Soler *et al.*, 2005) Furthermore, haploinsufficient IGF1R+/− mice exhibited altered vascular function (Abbas *et al.*, 2011), and IGF1R activation was shown to be critical during embryonic vascularization of the lungs (Han *et al.*, 2003). In cancer, IGF1–IGF1R signalling has been associated with increased tumour angiogenesis, and this is likely also mediated by indirect effects on the vasculature, e.g. through VEGF induction.

In addition to sprouting of ECs from pre-existing vessels, tumour angiogenesis is also facilitated by the incorporation of circulating endothelial progenitor cells (EPCs), usually derived from bone-marrow (Asahara *et al.*, 1999). Both IGF1 and insulin have been implicated in promoting EPC function and homing to sites of neovascularization (Sukhanov *et al.*, 2007; Thum *et al.*, 2007; Humpert *et al.*, 2008). In cancer, hypoxia-induced expression of stromal derived factor 1 (SDF1) and VEGF contribute to the mobilization of bone-marrow-derived progenitor cells that may incorporate in nascent vessels (Asahara *et al.*, 1999; van Beijnum *et al.*, 2015; Huijbers *et al.*, 2016). As such, this process contributes to tumour angiogenesis and progression, as well as therapy resistance (van Beijnum *et al.*, 2015; Huijbers *et al.*, 2016). Insulin and insulin analogues stimulated the outgrowth of EPCs *in vitro*, in a dose-dependent manner (Humpert *et al.*, 2008). Interestingly, inhibiting IR had no significant effect on this process, while blockade of IGF1R completely abrogated insulin-stimulated EPC outgrowth (Humpert *et al.*, 2008). *In vivo* perfusion of mice with IGF1 resulted in increased EPC numbers in the blood (Sukhanov *et al.*, 2007). This was accompanied by an increase in eNOS activity, which is essential for IGF1-mediated EPC mobilization (Sukhanov *et al.*, 2007; Thum *et al.*, 2007). A definitive role for IGF1R in EPC mobilization and function was demonstrated by Thum *et al.* (2007). Growth hormone treatment of elderly individuals increased circulating IGF1 levels as well as EPC numbers. These EPCs express IGF1R and blockade of this receptor counteracted the effects of IGF1 on EPC migration, colony formation and incorporation in vessels (Thum *et al.*, 2007). Thus, by stimulating not only vascular growth but also the incorporation of progenitor cells into growing blood vessels, IGF–IGF1R signalling can contribute to sustaining tumour angiogenesis.

(2) Insulin-like growth factor 2 (IGF2) receptor and angiogenesis

In contrast to IGF1R and IR, the IGF2R or mannose-6-phosphate receptor, is a single-pass transmembrane glycoprotein without tyrosine kinase activity. It shows affinity for IGF2, as well as for other mannose-6-phosphate-containing ligands but lacks affinity for IGF1 and insulin. It is generally seen as a regulatory receptor for IGF2 (Korner *et al.*, 1995; Livingstone, 2013) (Fig. 1) as circulating levels of IGF2 exceed that of IGF1 during human postnatal life (Bach, 2015). IGF2 binding to IGF2R has been suggested to lead to G(i) protein signalling to mediate cellular effects (Okamoto *et al.*, 1990), but this is still under debate (Korner *et al.*, 1995). As such, the role of IGF2R in tumour progression has been considered as

passive, modulating IGF2 availability for signalling through IR and IGF1R.

An initial role for IGF2R in angiogenesis was demonstrated by the discovery of the angiogenic factor proliferin, a member of the prolactin–growth hormone family (Jackson *et al.*, 1994; Volpert *et al.*, 1996), which was demonstrated to bind to IGF2R (Lee & Nathans, 1988). Placental angiogenesis is stimulated by proliferin, as was EC migration *in vitro* (Jackson *et al.*, 1994; Volpert *et al.*, 1996). Furthermore, a mutant form of IGF2 capable of only binding to IGF2R stimulates EC migration and chemotaxis (Volpert *et al.*, 1996; Groskopf *et al.*, 1997), which was suggested to be controlled by G(i) protein-mediated induction of the MAPK pathway (Groskopf *et al.*, 1997).

Direct angiogenic effects of IGF2 were demonstrated in the developing chick chorioallantoic membrane. Here, IGF2 dose-dependently stimulated capillary formation (Bae *et al.*, 1998). In addition, IGF2 increased EC migration, tube formation and invasion (Lee *et al.*, 2000). However, a direct involvement of IGF2R was not addressed in these studies, and the observed effects may have been mediated by IR and/or IGF1R. Direct involvement of IGF2/IGF2R signalling in angiogenesis was provided by Herr *et al.* (2003) who demonstrated that antibodies against IGF2R circumvented the induction of EC sprouting by IGF2. Pertussus toxin, an inhibitor of G-protein activation, abolished IGF2-induced sprout outgrowth, again suggesting involvement of G(i) signalling.

Not only insulin and IGF1, but also the IGF2–IGF2R pathway has been implicated in the process of EPC recruitment. EPCs express high levels of IGF2R, and IGF2 induced intracellular Ca^{2+} localization, chemotaxis, adhesion and invasion of EPCs. Effects were inhibited by mannose-6-phosphate, competing with IGF2 for IGF2R and neutralizing antibodies to IGF2R (Maeng *et al.*, 2009). A G(i) protein antagonist blocked these effects, again suggesting that IGF2R may be a functional signalling receptor (Maeng *et al.*, 2009). IGF2 is highly upregulated in hypoxic tissues (Kim *et al.*, 1998), and hypoxia has been appreciated as a driving mechanism of EPC recruitment (Takahashi *et al.*, 1999). *In vivo*, IGF2 combined with bone-marrow-derived EPCs resulted in maximal vascularization in a Matrigel plug assay. More importantly, physiological relevance and IGF2R importance were shown in a hind-limb ischemia model where EPC homing was inhibited by neutralizing IGF2R function (Maeng *et al.*, 2009).

IGF2 is frequently overexpressed in cancers due to loss of imprinting (Cui *et al.*, 2003). This, in combination with mutations in IGF2R resulting in reduced affinity for IGF2, leads to increased circulating and local IGF2 that can promote cellular growth through IR and IGF1R, and is associated with increased cancer risk and poor prognosis (reviewed by Livingstone, 2013). Interestingly, polymorphisms in IGF2R have been associated with increased malignant potential in different ways. By affecting transcript stability and translation of IGF2R, genetic variants of IGF2R coincided with increased secretion of procathepsin D and contributed to transforming growth factor β (TGFβ) activation (Zavras *et al.*, 2003; Kotsinas *et al.*, 2008). Both cathepsin D and TGFβ are known for their stimulating effects on tumour angiogenesis (Hu *et al.*, 2008; van Meeteren, Goumans & ten Dijke, 2011). Taken together, the involvement of IGF2R in tumour angiogenesis is not uncontroversial, but its potential contribution should not be neglected.

(3) Insulin receptor and angiogenesis

The pro-angiogenic effects of insulin have long been recognized in relation to its wound-healing properties, a process dependent on angiogenesis (Gurd, 1937). Later, the stimulatory effects of insulin on ECs were demonstrated (Nakao-Hayashi *et al.*, 1992). The intricate role of insulin in diabetic conditions further sheds light on its involvement in angiogenesis as insulin treatment in diabetic patients may lead to an initial worsening of diabetic retinopathy, reflective of excessive angiogenesis (Roysarkar *et al.*, 1993). Indeed, vascular endothelial specific knockout of IR resulted in a 57% decrease in hypoxia-induced retinal neovascularization (Kondo *et al.*, 2003). Subcutaneous insulin injection induced angiogenesis in mouse skin. Here, vessels were longer and more branched, and a higher presence of pericytes [alpha smooth muscle actin (αSMA) staining] was observed, suggesting vessel maturation (Liu, Petreaca & Martins-Green, 2009). In addition, insulin treatment of microvascular ECs led to increased migration and tube formation (Liu *et al.*, 2009; Rensing *et al.*, 2010). By contrast, others reported that microvascular ECs are relatively resistant to insulin *in vitro* due to a higher expression of IGF1R than IR (Nitert *et al.*, 2005; Johansson, Chisalita & Arnqvist, 2008). IR signalling affects angiogenesis through the PI3K/AKT pathway (Liu *et al.*, 2009) which induces the release of NO through phosphorylation of eNOS (Zeng & Quon, 1996; Michell *et al.*, 1999). NO is a critical factor in angiogenesis and plays a key role in EC survival, proliferation and migration (Cooke, 2003) and provides a positive feedback loop *via* the induction of VEGF expression (Dulak *et al.*, 2000).

The two isoforms of insulin receptor, IR-A and IR-B, can form homodimers as well as heterodimers (IR-A/B), and can form hybrid receptors with IGF1R, resulting in different affinities for IGF1, IGF2 and insulin (Fig. 1). Insulin receptor isoform expression is regulated developmentally, as well as in a tissue-specific manner (Seino & Bell, 1989; Mosthaf *et al.*, 1990). IR-B is highly expressed in liver and is considered to be the major metabolic receptor for insulin (Mosthaf *et al.*, 1990). By contrast, IR-A is regarded as the oncofetal form, with high expression during fetal development and cancer (Frasca *et al.*, 1999). Although the receptors differ in only 12 amino acids at the C-terminus of the α chain, their ligand binding and signalling properties are quite divergent. IR-A signalling primarily induces class I PI3K activation with mitogenic effects whereas IR-B signalling through class II PI3K ultimately results in glycogen synthesis (Leibiger *et al.*, 2001). Whether or not caused by a higher sequence similarity to IGF1R (Denley *et al.*, 2003), IR-A has a higher

affinity for IGFs, and a slightly higher affinity for insulin, as compared to IR-B (Frasca *et al.*, 1999). In addition, IR-B has prolonged autophosphorylation activity over IR-A and different internalization kinetics (Vogt *et al.*, 1991; Kellerer *et al.*, 1992). The exact mechanisms driving the alternative splicing have not been fully elucidated, though hormonal and metabolic factors may be involved as it was shown that insulin and high glucose levels can upregulate IR-B (Denley *et al.*, 2003).

We and others have demonstrated that tumour ECs overexpress IR relative to non-activated normal tissue ECs (van Beijnum *et al.*, 2006, 2015; Rensing *et al.*, 2010; P. Nowak-Sliwinska, J. R. van Beijnum, E. J. Huijbers, T. J. Wong & A. W. Griffioen, in preparation), most notably the IR-A isoform. Moreover, many cancer types overexpress this variant (Denley *et al.*, 2003; Belfiore *et al.*, 2009; Andres *et al.*, 2013). Tumour growth and angiogenesis were suppressed in xenograft tumours lacking IR, possibly mediated by reduced VEGF signalling (Zhang *et al.*, 2010). Overexpression of IR-A increased proliferation, colony formation, migration, invasion, and resistance to apoptosis in prostate cancer cells (Heidegger *et al.*, 2014), whereas overexpression of IR-B reduced proliferation and accelerated differentiation in colon cancer cells (Andres *et al.*, 2013). IR-A has a higher affinity for IGF2 than for IGF1 (Belfiore *et al.*, 2009), and overexpression of IR-A as well as its ligand IGF2 in tumours accelerates tumour growth and angiogenesis through autocrine and paracrine stimulatory loops (Denley *et al.*, 2003).

(4) Hybrid receptors and angiogenesis

Due to the high homology between the different receptors and their dimeric nature, hybrid receptors can be formed consisting of monomers of IR-A or IR-B and IGF1R (Fig. 1). The relative abundance of the individual monomers dictates the distribution of the hybrid receptors as a function of the molar fractions of each individual receptor (Bailyes *et al.*, 1997; Belfiore *et al.*, 2009), and as such will determine the eventual tissue response. In addition, other factors affect the precise signalling that is initiated upon receptor–ligand interactions (reviewed by Siddle, 2011, 2012). Among these factors are (*i*) differences in interactions of IGFR and IR with different intracellular adaptors and scaffolds, (*ii*) post-translational modifications, (*iii*) their internalization kinetics, and (*iv*) expression of receptor substrates in a particular cell or lipid raft domain.

