IGF signalling in germ cells and testicular germ cell tumours: roles and therapeutic approaches

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INTRODUCTION
The insulin and insulin-like growth factor (IGF) signalling system has been implicated in a vast array of both physiological and pathological cellular processes. The IGF family principally comprises three ligands (insulin, IGF1 and IGF2), three receptors (the insulin receptor (IR), the insulin-like growth factor 1 receptor (IGF1R) and the insulin-like growth factor 2 receptor (IGF2R)) in addition to six high affinity ligand binding proteins (IGFBP1-6), and accessory proteins such as IGFBP-specific proteases. The signalling through IR/IGFR is complex (see Fig. 1). IR and IGF1R are tyrosine kinase receptors that share a high degree of structural homology and can exist as either homo- or heterotetramers (hybrid receptors). Additional complexity arises through alternative splicing of the INSR gene resulting in two IR subunits IR-A and IR-B, which have differing affinities for insulin and the IGF ligands (Seino & Bell, 1989). This results in a total of seven different receptors. Insulin can signal through any receptor containing at least one IR subunit while IGF1 can signal through any receptor containing at least one IGF1R subunit. IGF2 can signal through the same receptors as IGF1 in addition to the IR-A homotetramer as well as the IGF2R homodimer (reviewed in Simpson et al., 2017). IGF2R is structurally unrelated to IR/IGF1R and possesses no tyrosine kinase activity or autophosphorylation sites. The IGF2R receptor can bind to G-proteins, however, and despite previous assertions that IGF2R functions only to control IGF2 ligand levels by acting as a sink receptor, it is possible that it can initiate downstream signalling (El-Shewy et al., 2007). While signalling through the IR-A/B and IR-B receptors mainly contributes to glucose homeostasis, signalling through the IGF1R hetero- and homotetramers generally leads to activation of both anti-apoptotic mechanisms resulting in increased cell survival and increased cellular proliferation and growth in normal and malignant tissues.

ROLE OF IGF SYSTEM IN TESTICULAR FUNCTION: LESSONS LEARNED FROM GENETIC MODELS
Signalling through the insulin and Igf receptors are absolutely required for sex determination in mice (Nef et al., 2003). There is complete XY sex reversal due to failure to upregulate Sry and a complete failure of the testicular genetic programme in mouse embryos with homozygous deletion of the Insr and Igf1r genes. Ovarian differentiation is also delayed in XX gonads in the same model, although the ovarian genetic programme is eventually initiated several days later than in control embryos (Pitetti et al., 2013a). It has subsequently been shown that specific ablation of the Insr and Igf1r genes in Sertoli cells drastically influences testis size, Sertoli cell number and sperm production, whereas deletion of these genes in just the germ cells themselves results in normal testicular function and size (Pitetti et al., 2013b). The same study showed that a disruption to the neonatal action of
IGF ligands can bind competitively to these receptors as shown. Bioavailability of IGF ligands is regulated by insulin-like growth factor binding proteins (IGFBPs). IGFBPs bound to ligands can also bind the acid labile subunit (ALS) in the bloodstream to form a ternary complex which is thought to be unable to cross the capillary endothelium unless partially dissociated. Insulin signalling primarily affects glucose metabolism (but can also regulate other functions including growth), signalling through the INSR-A homotetramer or any receptor containing IGF1R subunits results in downstream activation of pathways involved in proliferation, prevention of apoptosis and migration. The structurally unrelated IGF2R receptor binds IGF2, the functional significance of which is yet to be fully established but may act to regulate extracellular IGF2 levels.

**Figure 1** Insulin and IGF ligand specificity for INSR/IGF1R receptors. Each of the insulin receptors INSR-A, INSR-B and IGF1R transcripts encodes a single polypeptide chain, which undergoes proteolytic cleavage to produce an α and β subunit. Each αβ subunit forms homo- or heterotetramers. Insulin and the IGF ligands can bind competitively to these receptors as shown. Bioavailability of IGF ligands is regulated by insulin-like growth factor binding proteins 1–6 (IGFBPs). IGFBPs bound to ligands can also bind the acid labile subunit (ALS) in the bloodstream to form a ternary complex which is thought to be unable to cross the capillary endothelium unless partially dissociated. Insulin signalling primarily affects glucose metabolism (but can also regulate other functions including growth), signalling through the INSR-A homotetramer or any receptor containing IGF1R subunits results in downstream activation of pathways involved in proliferation, prevention of apoptosis and migration. The structurally unrelated IGF2R receptor binds IGF2, the functional significance of which is yet to be fully established but may act to regulate extracellular IGF2 levels.

follicle-stimulating hormone (FSH) on immature Sertoli cells occurred in the absence of Insr/Igf1r (IIR) signalling. A more recent study concluded that although foetal Leydig cell function is normal, adult Leydig cells fail to mature using the constitutive double knockout model (Neirijnck et al., 2018).

IGF1 has reversed testicular atrophy induced by cirrhosis of the liver in rats (in which IGF1 levels are reduced), resulting in full recovery of testicular weight and reversal of all histopathological abnormalities (Castilla-Cortazar et al., 2000). Although specific abrogation of IIR signalling in germ cells did not appear to impair testicular function in mice, there is evidence that germ cell function is profoundly affected by IGF signalling. In keeping with the recognized role of IGF1 as an inhibitor of apoptosis, in vitro organ culture of mouse testicular fragments supplemented with IGF1 increased the density of germ cells by decreasing apoptosis (Yao et al., 2017). Mice carrying a transgene-expressing IGFBP1 exhibited defects in spermatogenesis with altered production and quality of spermatozoa, attributed to the lack of bio-available IGF1 caused by increased binding of IGFBP1 (Froment et al., 2004). IGF1 administration was able to reverse the decrease in germ cell numbers observed in rats with surgically induced unilateral testicular atrophy (Bingol-Kologlu et al., 2010). Co-culture of Leydig cells with mouse spermatogonial stem cells (SSCs) and subsequent blockade of IIR signalling led to loss of expression of pluripotent genes in SSCs, supporting the idea that IGF1 produced by Leydig cells can maintain SSC pluripotency (Huang et al., 2009). IGF1 has also been shown to influence steroidogenesis in cultured Leydig cells (Lin et al., 1986). Insulin receptor substrate 2 (IRS2) is one of the key downstream effectors of IIR signalling and has itself been implicated in testicular development. Mice with homozygous deletion of the IRS2 have reduced testicular weight with lower numbers of Sertoli cells, spermatagonia and spermatocytes (Griffeth et al., 2013).