Hybrid receptors display differential affinity for IGFs and insulin (Fig. 1). Hybrid receptors consisting of IGF1R and IR-A have high affinity for IGF1 and IGF2 and markedly reduced affinity for insulin as compared to IR-A. Hybrids between IR-B and IGF1R lose their affinity for insulin, and have lower IGF affinities than IGF1R/IR-A hybrids (Pandini *et al.*, 2002). Hybrid IR-A/IR-B essentially function as IR-A (Janssen & Varewijck, 2014). IR-A-containing receptors function predominantly as mitogenic receptors whereas IR-B-containing receptors are thought to act mainly as metabolic mediators (Belfiore *et al.*, 2009).

ECs generally express more IGF1R than IR and hybrid IGF1R/IR have been found in HUVECs, however, incorporation of IR-A *versus* IR-B was not addressed (Chisalita & Arnqvist, 2004; Nitert *et al.*, 2005). IGF1 but not insulin was able to activate IGF1R/IR hybrids at low concentrations (Li *et al.*, 2005; Nitert *et al.*, 2005), suggesting that increased hybrid receptor expression reduces insulin sensitivity and enhances IGF responsiveness. Moreover, high IGF1R expression over IR drives the assembly of hybrid receptors and reduces the availability of IR homoreceptors, further contributing to a shift from insulin towards IGF reactivity with concomitant growth-promoting effects. Reduced insulin sensitivity can lead to hyperinsulinemia which has been associated with increased cancer progression through its actions on the pro-proliferative IR-A receptor. In addition, high insulin levels may in turn stimulate growth hormone receptor and IGF1 expression in the liver, positively feeding IGF signalling (Janssen & Varewijck, 2014).

The precise contribution of the hybrid receptors to cellular physiology is difficult to establish due to a lack of specific reagents. Antibodies distinguishing between IR-A and IR-B are not available, and most (therapeutic) monoclonal antibodies generated against IGF1R show no cross-reactivity with IR (Janssen & Varewijck, 2014). Although antibodies with reactivity towards both hybrid receptors and IGF1R or hybrid receptors and IR have been documented (Belfiore *et al.*, 2009), so far no selective targeting of hybrid receptors has been demonstrated. As such, the use of R-cells, mouse 3T3-like fibroblasts without IGF1R (Pandini *et al.*, 2002), in which human IR and/or IGF1R were introduced, have been instrumental in studying hybrid receptor characteristics. Nevertheless, this system precludes drawing firm conclusions on distribution and function *in vivo*.

(5) Insulin-like growth factor binding proteins (IGFBPs) and angiogenesis

IGFBPs regulate IGF bioavailability and stability through binding of IGFs in the circulation with high affinity, thereby modulating (either inhibiting or stimulating) IGF signalling. Furthermore, upon binding of IGFBPs, IGFs are protected from degradation which prolongs their half-life. Proteolytic cleavage of IGFBPs, e.g. through matrix metalloproteinases (MMPs), releases IGFs (Rajaram, Baylink & Mohan, 1997), although partial cleavage may retain IGF1 binding (Andress *et al.*, 1993; Lalou, Lassarre & Binoux, 1996; Firth & Baxter, 2002). In addition, native IGFBPs can also have independent cellular actions (Rajah *et al.*, 1995; Firth & Baxter, 2002; Wheatcroft & Kearney, 2009). Thus far, six IGFBPs have been identified (IGFBP1–6). IGFBP1, and -3 have been ascribed dual roles in tumour angiogenesis, which might be the result of divergent actions of direct cellular effects of these proteins *versus* IGF binding properties. High IGFBP1 levels have been associated with reduced cancer and cardiovascular disease risks, implying a vascular protective effect (Janssen *et al.*, 1998; Spoerri *et al.*, 1998). On the other hand, IGFBP1 is induced by hypoxia and was shown to induce EC migration (Kahn *et al.*, 2011).

Most circulating IGF is bound by IGFBP3, as such limiting IGF bioavailability. Through its heparin-binding domain, IGFBP3 can bind ECs independently of IGF binding (Janssen *et al.*, 1998; Hwa, Oh & Rosenfeld, 1999). IGFBP3 inhibited the proliferative effects of VEGF on ECs and reduced prostate cancer growth through inhibition of angiogenesis (Franklin, Ferry & Cohen, 2003; Liu *et al.*, 2007). By contrast, activation of sphingosine-1-phosphate by IGFBP3 in HUVECs protected the cells from serum starvation-induced apoptosis and enhanced cell migration (Takuwa *et al.*, 2010). Furthermore, IGFBP3 was shown to stimulate MMP2 and MMP9 activation and concomitantly tube formation of ECs (Granata *et al.*, 2007). Another pro-angiogenic effect of IGFBP3 is that it can directly stimulate EPCs or haematopoietic stem cells to develop into ECs and subsequently enhance cell migration and tube formation (Chang *et al.*, 2007).

IGFBP2 is recognized for its pro-angiogenic properties. Interestingly, one of its mechanisms involves nuclear translocation and VEGF expression-promoting activities (Azar *et al.*, 2011). In addition, IGFBP2 was shown to bind and activate $\alpha V\beta 3$ integrin on ECs and tumour cells, leading to the expression of VEGF *via* the PI3K pathway (Das *et al.*, 2012). Furthermore, IGFBP2 promoted EC–EPC interactions and cellular motility through binding with its tripeptide Arg-Gly-Asp (RGD) domain to $\alpha 5\beta 1$ integrin (Wang *et al.*, 2006; Feng *et al.*, 2015). However, in these studies the relative contributions of autocrine/paracrine *versus* intracellular functions of IGFBP2 were not clearly addressed. Interestingly, exogenously administered protease-resistant IGFBP2 inhibited cancer cell proliferation and angiogenesis *in vitro* and *in vivo* (Soh *et al.*, 2014).

The anti-angiogenic properties of IGFBP4 have been attributed to the C-terminal domain of the protein which shows anti-cathepsin B activity, and thus inhibits an important angiogenic lysosomal protease (Premzl, Turk & Kos, 2006; Das *et al.*, 2012; Moreno *et al.*, 2013). Furthermore, IGFBP4 reduced tube formation in human brain endothelial cells (HBECs), which was thought to be caused by the inhibition of VEGF-induced effects (Moreno *et al.*, 2006). IGFBP5 was also demonstrated to be an anti-angiogenic factor. IGFBP5 inhibits the activation of AKT and eNOS by VEGF, and thereby impairs proliferation, migration and tube formation in ECs (Rho *et al.*, 2008). The various inhibitory actions of IGFBP6 on tumour growth were initially mainly attributed to the inhibition of IGF2 (Bach, 2005). Later it was shown that IGFBP6 inhibited VEGF-induced angiogenesis both *in vitro* and *in vivo*, however, the exact mechanism behind this inhibitory function is unclear (Zhang *et al.*, 2012).

Although a series of IGFBP-related proteins, structurally related to the IGFBPs, have been identified and termed IGFBP7 and higher (Hwa *et al.*, 1999), their binding properties and contribution to the IGF system are not established. It is however, relevant in light of this review to point out that IGFBP7 specifically binds insulin, resulting in functional IR inhibition (Yamanaka *et al.*, 1997; Ruan *et al.*, 2016). Interestingly, this protein has been associated with angiogenesis and has also been named 'angiomodulin'. However, its exact role remains to be elucidated since stimulatory, as well as inhibitory effects have been described, that may possibly be concentration dependent (van Beijnum *et al.*, 2006; Tamura *et al.*, 2009; Zhu *et al.*, 2014; Zhao *et al.*, 2016).

III. THERAPEUTICS, RESISTANCE AND ANGIOGENESIS

(1) Targeting the insulin-like growth factor (IGF) axis

The dominant involvement of the IGF signalling pathway in cancer and tumour angiogenesis has led to the development of different therapeutics aimed at inhibiting its actions (Fig. 2; Table 1). An initial major concern however, was to circumvent the induction of hyperglycemia, insulin resistance

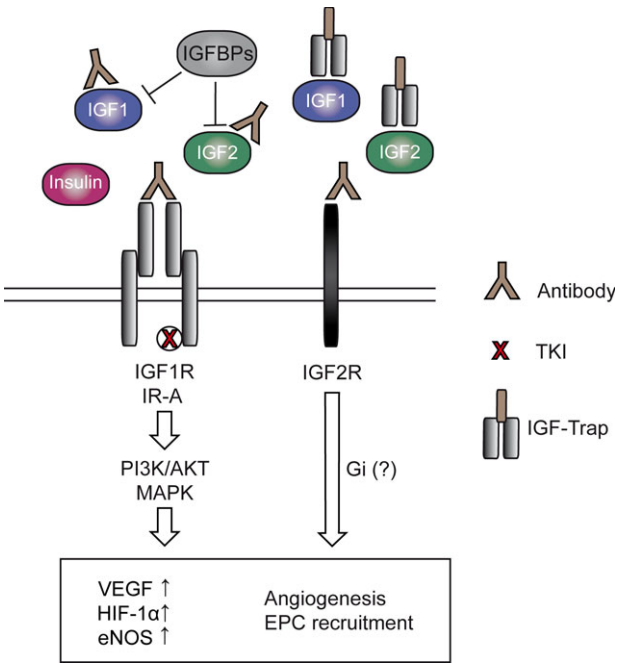

Fig. 2. Insulin-like growth factor (IGF) signalling axis therapeutics and anti-angiogenic actions. To inhibit signalling along the IGF axis, different strategies have been developed: (*i*) antibodies targeting the IGF1 receptor (IGF1R) and its ligands IGF1 and IGF2, (*ii*) receptor tyrosine kinase inhibitors (TKIs) and (*iii*) recombinant soluble IGF1R (sIGF1R) fused to an antibody heavy chain Fc region (IGF-Trap). Downstream signalling of the receptors induces angiogenesis and endothelial progenitor cell (EPC) recruitment, in part through induction of vascular endothelial growth factor (VEGF) signalling. eNOS, endothelial nitric oxide (NO) synthase; Gi, inhibitory G protein second messenger; HIF-1α, hypoxia-inducible factor 1α; IGFBP, IGF binding protein; IR-A, insulin receptor A; MAPK, mitogen-activated protein kinase; PI3K/AKT, phosphatidylinositol 3-kinase/AKT serine threonine kinase; VEGF, vascular endothelial growth factor.

Table 1. Drugs targeting the insulin-like growth factor (IGF) axis

Name	Target	Alternative name	Type
Medi-573	IGF1/2		Ab
BI 836845	IGF1/2		Ab
Figitumumab	IGF1R	CP-751,871	Ab
Dalotuzumab	IGF1R	MK-0646; h7C10	Ab
Cixutumumab	IGF1R	IMC-A12	Ab
Ganitumab	IGF1R	AMG-479	Ab
R1507	IGF1R	RO4858696	Ab
Robatumumab	IGF1R	SCH 717454	Ab
AVE-1642	IGF1R		Ab
BIIB022	IGF1R		Ab
XL-228	IGF1R		TKI
Linsitinib	IGF1R/IR	OSI-906	TKI
KW-2450	IGF1R/IR		TKI
AXL1717	IGF1R/IR	Picropodophyllin (PPP)	TKI
BMS-754807	IGF1R/IR		TKI

IGF-axis-targeting agents in clinical trials (www.clinicaltrials.gov). Modified from Bowers *et al.* (2015); Brahmkhatri, Prasanna & Atreya (2015); Iams & Lovly (2015) and King *et al.* (2014).
Ab, antibody; IGF1R, IGF1 receptor; IR, insulin receptor; TKI, tyrosine kinase inhibitor.

and hyperinsulinemia from inhibiting the IR (Gualberto & Pollak, 2009; Pollak, 2012; King *et al.*, 2014). Different IGF-axis targeted therapeutics have been developed over time [reviewed by Bowers *et al.* (2015); Gao *et al.* (2012)], which potentially give rise to different glucose homeostasis related toxicities. Tyrosine kinase inhibitors (TKIs) have been developed that target the receptors, however, these may induce hyperglycemia *via* the direct inhibition of IR (Gualberto & Pollak, 2009; King *et al.*, 2014). Anti-IGF1R antibodies generally do not cross-react with IR, but may interact with the hybrid receptors. They impair glucose tolerance through induction of circulating growth hormone, IGF1 and IGFBPs in response to blockade of pituitary IGF1R (King *et al.*, 2014). Scavengers neutralizing IGF1 and IGF2 such as antibodies (Haluska *et al.*, 2014), IGF-Trap (a soluble part of the IGF1R fused to the Fc portion of an IgG and capable of scavenging both IGF1 and IGF2 but not insulin; Wang *et al.*, 2015) and protease-resistant IGFBPs (Rosenzweig & Atreya, 2010), have generally less effect on glucose homeostasis (Gualberto & Pollak, 2009; King *et al.*, 2014).

Anti-receptor antibodies, ligand scavengers and small-molecule TKIs have all shown promising results in preclinical studies, but none of the agents lived up to this promise in clinical trials [reviewed by Gao *et al.* (2012); King *et al.* (2014)]. The intrinsic complexity of the pathway may have enabled escape signalling to take place; additionally poor study design and patient selection may have contributed. Sensitivity to anti-IGF1R therapy, e.g. by measuring IGF levels or screening for activating mutations downstream of the receptor was not taken into account (Pollak, 2012; Janssen & Varewijck, 2014; Bowers *et al.*, 2015). Nevertheless, while not yet successful as a monotherapy [except for in sarcoma-type tumours (Baserga, 2013)], targeting IGF1 signalling as part of a carefully designed combination therapy may still hold promise.

Below we discuss several resistance and escape mechanisms that may contribute to inadequate therapeutic success of interference with the IGF1 signalling axis, with a focus on implications for angiogenesis inhibition.

(2) Resistance mechanisms within the family

Different explanations for failure of IGF-axis targeted therapies have been postulated, which can be roughly divided into intrinsic resistance and adaptive/evasive resistance. First, IGF-independent growth, when tumours primarily rely on other growth factors, may pose an intrinsic resistance mechanism. Second, subcellular IGF1R localization may also contribute to intrinsic resistance to anti-IGF signalling therapeutics. For example, aberrant IGF1R glycosylation, impairing insertion into the plasma membrane (Kim *et al.*, 2012), and nuclear localization of IGF1R (Asmane *et al.*, 2012) prevent antibody–receptor interactions and were associated with reduced responsiveness. Although one might expect that this would decrease responsiveness to IGFs and thus impair the malignant potential of tumours, this was not reported. Apparently, not only the relative balance of, but also the dependence on, the different receptors in the IGF(R) axis will determine responsivity to IGFs. Third, constitutively activated PI3K/AKT or Ras/MAPK pathways that function downstream of the IGF and IR receptors, will sustain signalling even in the presence of receptor inhibitors. In addition, redundancy in downstream signalling cascades, shared with other receptor tyrosine kinases (RTKs), may limit inhibitory action of IGF axis therapeutics (King *et al.*, 2014).

Cells expressing a low ratio of IGF1R:IR are more likely to show intrinsic resistance to IGF1R therapeutics (Buck *et al.*, 2010; Ulanet *et al.*, 2010; Garofalo *et al.*, 2011). Due to the high specificity of the targeted agents, the more prevalent hybrid IR/IGF1R receptors will not be inhibited, and signalling can proceed (Fig. 3). Very recently it was

reported that total IR expression correlates with resistance against the anti-IGF1R agent cixutumumab (Forest *et al.*, 2015). Moreover, the relative expression of IR-A *versus* IR-B will determine to what extent continued IR signalling contributes to either mitogenic signalling (preferred by IR-A) or metabolic signalling (preferred by IR-B) (Sacco *et al.*, 2009; Garofalo *et al.*, 2011, 2012; Giudice *et al.*, 2011). Since enhanced IR-A signalling is recognized to mediate growth-promoting effects during IGF1R blockade, co-targeting of IR-A and IGF1R is advocated (Salisbury & Macaulay, 2003; Buck *et al.*, 2010). OSI-906 is a TKI which inhibits both IR and IGF1R, and indeed proved more effective in inhibiting tumour growth than an anti-IGF1R antibody (Buck *et al.*, 2010). However, a recent phase 3 study in adrenocortical carcinoma did not report significant clinical benefit of OSI-906 (Fassnacht *et al.*, 2015).

Recently, circulating IGF1R has been reported in cancer patients (Xu *et al.*, 2014). Although its relevance and exact nature remain to be determined, it is hypothesized that this might contribute to resistance against IGF1R therapeutics. Circulating receptors can interfere with cellular interaction of anti-IGF1R antibodies. In addition, it was suggested that displacement of soluble receptor-bound IGFs by the antibodies increases bioavailability of IGFs and potential for cellular interactions (Janssen & Varewijck, 2014) (Fig. 3). However, solid experimental evidence for these mechanisms is lacking. Next to intrinsic unresponsiveness to blockade of the IGF signalling axis, adaptive/evasive resistance mechanisms have been elucidated that diminish efficacy. Upon antibody blockade of IGF1R, its cell surface expression is suppressed due to internalization, and the balance shifts to increased dominance of IR-A signalling, leading to therapy resistance (Buck *et al.*, 2010; Ulanet *et al.*, 2010; Garofalo *et al.*, 2011, 2012). In addition, IGF1R expression may be downregulated as a consequence of conventional anticancer therapy, e.g. by tamoxifen (Janssen & Varewijck, 2014), rendering anti-IGF1(R) therapeutics less effective. Furthermore, impaired signalling of IGF1R results in a compensatory feedback mechanism culminating in increased IGF1 production due to enhanced growth hormone secretion (Wan *et al.*, 2007; Moody *et al.*, 2014) (Fig. 3).

As an alternative strategy, antibody-mediated blockade of IGF1 and IGF2 has been explored, as this may circumvent resistance to receptor blockade. This antibody inhibited primary tumour growth in mice, albeit not metastatic colonization (Haluska *et al.*, 2014). Also, the IGF-Trap (Wang *et al.*, 2015) is currently under investigation. However, such scavengers can only block free, bioactive IGFs, after being released from their binding partners, the IGFBPs. Moreover, a reduction in circulating IGF1 results in activation of growth hormone secretion and subsequent IGF1 production by the liver (Belfiore *et al.*, 2009) which will result in restored tumour growth and therapy resistance. Selective blockade of IGF2 is not expected to have such feedback effects. Moreover, given the dominant expression of high IGF2-affinity hybrid receptor containing the IR-A isoform in cancer and tumour endothelium, in combination with the high circulating IGF2 levels in humans, IGF2 inhibition might promise a more effective strategy over IGF1 inhibition alone. Indeed, neutralization of IGF2 restored sensitivity to anti-IGF1R antibody *in vitro* (Forest *et al.*, 2015).

Taken together, the intricate interplay and diversity of responses of the different homo- and heterodimeric receptors precludes the specific therapeutic targeting of a

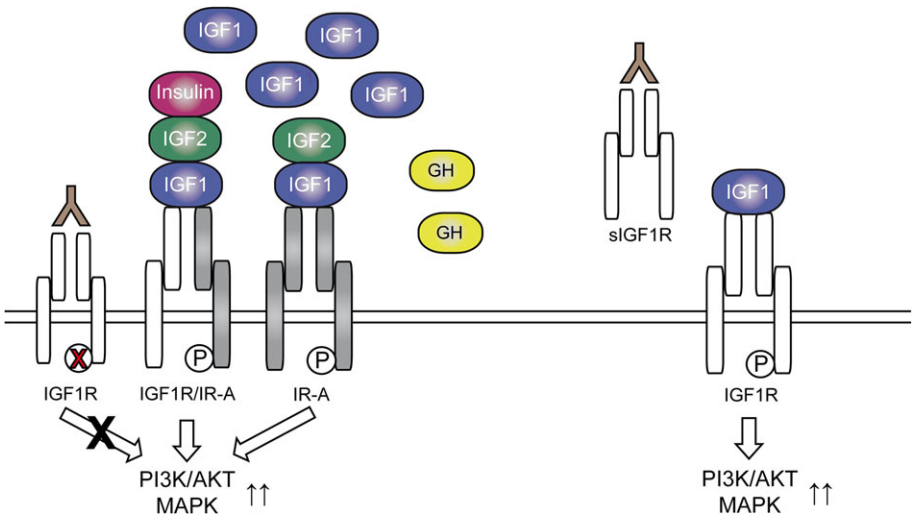

Fig. 3. Resistance to insulin-like growth factor (IGF) signalling axis inhibition. Antibody blockade or tyrosine kinase inhibitor (TKI)-mediated inhibition of IGF1 receptor (IGF1R) will downregulate IGF1R expression and result in predominant expression of insulin receptor A (IR-A) homodimers or IR-A/IGF1R hybrids that show enhanced responsiveness to insulin and IGFs and will promote mitogenic and pro-angiogenic signalling. In addition, pituitary IGF1R blockade will increase circulating growth hormone (GH) and IGF1. Alternatively, antibody blockade of soluble IGF1R (sIGF1R) will displace bound IGFs, making them available for cellular receptor interactions, and simultaneously reduce the availability of anti-IGF1R antibody for cellular receptor binding. MAPK, mitogen-activated protein kinase; PI3K/AKT, phosphatidylinositol 3-kinase/AKT serine threonine kinase.

single player in this system to control tumour growth and angiogenesis.

(3) Resistance mechanisms outside the family

Close relationships between IGF1R and IRs resulting in intrinsic or evasive resistance may not be the only explanation for reduced effectiveness of anti-IGF(1R) therapy. Extensive crosstalk also exists between IGF1R and growth hormone receptor (GHR), oestrogen receptor (ER), androgen receptor (AR), MET proto-oncogene receptor tyrosine kinase (c-MET), epidermal growth factor receptor (EGFR) and erb-b2 receptor tyrosine kinase HER2 signalling pathways (Janssen & Varewijck, 2014). Thus, other oncogenic signalling pathways can take over in the presence of IGF-axis blockade. In addition, interplay with important angiogenic signalling cascades has been described that can further amplify IGF signalling. Combining therapeutics directed against multiple activated cellular pathways therefore seems like an obvious choice.

Not only are the receptors of the IGF signalling axis capable of dimerizing with each other, interactions of IGF1R and IGF1 with other cell surface receptors have been described. In light of the pro-angiogenic actions of the IGF signalling axis, the interplay of IGF1R with integrins is highly relevant (Drake, Cheresh & Little, 1995; Clemmons & Maile, 2005; Beauvais & Rapraeger, 2010; Rapraeger, 2013; Rapraeger *et al.*, 2013) and provides additional therapeutic angles. $\alpha V\beta 3$ integrin, involved in cell migration, is virtually absent in quiescent endothelium, but highly upregulated in angiogenic ECs (Drake *et al.*, 1995). It has been shown that syndecan-1 (Sdc1), a heparan sulfate proteoglycan, clusters and activates IGF1R and $\alpha V\beta 3$ integrin (Beauvais & Rapraeger, 2010) (Fig. 4). Synstatin, a dominant-negative inhibitory peptide sequence of Sdc1, is capable of blocking the capture of $\alpha V\beta 3$ integrin and IGF1R and thereby inhibits the activation of IGF1R (Rapraeger, 2013). In addition to IGF1R, IGF1 itself can directly bind $\alpha V\beta 3$ and $\alpha 6\beta 4$ integrins, thereby inducing ternary complex formation of IGF1R, IGF1 and integrins (Saegusa *et al.*, 2009; Fujita *et al.*, 2012; Fujita, Takada & Takada, 2013). Thus, escape signalling involving direct activation of integrins by IGF1 may be induced by blocking IGF1R. Indeed, this mechanism was found to be responsible for induced resistance against IGF1R antibodies in head and neck squamous cell carcinoma (HNSCC) and non-small cell lung cancer (NSCLC) (Shin *et al.*, 2013). Taken together, co-targeting of integrins may potentiate the effects of anti-IGF1R therapeutics or prevent the induction of therapy resistance.

As discussed above, induction of VEGF expression is a recognized mechanism by which signalling along the IGF/IGFR axis contributes to angiogenesis. Different accessory proteins and membrane receptors have linked IGF1 signalling to angiogenesis *via* VEGF. One of these is CD147, or basigin, a glycoprotein commonly overexpressed in tumour cells and involved in promoting tumour progression (Millimaggi *et al.*, 2007). Tumour cell-derived vesicles containing CD147 promoted an angiogenic

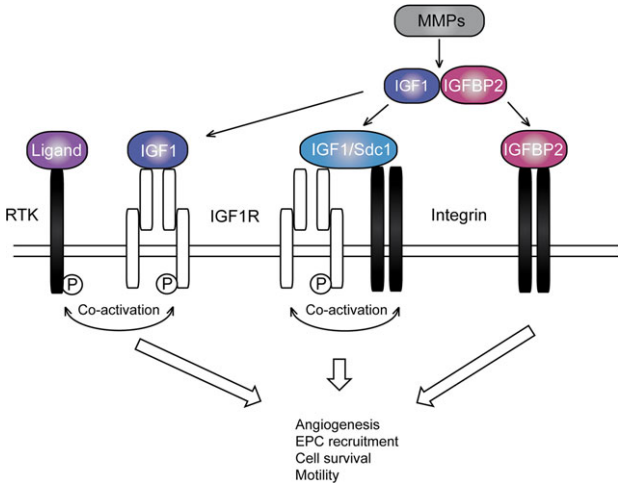

Fig. 4. Interplay between insulin-like growth factors (receptors) [IGF(R)s] and angiogenic signalling. IGF1R and other receptor tyrosine kinases (RTKs) can assemble and co-activate each other leading to enhanced pro-angiogenic signalling. IGF1 and syndecan-1 (Sdc1) can both cluster IGF1R and integrins, resulting in co-activation of both membrane proteins, resulting in an amplified pro-angiogenic response. Integrins can also be directly stimulated by IGF binding protein 2 (IGFBP2). Matrix metalloproteinases (MMPs) can disrupt the interaction between IGFs and IGFBP2, enhancing the bioavailability of both proteins further sustaining angiogenic actions. EPC, endothelial progenitor cell.

phenotype in endothelial cells *in vitro* (Millimaggi *et al.*, 2007). Others subsequently demonstrated that hypoxia-induced expression of IGF1, VEGF and CD147 contributed to a positive feedback mechanism with reciprocal induction of expression, and inhibition of CD147 subsequently reduced IGF1-driven angiogenesis (Chen *et al.*, 2012). Another mechanism of VEGF induction *via* the IGF/IGFR axis is mediated by mucin-1 (Muc1), a known epithelial tumour marker. In breast cancer cell lines, Muc1 induces IGF1R phosphorylation and subsequent VEGFR expression (Woo *et al.*, 2012). These data further exemplify the independent actions of IGFR/IR ligands that may contribute to tumour progression and angiogenesis, even in the presence of agents that block IGFR/IR signalling, and further stresses the notion that sole inhibition of this pathway is likely not sufficient for cancer management.

Very recently, it was shown that IGF1R as well as IR-A can interact with discoidin domain receptors (DDRs) (Morcavallo *et al.*, 2011; Malaguarnera *et al.*, 2015). DDRs are receptor tyrosine kinases with collagens as their ligand. As such, they are involved in matrix adhesion and migration (Borza & Pozzi, 2014; Leitinger, 2014), and are frequently overexpressed in cancer (Valiathan *et al.*, 2012). DDRs are essential for vascular development (Morcavallo *et al.*, 2011), and knockdown of DDR1 ligand collagen 4A5 inhibited EC proliferation and tube formation (Xiao *et al.*, 2015). Interaction between IGF1R and DDR1 was induced upon IGF1 stimulation, and promoted cell proliferation and migration (Malaguarnera *et al.*, 2015), whereas IR-A

Table 2. Ongoing clinical trials of combinations of insulin-like growth factor (IGF) axis and vascular endothelial growth factor (VEGF) axis targeting

Trial	Drug	Type	Drug	Type	Condition	Phase
NCT00729833	Figitumumab	Ab	Sunitinib	TKI	Advanced solid tumours	1
NCT01008566	Cixutumumab	Ab	Sorafenib	TKI	Advanced liver cancer	1
NCT01263782	Cixutumumab	Ab	Bevacizumab	Ab	Non-small cell lung cancer	2
NCT00906373	Cixutumumab	Ab	Sorafenib	TKI	Advanced liver cancer	2
NCT00955305	Cixutumumab	Ab	Bevacizumab	Ab	Recurrent non-small cell lung cancer	2
NCT00974896	Ganitumab	Ab	Sorafenib	TKI	Advanced solid tumours	1
NCT00811993	R1507	Ab	Bevacizumab	Ab	Advanced solid tumours	1
NCT00811993	R1507	Ab	Sorafenib	TKI	Advanced solid tumours	1
NCT01498952	MEDI-573	Ab	Sorafenib	TKI	Metastatic/unresectable hepatocellular carcinoma	1
NCT00791544	AVE-1642	Ab	Sorafenib	TKI	Advanced or metastatic liver carcinoma	1/2
NCT00956436	BIIB022	Ab	Sorafenib	TKI	Hepatocellular carcinoma	1
NCT01334710	Linsitinib	TKI	Sorafenib	TKI	Advanced hepatocellular carcinoma	2

Ab, antibody; TKI, tyrosine kinase inhibitor.

stimulation by IGF2 induced DDR1 and DDR2 activation (Morcavallo *et al.*, 2011). Together, these studies provide an alternative route for growth- and angiogenesis-promoting effects not targeted by inhibitors of IGF1R.

(4) Anti-angiogenic therapy and IGF axis inhibition – brotherly love?

Since IGFs and IGFRs appear to be rather promiscuous proteins in promoting tumour progression and angiogenesis through interactions with and activation of other cellular receptors and effectors, induction of evasive resistance to targeted therapeutics is not surprising. Although these mechanisms apply to tumour cells and tumour ECs alike, the direct exposure of ECs to IGFs and insulin in the blood in combination with the overexpression of IR-A in tumour ECs underscore the impact of such resistance for both anti-tumour and anti-angiogenic effects. Moreover, global pathway analysis of gene expression signatures of IGFR inhibitor-resistant cell lines in comparison to sensitive cell lines revealed an enrichment for genes involved in VEGF signalling, axon guidance, hypoxia response and angiogenesis, suggesting that upon blocking IGF1R alternative mechanisms are activated that favour tumour vasculature growth and expansion (Garofalo *et al.*, 2011, 2012).

Combining anti-angiogenic therapeutics and IGF axis inhibitors has proven beneficial, albeit predominantly in preclinical models. Tumours expressing a dominant-negative isoform of IGF1R were more sensitive to VEGF blockade by bevacizumab (Avastin) and displayed reduced tumour growth and angiogenesis (Li *et al.*, 2011). Analogously, combined blocking of VEGF and IGF1R with bevacizumab and cixutumumab (IMC-A12) was more effective in inhibiting ovarian cancer growth than either antibody alone (Shao *et al.*, 2012). Interestingly, in this study, it was shown that blockade of IGF1R did overcome resistance to VEGF blockade (Shao *et al.*, 2012). At the time of writing, clinical trials combining anti-angiogenic agents and IGF(R) targeting are ongoing, but no results have been published so far (Table 2). For a recent overview of published clinical trials involving IGF1R pathway inhibition, see Iams & Lovly (2015).

Novel therapeutic moieties appearing on the horizon may also offer improved therapeutic efficacy over single treatments. For instance, a bispecific antibody fusion molecule targeting both IGF1R and VEGF crosslinks both targets and ensures internalization and degradation, and is a potent inhibitor of tumour growth in different xenograft models when compared to individual anti-IGF1R and anti-VEGF therapeutics. Moreover, this so-called bi-AbCap inhibited tumour growth and angiogenesis more effectively than combining anti-IGF1R and anti-VEGF antibodies (Shen *et al.*, 2015). In another study, adnectins, engineered target-binding protein therapeutics (Lipovsek, 2011), designed to inhibit VEGFR2 and IGF1R, demonstrated a potentiating effect on inhibiting tumour growth and angiogenesis in a Ewing sarcoma model (Ackermann *et al.*, 2012).

Although the combination therapies show more potent tumour growth inhibition, many of the published studies do not demonstrate superiority of the combination over the single therapies. Additional insight into the synergistic actions of IGFs with other growth factors on tumour angiogenesis and hence their therapeutic targetability could be of value for improving combined targeting. Furthermore, evidence-based therapeutic design, or stochastic modelling-based combination definition (Weiss *et al.*, 2015*a*,*b*; Nowak-Sliwinska *et al.*, 2016) is a powerful method to identify drugs that act synergistically with IGF(R) targeting agents. We expect that biomarker analysis, giving insight into the relative expression of the players in this signalling axis, will also aid in designing more effective combinations of inhibitors. Moreover, combining IGF/IGFR/IR therapeutics with other agents targeted at the tumour and endothelium will undoubtedly improve therapeutic outcome.

IV. CONCLUSIONS

(1) The IGF signalling pathway is not only a driver of malignant transformation but also intricately involved in sustaining tumour angiogenesis.

(2) The initial excitement surrounding IGF-axis-interfering drugs for targeting tumour growth has largely subsided due to disappointing clinical results. The extensively documented resistance to IGF-axis-targeted therapeutics can be mainly attributed to the intricate complexity of the pathway. Most notably, crosstalk with angiogenesis-stimulating factors, with pivotal roles for integrins and VEGF, allows for escape of therapeutic repression.

(3) Several strategies have been proposed for overcoming resistance including novel dual-targeting therapeutic moieties. Such inventions might drive the development of other novel agents co-targeting IGFR/IR and its interaction partners essential for tumour growth and angiogenesis such as $\alpha V\beta 3$ integrin. In addition, there is also opportunity for more effective interference in the IGF signalling axis itself.

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VI. REFERENCES

Abbas, A., Imrie, H., Viswambharan, H., Sukumar, P., Rajwani, A., Cubbon, R. M., Gage, M., Smith, J., Galloway, S., Yuldeshava, N., Kahn, M., Xuan, S., Grant, P. J., Channon, K. M., Beech, D. J., Wheatcroft, S. B. & Kearney, M. T. (2011). The insulin-like growth factor-1 receptor is a negative regulator of nitric oxide bioavailability and insulin sensitivity in the endothelium. *Diabetes* **60**, 2169–2178.

Ackermann, M., Morse, B. A., Delventhal, V., Carvajal, I. M. & Konerding, M. A. (2012). Anti-VEGFR2 and anti-IGF-1R-Adnectins inhibit Ewing's sarcoma A673-xenograft growth and normalize tumor vascular architecture. *Angiogenesis* **15**, 685–695.

Andres, S. F., Simmons, J. G., Mah, A. T., Santoro, M. A., Van, L. L. & Lund, P. K. (2013). Insulin receptor isoform switching in intestinal stem cells, progenitors, differentiated lineages and tumors: evidence that IR-B limits proliferation. *Journal of Cell Science* **126**, 5645–5656.

Andress, D. L., Loop, S. M., Zapf, J. & Kiefer, M. C. (1993). Carboxy-truncated insulin-like growth factor binding protein-5 stimulates mitogenesis in osteoblast-like cells. *Biochemical and Biophysical Research Communications* **195**, 25–30.

Andrieu-Soler, C., Berdugo, M., Doat, M., Courtois, Y., BenEzra, D. & Behar-Cohen, F. (2005). Downregulation of IRS-1 expression causes inhibition of corneal angiogenesis. *Investigative Ophthalmology & Visual Science* **46**, 4072–4078.

Asahara, T., Masuda, H., Takahashi, T., Kalka, C., Pastore, C., Silver, M., Kearne, M., Magner, M. & Isner, J. M. (1999). Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circulation Research* **85**, 221–228.

Asmane, I., Watkin, E., Alberti, L., Duc, A., Marec-Berard, P., Ray-Coquard, I., Cassier, P., Decouvelaere, A. V., Ranchere, D., Kurtz, J. E., Bergerat, J. P. & Blay, J. Y. (2012). Insulin-like growth factor type 1 receptor (IGF-1R) exclusive nuclear staining: a predictive biomarker for IGF-1R monoclonal antibody (Ab) therapy in sarcomas. *European Journal of Cancer* **48**, 3027–3035.

Azar, W. J., Azar, S. H., Higgins, S., Hu, J. F., Hoffman, A. R., Newgreen, D. F., Werther, G. A. & Russo, V. C. (2011). IGFBP-2 enhances VEGF gene promoter activity and consequent promotion of angiogenesis by neuroblastoma cells. *Endocrinology* **152**, 3332–3342.

Bach, L. A. (2005). IGFBP-6 five years on; not so 'forgotten'? *Growth Hormone & IGF Research* **15**, 185–192.

Bach, L. A. (2015). Endothelial cells and the IGF system. *Journal of Molecular Endocrinology* **54**, R1–R13.

Bae, M. H., Lee, M. J., Bae, S. K., Lee, O. H., Lee, Y. M., Park, B. C. & Kim, K. W. (1998). Insulin-like growth factor II (IGF-II) secreted from HepG2 human hepatocellular carcinoma cells shows angiogenic activity. *Cancer Letters* **128**, 41–46.

Bailyes, E. M., Nave, B. T., Soos, M. A., Orr, S. R., Hayward, A. C. & Siddle, K. (1997). Insulin receptor/IGF-I receptor hybrids are widely distributed in mammalian tissues: quantification of individual receptor species by selective immunoprecipitation and immunoblotting. *Biochemical Journal* **327**(Pt. 1), 209–215.

Baserga, R. (2013). The decline and fall of the IGF-I receptor. *Journal of Cellular Physiology* **228**, 675–679.

Beauvais, D. M. & Rapraeger, A. C. (2010). Syndecan-1 couples the insulin-like growth factor-1 receptor to inside-out integrin activation. *Journal of Cell Science* **123**, 3796–3807.

van Beijnum, J. R., Dings, R. P., van der Linden, E., Zwaans, B. M., Ramaekers, F. C., Mayo, K. H. & Griffioen, A. W. (2006). Gene expression of tumor angiogenesis dissected: specific targeting of colon cancer angiogenic vasculature. *Blood* **108**, 2339–2348.

van Beijnum, J. R. & Griffioen, A. W. (2005). In silico analysis of angiogenesis associated gene expression identifies angiogenic stage related profiles. *Biochimica et Biophysica Acta* **1755**, 121–134.

van Beijnum, J. R., Nowak-Sliwinska, P., Huijbers, E. J., Thijssen, V. L. & Griffioen, A. W. (2015). The great escape; the hallmarks of resistance to antiangiogenic therapy. *Pharmacological Reviews* **67**, 441–461.

Belfiore, A., Frasca, F., Pandini, G., Sciacca, L. & Vigneri, R. (2009). Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease. *Endocrine Reviews* **30**, 586–623.

Bid, H. K., London, C. A., Gao, J., Zhong, H., Hollingsworth, R. E., Fernandez, S., Mo, X. & Houghton, P. J. (2013). Dual targeting of the type 1 insulin-like growth factor receptor and its ligands as an effective antiangiogenic strategy. *Clinical Cancer Research* **19**, 2984–2994.

Borza, C. M. & Pozzi, A. (2014). Discoidin domain receptors in disease. *Matrix Biology* **34**, 185–192.

Bowers, L. W., Rossi, E. L., O'Flanagan, C. H., deGraffenried, L. A. & Hursting, S. D. (2015). The role of the insulin/IGF system in cancer: lessons learned from clinical trials and the energy balance-cancer link. *Frontiers in Endocrinology (Lausanne)* **6**, 77.

Brahmkhatri, V. P., Prasanna, C. & Atreya, H. S. (2015). Insulin-like growth factor system in cancer: novel targeted therapies. *Biomed Research International* **2015**, 538019.

Buck, E., Gokhale, P. C., Koujak, S., Brown, E., Eyzaguirre, A., Tao, N., Rosenfeld-Franklin, M., Lerner, L., Chiu, M. I., Wild, R., Epstein, D., Pachter, J. A. & Miglarese, M. R. (2010). Compensatory insulin receptor (IR) activation on inhibition of insulin-like growth factor-1 receptor (IGF-1R): rationale for cotargeting IGF-1R and IR in cancer. *Molecular Cancer Therapeutics* **9**, 2652–2664.

Carmeliet, P. & Jain, R. K. (2011). Molecular mechanisms and clinical applications of angiogenesis. *Nature* **473**, 298–307.

Chang, K. H., Chan-Ling, T., McFarland, E. L., Afzal, A., Pan, H., Baxter, L. C., Shaw, L. C., Caballero, S., Sengupta, N., Li, C. S., Sullivan, S. M. & Grant, M. B. (2007). IGF binding protein-3 regulates hematopoietic stem cell and endothelial precursor cell function during vascular development. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 10595–10600.

Chao, W. & D'Amore, P. A. (2008). IGF2: epigenetic regulation and role in development and disease. *Cytokine & Growth Factor Reviews* **19**, 111–120.

Che, W., Lerner-Marmarosh, N., Huang, Q., Osawa, M., Ohta, S., Yoshizumi, M., Glassman, M., Lee, J. D., Yan, C., Berk, B. C. & Abe, J. (2002). Insulin-like growth factor-1 enhances inflammatory responses in endothelial cells: role of Gab1 and MEKK3 in TNF-alpha-induced c-Jun and NF-kappaB activation and adhesion molecule expression. *Circulation Research* **90**, 1222–1230.

Chen, Y., Gou, X., Ke, X., Cui, H. & Chen, Z. (2012). Human tumor cells induce angiogenesis through positive feedback between CD147 and insulin-like growth factor-I. *PLoS One* **7**, e40965.

Chisalita, S. I. & Arnqvist, H. J. (2004). Insulin-like growth factor I receptors are more abundant than insulin receptors in human micro- and macrovascular endothelial cells. *American Journal of Physiology – Endocrinology and Metabolism* **286**, E896–E901.

Clemmons, D. R. & Maile, L. A. (2005). Interaction between insulin-like growth factor-I receptor and alphaVbeta3 integrin linked signaling pathways: cellular responses to changes in multiple signaling inputs. *Molecular Endocrinology* **19**, 1–11.

Cooke, J. P. (2003). NO and angiogenesis. *Atherosclerosis Supplements* **4**, 53–60.

Cui, H., Cruz-Correa, M., Giardiello, F. M., Hutcheon, D. F., Kafonek, D. R., Brandenburg, S., Wu, Y., He, X., Powe, N. R. & Feinberg, A. P. (2003). Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. *Science* **299**, 1753–1755.

Cursiefen, C., Viaud, E., Bock, F., Geudelin, B., Ferry, A., Kadlecova, P., Levy, M., Al, M. S., Colin, S., Thorin, E., Majo, F., Frueh, B., Wilhelm, F., Meyer-Ter-Vehn, T., Geerling, G., Bohringer, D., Reinhard, T., Meller, D., Pleyer, U., Bachmann, B. & Seitz, B. (2014). Aganirsen antisense oligonucleotide eye drops inhibit keratitis-induced corneal neovascularization and reduce need for transplantation: the I-CAN study. *Ophthalmology* **121**, 1683–1692.

Das, S. K., Bhutia, S. K., Kegelman, T. P., Peachy, L., Oyesanya, R. A., Dasgupta, S., Sokhi, U. K., Azab, B., Dash, R., Quinn, B. A., Kim, K., Barral, P. M., Su, Z. Z., Boukerche, H., Sarkar, D. & Fisher, P. B. (2012).

MDA-9/syntenin: a positive gatekeeper of melanoma metastasis. *Frontiers in Bioscience (Landmark Edition)* **17**, 1–15.

Delafontaine, P., Song, Y. H. & Li, Y. (2004). Expression, regulation, and function of IGF-1, IGF-1R, and IGF-1 binding proteins in blood vessels. *Arteriosclerosis, Thrombosis, and Vascular Biology* **24**, 435–444.

Denley, A., Wallace, J. C., Cosgrove, L. J. & Forbes, B. E. (2003). The insulin receptor isoform exon 11- (IR-A) in cancer and other diseases: a review. *Hormone and Metabolic Research* **35**, 778–785.

Drake, C. J., Cheresh, D. A. & Little, C. D. (1995). An antagonist of integrin alpha v beta 3 prevents maturation of blood vessels during embryonic neovascularization. *Journal of Cell Science* **108**(Pt. 7), 2655–2661.

Dulak, J., Jozkowicz, A., Dembinska-Kiec, A., Guevara, I., Zdzienicka, A., Zmudzinska-Grochot, D., Florek, I., Wojtowicz, A., Szuba, A. & Cooke, J. P. (2000). Nitric oxide induces the synthesis of vascular endothelial growth factor by rat vascular smooth muscle cells. *Arteriosclerosis, Thrombosis, and Vascular Biology* **20**, 659–666.

Fassnacht, M., Berruti, A., Baudin, E., Demeure, M. J., Gilbert, J., Haak, H., Kroiss, M., Quinn, D. I., Hesseltine, E., Ronchi, C. L., Terzolo, M., Choueiri, T. K., Poondru, S., Fleege, T., Rorig, R., Chen, J., Stephens, A. W., Worden, F. & Hammer, G. D. (2015). Linsitinib (OSI-906) versus placebo for patients with locally advanced or metastatic adrenocortical carcinoma: a double-blind, randomised, phase 3 study. *Lancet Oncology* **16**, 426–435.

Feng, N., Zhang, Z., Wang, Z., Zheng, H., Qu, F., He, X. & Wang, C. (2015). Insulin-like growth factor binding protein-2 promotes adhesion of endothelial progenitor cells to endothelial cells via integrin alpha5beta1. *Jorunal of Molecular Neuroscience* **3**, 426–434.

Firth, S. M. & Baxter, R. C. (2002). Cellular actions of the insulin-like growth factor binding proteins. *Endocrine Reviews* **23**, 824–854.

Forest, A., Amatulli, M., Ludwig, D. L., Damoci, C. B., Wang, Y., Burns, C. A., Donoho, G. P., Zanella, N., Fiebig, H. H., Prewett, M. C., Surguladze, D., DeLigio, J. T., Houghton, P. J., Smith, M. A. & Novosiadly, R. (2015). Intrinsic resistance to cixutumumab is conferred by distinct isoforms of the insulin receptor. *Molecular Cancer Research* **12**, 1615–1626.

Franklin, S. L., Ferry, R. J. Jr. & Cohen, P. (2003). Rapid insulin-like growth factor (IGF)-independent effects of IGF binding protein-3 on endothelial cell survival. *Journal of Clinical Endocrinology & Metabolism* **88**, 900–907.

Frasca, F., Pandini, G., Scalia, P., Sciacca, L., Mineo, R., Costantino, A., Goldfine, I. D., Belfiore, A. & Vigneri, R. (1999). Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. *Molecular and Cellular Biology* **19**, 3278–3288.

Frattali, A. L. & Pessin, J. E. (1993). Relationship between alpha subunit ligand occupancy and beta subunit autophosphorylation in insulin/insulin-like growth factor-1 hybrid receptors. *Journal of Biological Chemistry* **268**, 7393–7400.

Fujita, M., Ieguchi, K., Davari, P., Yamaji, S., Taniguchi, Y., Sekiguchi, K., Takada, Y. K. & Takada, Y. (2012). Cross-talk between integrin alpha6beta4 and insulin-like growth factor-1 receptor (IGF1R) through direct alpha6beta4 binding to IGF1 and subsequent alpha6beta4-IGF1-IGF1R ternary complex formation in anchorage-independent conditions. *Journal of Biological Chemistry* **287**, 12491–12500.

Fujita, M., Takada, Y. K. & Takada, Y. (2013). Insulin-like growth factor (IGF) signaling requires alphavbeta3-IGF1-IGF type 1 receptor (IGF1R) ternary complex formation in anchorage independence, and the complex formation does not require IGF1R and Src activation. *Journal of Biological Chemistry* **288**, 3059–3069.

Gao, J., Chang, Y. S., Jallal, B. & Viner, J. (2012). Targeting the insulin-like growth factor axis for the development of novel therapeutics in oncology. *Cancer Research* **72**, 3–12.

Garofalo, C., Manara, M. C., Nicoletti, G., Marino, M. T., Lollini, P. L., Astolfi, A., Pandini, G., Lopez-Guerrero, J. A., Schaefer, K. L., Belfiore, A., Picci, P. & Scotlandi, K. (2011). Efficacy of and resistance to anti-IGF-1R therapies in Ewing's sarcoma is dependent on insulin receptor signaling. *Oncogene* **30**, 2730–2740.

Garofalo, C., Mancarella, C., Grilli, A., Manara, M. C., Astolfi, A., Marino, M. T., Conte, A., Sigismund, S., Care, A., Belfiore, A., Picci, P. & Scotlandi, K. (2012). Identification of common and distinctive mechanisms of resistance to different anti-IGF-IR agents in Ewing's sarcoma. *Molecular Endocrinology* **26**, 1603–1616.

Giudice, J., Leskow, F. C., Arndt-Jovin, D. J., Jovin, T. M. & Jares-Erijman, E. A. (2011). Differential endocytosis and signaling dynamics of insulin receptor variants IR-A and IR-B. *Journal of Cell Science* **124**, 801–811.

Granata, R., Trovato, L., Lupia, E., Sala, G., Settanni, F., Camussi, G., Ghidoni, R. & Ghigo, E. (2007). Insulin-like growth factor binding protein-3 induces angiogenesis through IGF-I- and SphK1-dependent mechanisms. *Journal of Thrombosis and Haemostasis* **5**, 835–845.

Grant, M. B., Caballero, S. & Millard, W. J. (1993). Inhibition of IGF-I and b-FGF stimulated growth of human retinal endothelial cells by the somatostatin analogue, octreotide: a potential treatment for ocular neovascularization. *Regulatory Peptides* **48**, 267–278.

Griffioen, A. W. & Molema, G. (2000). Angiogenesis: potentials for pharmacologic intervention in the treatment of cancer, cardiovascular diseases, and chronic inflammation. *Pharmacological Reviews* **52**, 237–268.

Groskopf, J. C., Syu, L. J., Saltiel, A. R. & Linzer, D. I. (1997). Proliferin induces endothelial cell chemotaxis through a G protein-coupled, mitogen-activated protein kinase-dependent pathway. *Endocrinology* **138**, 2835–2840.

Gualberto, A. & Pollak, M. (2009). Emerging role of insulin-like growth factor receptor inhibitors in oncology: early clinical trial results and future directions. *Oncogene* **28**, 3009–3021.

Gurd, F. B. (1937). Postoperative use of insulin in the nondiabetic: with special reference to wound healing. *Annals of Surgery* **106**, 761–769.

Haluska, P., Menefee, M., Plimack, E. R., Rosenberg, J., Northfelt, D., LaVallee, T., Shi, L., Yu, X. Q., Burke, P., Huang, J., Viner, J., McDevitt, J. & LoRusso, P. (2014). Phase I dose-escalation study of MEDI-573, a bispecific, antiligand monoclonal antibody against IGFI and IGFII, in patients with advanced solid tumors. *Clinical Cancer Research* **20**, 4747–4757.

Han, R. N., Post, M., Tanswell, A. K. & Lye, S. J. (2003). Insulin-like growth factor-I receptor-mediated vasculogenesis/angiogenesis in human lung development. *American Journal of Respiratory Cell and Molecular Biology* **28**, 159–169.

Heidegger, I., Kern, J., Ofer, P., Klocker, H. & Massoner, P. (2014). Oncogenic functions of IGF1R and INSR in prostate cancer include enhanced tumor growth, cell migration and angiogenesis. *Oncotarget* **5**, 2723–2735.

Herr, F., Liang, O. D., Herrero, J., Lang, U., Preissner, K. T., Han, V. K. & Zygmunt, M. (2003). Possible angiogenic roles of insulin-like growth factor II and its receptors in uterine vascular adaptation to pregnancy. *Journal of Clinical Endocrinology & Metabolism* **88**, 4811–4817.

Hu, L., Roth, J. M., Brooks, P., Luty, J. & Karpatkin, S. (2008). Thrombin up-regulates cathepsin D which enhances angiogenesis, growth, and metastasis. *Cancer Research* **68**, 4666–4673.

Huijbers, E. J., van Beijnum, J. R., Thijssen, V. L., Sabrkhany, S., Nowak-Sliwinska, P. & Griffioen, A. W. (2016). Role of the tumor stroma in resistance to anti-angiogenic therapy. *Drug Resistance Updates* **25**, 26–37.

Humpert, P. M., Djuric, Z., Zeuge, U., Oikonomou, D., Seregin, Y., Laine, K., Eckstein, V., Nawroth, P. P. & Bierhaus, A. (2008). Insulin stimulates the clonogenic potential of angiogenic endothelial progenitor cells by IGF-1 receptor-dependent signaling. *Molecular Medicine* **14**, 301–308.

Hwa, V., Oh, Y. & Rosenfeld, R. G. (1999). The insulin-like growth factor-binding protein (IGFBP) superfamily. *Endocrine Reviews* **20**, 761–787.

Iams, W. T. & Lovly, C. M. (2015). Molecular pathways: clinical applications and future direction of insulin-like growth factor-1 receptor pathway blockade. *Clinical Cancer Research* **21**, 4270–4277.

Jackson, D., Volpert, O. V., Bouck, N. & Linzer, D. I. (1994). Stimulation and inhibition of angiogenesis by placental proliferin and proliferin-related protein. *Science* **266**, 1581–1584.

Jain, R. K. (2014). Antiangiogenesis strategies revisited: from starving tumors to alleviating hypoxia. *Cancer Cell* **26**, 605–622.

Janssen, J. A., Stolk, R. P., Pols, H. A., Grobbee, D. E. & Lamberts, S. W. (1998). Serum total IGF-I, free IGF-I, and IGFB-1 levels in an elderly population: relation to cardiovascular risk factors and disease. *Arteriosclerosis, Thrombosis, and Vascular Biology* **18**, 277–282.

Janssen, J. A. & Varewijck, A. J. (2014). IGF-IR targeted therapy: past, present and future. *Frontiers in Endocrinology (Lausanne)* **5**, 224.

Johansson, G. S., Chisalita, S. I. & Arnqvist, H. J. (2008). Human microvascular endothelial cells are sensitive to IGF-I but resistant to insulin at the receptor level. *Molecular and Cellular Endocrinology* **296**, 58–63.

Kahn, M. B., Yuldasheva, N. Y., Cubbon, R. M., Smith, J., Rashid, S. T., Viswambharan, H., Imrie, H., Abbas, A., Rajwani, A., Aziz, A., Baliga, V., Sukumar, P., Gage, M., Kearney, M. T. & Wheatcroft, S. B. (2011). Insulin resistance impairs circulating angiogenic progenitor cell function and delays endothelial regeneration. *Diabetes* **60**, 1295–1303.

Kellerer, M., Lammers, R., Ermel, B., Tippmer, S., Vogt, B., Obermaier-Kusser, B., Ullrich, A. & Haring, H. U. (1992). Distinct alpha-subunit structures of human insulin receptor A and B variants determine differences in tyrosine kinase activities. *Biochemistry* **31**, 4588–4596.

Kerbel, R. & Folkman, J. (2002). Clinical translation of angiogenesis inhibitors. *Nature Reviews Cancer* **2**, 727–739.

Kim, K. W., Bae, S. K., Lee, O. H., Bae, M. H., Lee, M. J. & Park, B. C. (1998). Insulin-like growth factor II induced by hypoxia may contribute to angiogenesis of human hepatocellular carcinoma. *Cancer Research* **58**, 348–351.

Kim, J. G., Kang, M. J., Yoon, Y. K., Kim, H. P., Park, J., Song, S. H., Han, S. W., Park, J. W., Kang, G. H., Kang, K. W., Oh, D. Y., Im, S. A., Bang, Y. J., Yi, E. C. & Kim, T. Y. (2012). Heterodimerization of glycosylated insulin-like growth factor-1 receptors and insulin receptors in cancer cells sensitive to anti-IGF1R antibody. *PLoS One* **7**, e33322.

King, H., Aleksic, T., Haluska, P. & Macaulay, V. M. (2014). Can we unlock the potential of IGF-1R inhibition in cancer therapy? *Cancer Treatment Reviews* **40**, 1096–1105.

Kondo, T., Vicent, D., Suzuma, K., Yanagisawa, M., King, G. L., Holzenberger, M. & Kahn, C. R. (2003). Knockout of insulin and IGF-1 receptors on vascular endothelial cells protects against retinal neovascularization. *Journal of Clinical Investigation* **111**, 1835–1842.

Korner, C., Nurnberg, B., Uhde, M. & Braulke, T. (1995). Mannose 6-phosphate/insulin-like growth factor II receptor fails to interact with G-proteins. Analysis of mutant cytoplasmic receptor domains. *Journal of Biological Chemistry* **270**, 287–295.

Kotsinas, A., Evangelou, K., Sideridou, M., Kotzamanis, G., Constantinides, C., Zavras, A. I., Douglass, C. W., Papavassiliou, A. G. & Gorgoulis, V. G. (2008). The 3' UTR IGF2R-A2/B2 variant is associated with increased tumor growth and advanced stages in non-small cell lung cancer. *Cancer Letters* **259**, 177–185.

Kurmasheva, R. T., Dudkin, L., Billups, C., Debelenko, L. V., Morton, C. L. & Houghton, P. J. (2009). The insulin-like growth factor-1 receptor-targeting antibody, CP-751,871, suppresses tumor-derived VEGF and synergizes with rapamycin in models of childhood sarcoma. *Cancer Research* **69**, 7662–7671.

Lalou, C., Lassarre, C. & Binoux, M. (1996). Isolation and characterization of proteolytic fragments of insulin-like growth factor-binding protein-3. *Hormone Research* **45**, 156–159.

Lee, O. H., Bae, S. K., Bae, M. H., Lee, Y. M., Moon, E. J., Cha, H. J., Kwon, Y. G. & Kim, K. W. (2000). Identification of angiogenic properties of insulin-like growth factor II in in vitro angiogenesis models. *British Journal of Cancer* **82**, 385–391.

Lee, S. J. & Nathans, D. (1988). Proliferin secreted by cultured cells binds to mannose 6-phosphate receptors. *Journal of Biological Chemistry* **263**, 3521–3527.

Leibiger, B., Leibiger, I. B., Moede, T., Kemper, S., Kulkarni, R. N., Kahn, C. R., de Vargas, L. M. & Berggren, P. O. (2001). Selective insulin signaling through A and B insulin receptors regulates transcription of insulin and glucokinase genes in pancreatic beta cells. *Molecular Cell* **7**, 559–570.

Leitinger, B. (2014). Discoidin domain receptor functions in physiological and pathological conditions. *International Review of Cell and Molecular Biology* **310**, 39–87.

Li, H., Adachi, Y., Yamamoto, H., Min, Y., Ohashi, H., Ii, M., Arimura, Y., Endo, T., Lee, C. T., Carbone, D. P., Imai, K. & Shinomura, Y. (2011). Insulin-like growth factor-I receptor blockade reduces tumor angiogenesis and enhances the effects of bevacizumab for a human gastric cancer cell line, MKN45. *Cancer* **117**, 3135–3147.

Li, G., Barrett, E. J., Wang, H., Chai, W. & Liu, Z. (2005). Insulin at physiological concentrations selectively activates insulin but not insulin-like growth factor I (IGF-I) or insulin/IGF-I hybrid receptors in endothelial cells. *Endocrinology* **146**, 4690–4696.

Lipovsek, D. (2011). Adnectins: engineered target-binding protein therapeutics. *Protein Engineering, Design & Selection* **24**, 3–9.

Liu, B., Lee, K. W., Anzo, M., Zhang, B., Zi, X., Tao, Y., Shiry, L., Pollak, M., Lin, S. & Cohen, P. (2007). Insulin-like growth factor-binding protein-3 inhibition of prostate cancer growth involves suppression of angiogenesis. *Oncogene* **26**, 1811–1819.

Liu, Y., Petreaca, M. & Martins-Green, M. (2009). Cell and molecular mechanisms of insulin-induced angiogenesis. *Journal of Cellular and Molecular Medicine* **13**, 4492–4504.

Livingstone, C. (2013). IGF2 and cancer. *Endocrine-Related Cancer* **20**, R321–R339.

Maeng, Y. S., Choi, H. J., Kwon, J. Y., Park, Y. W., Choi, K. S., Min, J. K., Kim, Y. H., Suh, P. G., Kang, K. S., Won, M. H., Kim, Y. M. & Kwon, Y. G. (2009). Endothelial progenitor cell homing: prominent role of the IGF2-IGF2R-PLCbeta2 axis. *Blood* **113**, 233–243.

Malaguarnera, R., Nicolosi, M. L., Sacco, A., Morcavallo, A., Vella, V., Voci, C., Spatuzza, M., Xu, S. Q., Iozzo, R. V., Vigneri, R., Morrione, A. & Belfiore, A. (2015). Novel cross talk between IGF-IR and DDR1 regulates IGF-IR trafficking, signaling and biological responses. *Oncotarget* **6**, 16084–16105.

van Meeteren, L. A., Goumans, M. J. & ten Dijke, P. (2011). TGF-beta receptor signaling pathways in angiogenesis; emerging targets for anti-angiogenesis therapy. *Current Pharmaceutical Biotechnology* **12**, 2108–2120.

Merimee, T. J., Zapf, J. & Froesch, E. R. (1983). Insulin-like growth factors. Studies in diabetics with and without retinopathy. *New England Journal of Medicine* **309**, 527–530.

Michell, B. J., Griffiths, J. E., Mitchelhill, K. I., Rodriguez-Crespo, I., Tiganis, T., Bozinovski, S., de Montellano, P. R., Kemp, B. E. & Pearson, R. B. (1999). The Akt kinase signals directly to endothelial nitric oxide synthase. *Current Biology* **9**, 845–848.

Millimaggi, D., Mari, M., D'Ascenzo, S., Carosa, E., Jannini, E. A., Zucker, S., Carta, G., Pavan, A. & Dolo, V. (2007). Tumor vesicle-associated CD147 modulates the angiogenic capability of endothelial cells. *Neoplasia* **9**, 349–357.

Moody, G., Beltran, P. J., Mitchell, P., Cajulis, E., Chung, Y. A., Hwang, D., Kendall, R., Radinsky, R., Cohen, P. & Calzone, F. J. (2014). IGF1R blockade with ganitumab results in systemic effects on the GH-IGF axis in mice. *Journal of Endocrinology* **221**, 145–155.

Morcavallo, A., Gaspari, M., Pandini, G., Palummo, A., Cuda, G., Larsen, M. R., Vigneri, R. & Belfiore, A. (2011). Research resource: new and diverse substrates for the insulin receptor isoform A revealed by quantitative proteomics after stimulation with IGF-II or insulin. *Molecular Endocrinology* **25**, 1456–1468.

Moreno, M. J., Ball, M., Andrade, M. F., McDermid, A. & Stanimirovic, D. B. (2006). Insulin-like growth factor binding protein-4 (IGFBP-4) is a novel anti-angiogenic and anti-tumorigenic mediator secreted by dibutyryl cyclic AMP (dB-cAMP)-differentiated glioblastoma cells. *Glia* **53**, 845–857.

Moreno, M. J., Ball, M., Rukhlova, M., Slinn, J., L'abbe, D., Iqbal, U., Monette, R., Hagedorn, M., O'Connor-McCourt, M. D., Durocher, Y. & Stanimirovic, D. B. (2013). IGFBP-4 anti-angiogenic and anti-tumorigenic effects are associated with anti-cathepsin B activity. *Neoplasia* **15**, 554–567.

Mosthaf, L., Grako, K., Dull, T. J., Coussens, L., Ullrich, A. & McClain, D. A. (1990). Functionally distinct insulin receptors generated by tissue-specific alternative splicing. *EMBO Journal* **9**, 2409–2413.

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

Nitert, M. D., Chisalita, S. I., Olsson, K., Bornfeldt, K. E. & Arnqvist, H. J. (2005). IGF-I/insulin hybrid receptors in human endothelial cells. *Molecular and Cellular Endocrinology* **229**, 31–37.

Nowak-Sliwinska, P., Weiss, A., Ding, X., Dyson, P. J., van den Bergh, H., Griffioen, A. W. & Ho, C. M. (2016). Optimization of drug combinations using Feedback System Control. *Nature Protocols* **11**, 302–315.

Okamoto, T., Katada, T., Murayama, Y., Ui, M., Ogata, E. & Nishimoto, I. (1990). A simple structure encodes G protein-activating function of the IGF-II/mannose 6-phosphate receptor. *Cell* **62**, 709–717.

Otsubo, T., Hida, Y., Ohga, N., Sato, H., Kai, T., Matsuki, Y., Takasu, H., Akiyama, K., Maishi, N., Kawamoto, T., Shinohara, N., Nonomura, K. & Hida, K. (2014). Identification of novel targets for antiangiogenic therapy by comparing the gene expressions of tumor and normal endothelial cells. *Cancer Science* **105**, 560–567.

Pan, Y., Liang, H., Liu, H., Li, D., Chen, X., Li, L., Zhang, C. Y. & Zen, K. (2014). Platelet-secreted microRNA-223 promotes endothelial cell apoptosis induced by advanced glycation end products via targeting the insulin-like growth factor 1 receptor. *Journal of Immunology* **192**, 437–446.

Pandini, G., Frasca, F., Mineo, R., Sciacca, L., Vigneri, R. & Belfiore, A. (2002). Insulin/insulin-like growth factor I hybrid receptors have different biological characteristics depending on the insulin receptor isoform involved. *Journal of Biological Chemistry* **277**, 39684–39695.

Pollak, M. (2012). The insulin receptor/insulin-like growth factor receptor family as a therapeutic target in oncology. *Clinical Cancer Research* **18**, 40–50.

Premzl, A., Turk, V. & Kos, J. (2006). Intracellular proteolytic activity of cathepsin B is associated with capillary-like tube formation by endothelial cells in vitro. *Journal of Cellular Biochemistry* **97**, 1230–1240.

Rajah, R., Katz, L., Nunn, S., Solberg, P., Beers, T. & Cohen, P. (1995). Insulin-like growth factor binding protein (IGFBP) proteases: functional regulators of cell growth. *Progress in Growth Factor Research* **6**, 273–284.

Rajaram, S., Baylink, D. J. & Mohan, S. (1997). Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. *Endocrine Reviews* **18**, 801–831.

Rapraeger, A. C. (2013). Synstatin: a selective inhibitor of the syndecan-1-coupled IGF1R-alphavbeta3 integrin complex in tumorigenesis and angiogenesis. *FEBS Journal* **280**, 2207–2215.

Rapraeger, A. C., Ell, B. J., Roy, M., Li, X., Morrison, O. R., Thomas, G. M. & Beauvais, D. M. (2013). Vascular endothelial-cadherin stimulates syndecan-1-coupled insulin-like growth factor-1 receptor and cross-talk between alphaVbeta3 integrin and vascular endothelial growth factor receptor 2 at the onset of endothelial cell dissemination during angiogenesis. *FEBS Journal* **280**, 2194–2206.

Reidy, D. L., Vakiani, E., Fakih, M. G., Saif, M. W., Hecht, J. R., Goodman-Davis, N., Hollywood, E., Shia, J., Schwartz, J., Chandrawansa, K., Dontabhaktuni, A., Youssoufian, H., Solit, D. B. & Saltz, L. B. (2010). Randomized, phase II study of the insulin-like growth factor-1 receptor inhibitor IMC-A12, with or without cetuximab, in patients with cetuximab- or panitumumab-refractory metastatic colorectal cancer. *Journal of Clinical Oncology* **28**, 4240–4246.

Rensing, K. L., Houttuijn Bloemendaal, F. M., Weijers, E. M., Richel, D. J., Buller, H. R., Koolwijk, P., van der Loos, C. M., Twickler, T. B. & von der Thusen, J. H. (2010). Could recombinant insulin compounds contribute to adenocarcinoma progression by stimulating local angiogenesis? *Diabetologia* **53**, 966–970.

Rho, S. B., Dong, S. M., Kang, S., Seo, S. S., Yoo, C. W., Lee, D. O., Woo, J. S. & Park, S. Y. (2008). Insulin-like growth factor-binding protein-5 (IGFBP-5) acts as a tumor suppressor by inhibiting angiogenesis. *Carcinogenesis* **29**, 2106–2111.

Rosenzweig, S. A. & Atreya, H. S. (2010). Defining the pathway to insulin-like growth factor system targeting in cancer. *Biochemical Pharmacology* **80**, 1115–1124.

Roysarkar, T. K., Gupta, A., Dash, R. J. & Dogra, M. R. (1993). Effect of insulin therapy on progression of retinopathy in noninsulin-dependent diabetes mellitus. *American Journal of Ophthalmology* **115**, 569–574.

Ruan, W., Kang, Z., Li, Y., Sun, T., Wang, L., Liang, L., Lai, M. & Wu, T. (2016). Interaction between IGFBP7 and insulin: a theoretical and experimental study. *Scientific Reports* **6**, 19586.

Sacco, A., Morcavallo, A., Pandini, G., Vigneri, R. & Belfiore, A. (2009). Differential signaling activation by insulin and insulin-like growth factors I and II upon binding to insulin receptor isoform A. *Endocrinology* **150**, 3594–3602.

Saegusa, J., Yamaji, S., Ieguchi, K., Wu, C. Y., Lam, K. S., Liu, F. T., Takada, Y. K. & Takada, Y. (2009). The direct binding of insulin-like growth factor-1 (IGF-1) to integrin alphavbeta3 is involved in IGF-1 signaling. *Journal of Biological Chemistry* **284**, 24106–24114.

Salisbury, A. J. & Macaulay, V. M. (2003). Development of molecular agents for IGF receptor targeting. *Hormone and Metabolic Research* **35**, 843–849.

Schmitz, S., Kaminsky-Forrett, M. C., Henry, S., Zanetta, S., Geoffrois, L., Bompas, E., Moxhon, A., Mignion, L., Guigay, J., Knoops, L., Hamoir, M. & Machiels, J. P. (2012). Phase II study of figitumumab in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck: clinical activity and molecular response (GORTEC 2008-02). *Annals of Oncology* **23**, 2153–2161.

Seino, S. & Bell, G. I. (1989). Alternative splicing of human insulin receptor messenger RNA. *Biochemical and Biophysical Research Communications* **159**, 312–316.

Sell, C., Rubini, M., Rubin, R., Liu, J. P., Efstratiadis, A. & Baserga, R. (1993). Simian virus 40 large tumor antigen is unable to transform mouse embryonic fibroblasts lacking type 1 insulin-like growth factor receptor. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 11217–11221.

Shao, M., Hollar, S., Chambliss, D., Schmitt, J., Emerson, R., Chelladurai, B., Perkins, S., Ivan, M. & Matei, D. (2012). Targeting the insulin growth factor and the vascular endothelial growth factor pathways in ovarian cancer. *Molecular Cancer Therapeutics* **11**, 1576–1586.

Shen, Y., Zeng, L., Novosyadlyy, R., Forest, A., Zhu, A., Korytko, A., Zhang, H., Eastman, S. W., Topper, M., Hindi, S., Covino, N., Persaud, K., Kang, Y., Burtrum, D., Surguladze, D., Prewett, M., Chintharlapalli, S., Wroblewski, V. J., Shen, J., Balderes, P., Zhu, Z., Snavely, M. & Ludwig, D. L. (2015). A bi-functional antibody-receptor domain fusion protein simultaneously targeting IGF-IR and VEGF for degradation. *MAbs* **7**, 931–945.

Shigematsu, S., Yamauchi, K., Nakajima, K., Iijima, S., Aizawa, T. & Hashizume, K. (1999). IGF-1 regulates migration and angiogenesis of human endothelial cells. *Endocrine Journal* **46**(Suppl), S59–S62.

Shin, D. H., Lee, H. J., Min, H. Y., Choi, S. P., Lee, M. S., Lee, J. W., Johnson, F. M., Mehta, K., Lippman, S. M., Glisson, B. S. & Lee, H. Y. (2013). Combating resistance to anti-IGFR antibody by targeting the integrin beta3-Src pathway. *Journal of the National Cancer Institute* **105**, 1558–1570.

Siddle, K. (2011). Signalling by insulin and IGF receptors: supporting acts and new players. *Journal of Molecular Endocrinology* **47**, R1–R10.

Siddle, K. (2012). Molecular basis of signaling specificity of insulin and IGF receptors: neglected corners and recent advances. *Frontiers in Endocrinology (Lausanne)* **3**, 34.

Slomiany, M. G. & Rosenzweig, S. A. (2006). Hypoxia-inducible factor-1-dependent and -independent regulation of insulin-like growth factor-1-stimulated vascular endothelial growth factor secretion. *Journal of Pharmacology and Experimental Therapeutics* **318**, 666–675.

Soh, C. L., McNeil, K., Owczarek, C. M., Hardy, M. P., Fabri, L. J., Pearse, M., Delaine, C. A. & Forbes, B. E. (2014). Exogenous administration of protease-resistant, non-matrix-binding IGFBP-2 inhibits tumour growth in a murine model of breast cancer. *British Journal of Cancer* **110**, 2855–2864.

Spoerri, P. E., Ellis, E. A., Tarnuzzer, R. W. & Grant, M. B. (1998). Insulin-like growth factor: receptor and binding proteins in human retinal endothelial cell cultures of diabetic and non-diabetic origin. *Growth Hormone & IGF Research* **8**, 125–132.

St Croix, C. B., Rago, C., Velculescu, V., Traverso, G., Romans, K. E., Montgomery, E., Lal, A., Riggins, G. J., Lengauer, C., Vogelstein, B. & Kinzler, K. W. (2000). Genes expressed in human tumor endothelium. *Science* **289**, 1197–1202.

Sukhanov, S., Higashi, Y., Shai, S. Y., Vaughn, C., Mohler, J., Li, Y., Song, Y. H., Titterington, J. & Delafontaine, P. (2007). IGF-1 reduces inflammatory responses, suppresses oxidative stress, and decreases atherosclerosis progression in ApoE-deficient mice. *Arteriosclerosis, Thrombosis, and Vascular Biology* **27**, 2684–2690.

Takahashi, T., Kalka, C., Masuda, H., Chen, D., Silver, M., Kearney, M., Magner, M., Isner, J. M. & Asahara, T. (1999). Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nature Medicine* **5**, 434–438.

Takuwa, Y., Du, W., Qi, X., Okamoto, Y., Takuwa, N. & Yoshioka, K. (2010). Roles of sphingosine-1-phosphate signaling in angiogenesis. *World Journal of Biological Chemistry* **1**, 298–306.

Tamura, K., Hashimoto, K., Suzuki, K., Yoshie, M., Kutsukake, M. & Sakurai, T. (2009). Insulin-like growth factor binding protein-7 (IGFBP7) blocks vascular endothelial cell growth factor (VEGF)-induced angiogenesis in human vascular endothelial cells. *European Journal of Pharmacology* **610**, 61–67.

Thum, T., Hoeber, S., Froese, S., Klink, I., Stichtenoth, D. O., Galuppo, P., Jakob, M., Tsikas, D., Anker, S. D., Poole-Wilson, P. A., Borlak, J., Ertl, G. & Bauersachs, J. (2007). Age-dependent impairment of endothelial progenitor cells is corrected by growth-hormone-mediated increase of insulin-like growth-factor-1. *Circulation Research* **100**, 434–443.

Ulanet, D. B., Ludwig, D. L., Kahn, C. R. & Hanahan, D. (2010). Insulin receptor functionally enhances multistage tumor progression and conveys intrinsic resistance to IGF-1R targeted therapy. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 10791–10798.

Valiathan, R. R., Marco, M., Leitinger, B., Kleer, C. G. & Fridman, R. (2012). Discoidin domain receptor tyrosine kinases: new players in cancer progression. *Cancer Metastasis Reviews* **31**, 295–321.

Vogt, B., Carrascosa, J. M., Ermel, B., Ullrich, A. & Haring, H. U. (1991). The two isotypes of the human insulin receptor (HIR-A and HIR-B) follow different internalization kinetics. *Biochemical and Biophysical Research Communications* **177**, 1013–1018.

Volpert, O., Jackson, D., Bouck, N. & Linzer, D. I. (1996). The insulin-like growth factor II/mannose 6-phosphate receptor is required for proliferin-induced angiogenesis. *Endocrinology* **137**, 3871–3876.

Wan, X., Harkavy, B., Shen, N., Grohar, P. & Helman, L. J. (2007). Rapamycin induces feedback activation of Akt signaling through an IGF-1R-dependent mechanism. *Oncogene* **26**, 1932–1940.

Wang, G. K., Hu, L., Fuller, G. N. & Zhang, W. (2006). An interaction between insulin-like growth factor-binding protein 2 (IGFBP2) and integrin alpha5 is essential for IGFBP2-induced cell mobility. *Journal of Biological Chemistry* **281**, 14085–14091.

Wang, N., Rayes, R. F., Elahi, S. M., Lu, Y., Hancock, M. A., Massie, B., Rowe, G. E., Aomari, H., Hossain, S., Durocher, Y., Pinard, M., Tabaries, S., Siegel, P. M. & Brodt, P. (2015). The IGF-Trap: novel inhibitor of carcinoma growth and metastasis. *Molecular Cancer Therapeutics* **14**, 982–993.

Weiss, A., Berndsen, R. H., Ding, X., Ho, C. M., Dyson, P. J., van den Bergh, H., Griffioen, A. W. & Nowak-Sliwinska, P. (2015*a*). A streamlined search technology for identification of synergistic drug combinations. *Scientific Reports* **5**, 14508.

Weiss, A., Ding, X., van Beijnum, J. R., Wong, I., Wong, T. J., Berndsen, R. H., Dormond, O., Dallinga, M., Shen, L., Schlingemann, R. O., Pili, R., Ho, C. M., Dyson, P. J., van den Bergh, H., Griffioen, A. W. & Nowak-Sliwinska, P. (2015*b*). Rapid optimization of drug combinations for the optimal angiostatic treatment of cancer. *Angiogenesis* **18**, 233–244.

Wheatcroft, S. B. & Kearney, M. T. (2009). IGF-dependent and IGF-independent actions of IGF-binding protein-1 and -2: implications for metabolic homeostasis. *Trends Endocrinology and Metabolism* **20**, 153–162.

Woo, J. K., Choi, Y., Oh, S. H., Jeong, J. H., Choi, D. H., Seo, H. S. & Kim, C. W. (2012). Mucin 1 enhances the tumor angiogenic response by activation of the AKT signaling pathway. *Oncogene* **31**, 2187–2198.

Xiao, Q., Jiang, Y., Liu, Q., Yue, J., Liu, C., Zhao, X., Qiao, Y., Ji, H., Chen, J. & Ge, G. (2015). Minor type IV collagen alpha5 chain promotes cancer progression through discoidin domain receptor-1. *PLoS Genetics* **11**, e1005249.

Xu, J. W., Wang, T. X., You, L., Zheng, L. F., Shu, H., Zhang, T. P. & Zhao, Y. P. (2014). Insulin-like growth factor 1 receptor (IGF-1R) as a target of MiR-497 and plasma IGF-1R levels associated with TNM stage of pancreatic cancer. *PLoS One* **9**, e92847.

Yamanaka, Y., Wilson, E. M., Rosenfeld, R. G. & Oh, Y. (1997). Inhibition of insulin receptor activation by insulin-like growth factor binding proteins. *Journal of Biological Chemistry* **272**, 30729–30734.

Zavras, A. I., Pitiphat, W., Wu, T., Cartsos, V., Lam, A., Douglass, C. W. & Diehl, S. R. (2003). Insulin-like growth factor II receptor gene-167 genotype increases the risk of oral squamous cell carcinoma in humans. *Cancer Research* **63**, 296–297.

Zeng, G. & Quon, M. J. (1996). Insulin-stimulated production of nitric oxide is inhibited by wortmannin. Direct measurement in vascular endothelial cells. *Journal of Clinical Investigation* **98**, 894–898.

Zhang, H., Fagan, D. H., Zeng, X., Freeman, K. T., Sachdev, D. & Yee, D. (2010). Inhibition of cancer cell proliferation and metastasis by insulin receptor downregulation. *Oncogene* **29**, 2517–2527.

Zhang, C., Lu, L., Li, Y., Wang, X., Zhou, J., Liu, Y., Fu, P., Gallicchio, M. A., Bach, L. A. & Duan, C. (2012). IGF binding protein-6 expression in vascular endothelial cells is induced by hypoxia and plays a negative role in tumor angiogenesis. *International Journal of Cancer* **130**, 2003–2012.

Zhao, W., Wang, J., Zhu, B., Duan, Y., Chen, F., Nian, W., Sun, J., Zhang, B., Tong, Z. & Chen, Z. (2016). IGFBP7 functions as a potential lymphangiogenesis inducer in non-small cell lung carcinoma. *Oncology Reports* **35**, 1483–1492.

Zhu, S., Xu, F., Zhang, J., Ruan, W. & Lai, M. (2014). Insulin-like growth factor binding protein-related protein 1 and cancer. *Clinica Chimica Acta* **431**, 23–32.

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