In a zebrafish model, either ectopic overexpression of IGF-I or dominant negative expression of IGF receptors in primordial germ cells (the putative cells of origin in TGCT) leads to defects in migration of these cells to the genital ridge (Sang et al., 2008). A separate study found that knocking down the IGFR1b gene in zebrafish embryos resulted in both mismigration and elimination of primordial germ cells (Schlueter et al., 2007). In C.elegans, mutations in the daf-2 gene (the single gene encoding an insulin/insulin-like growth factor receptor in this species) led to infertility (Tissenbaum & Ruvkun, 1998) and a study using a conditional daf-2 allele demonstrated the necessity of IIR signalling for larval germ cell proliferation by promoting cell cycle progression (Michaelson et al., 2010). Together, these studies provide evidence for an essential role of IGF signalling in supporting the normal development of germ cells.

**ROLE OF IGF SYSTEM IN CANCER RISK**

There are several lines of long-standing evidence linking higher serum levels of IGF1 (associated with increased growth) and decreased serum levels of some IGFBPs (associated with suppressing growth through binding IGF1) with additional cancer risk (reviewed in Crowe et al., 2011). The congenital overgrowth disorder, Beckwith–Wiedemann syndrome, is associated with increased cancer risk and is frequently associated with disrupted imprinting of the IGF2 gene. The gene encoding the potent mitogen IGF2 is imprinted in normal somatic cells, with
only the paternal allele being expressed. Loss of imprinting in this chromosomal location results in increased IGF2 levels by being biallelically expressed (Mussa et al., 2016).

It is noted that both height and body mass index (BMI) correlate with higher cancer risk and with increased circulating IGF1 levels and/or decreased IGFBP levels (Nunney, 2018). Consistent with these findings, patients with congenital secondary IGF1 deficiency are less likely to develop cancer (Steuerman et al., 2011), while mice with reduced circulating Igf1 levels experience delayed onset of mammary tumours compared to controls (Wu et al., 2003). In accordance with other tumour types, height has also been reported as a risk factor for testicular germ cell tumours (TGCTs) (Rasmussen et al., 2003; Richiardi et al., 2003; McGlynn et al., 2007), although there is no direct evidence that circulating IGFL1 levels are linked to a higher risk of developing TGCT. However, the chromosomal disorder Klinefelter syndrome (47 XXY) is associated with increased height (Aksglaede et al., 2008) and an increased risk of mediastinal germ cell tumours (Nichols et al., 1987) but serum IGFL1 and IGFBP3 levels in the normal range (Aksglaede et al., 2008).

Mice with elevated growth hormone (GH)/Igf1 serum concentration had a higher incidence and reduced latency of mammary tumours but only in the context of a high fat diet (Gahete et al., 2014). Modulating factors such as diet perhaps explains the lack of concordance in the literature when trying to assess the proportion of risk attributable to circulating IGF1 concentration and these factors may explicate the lack of such a relationship in TGCT. A large meta-analysis examining the effects of circulating IGFL1 and IGFBP3 levels on the risk of developing several common cancers detected an association between increased IGFL1 concentration and prostate, colorectal and pre-menopausal breast cancer risk, while perhaps surprisingly, increased IGFBP3 levels were associated with risk of pre-menopausal breast cancer. This finding challenges the assumption that IGFBP3 only exerts its effects on cancer risk by regulating bioavailability of IGFL1. This study did not detect a protective effect of lower IGFBP3 levels overall; however, when one of the lung cancer cohorts was removed (that recruited only heavy smokers and asbestos workers), the risk of lung cancer was significantly decreased in individuals with higher IGFBP3 concentration (Renehan et al., 2004). Overall, serum levels of IGFL1 ligands have a modest effect on cancer risk and may need very large association or meta-studies to detect them. An alternative mechanism for IIR activity to influence cancer risk would be altered expression of the IGFL1 receptor in the target organ. In this regard, it is interesting to note that Igf1r concentration was higher in cryptorchid than normal testes post-puberty in an induced rat model (Antich et al., 1995). Cryptorchidism is a well-known risk factor for TGCT (Banks et al., 2013); however, the status of IGFL1 expression is unknown in this condition in humans. Polymorphisms within IGFL1-related genes have also been associated with risk of several cancers including breast and prostate although these are not necessarily linked to differences in circulating IGFL levels (Al-Zahrani et al., 2006; Canzian et al., 2006; Cao et al., 2014a; Jung et al., 2017). There is, however, no positive evidence linking polymorphisms in IGFL1 genes to testicular cancer risk (Chia et al., 2008; Loveday et al., 2018).

**ROLE OF IGF SYSTEM IN ONCogenesis**

Increased expression of many components of the IGFL family has been invoked in tumourigenic mechanisms. IGFL1, IGFL2 and IGFL2 are frequently overexpressed in a large number of tumour types (Papa et al., 1993; Bergmann et al., 1995; Sekiy-Otu et al., 1995; Steller et al., 1996; Weber et al., 2002). Insulin-like growth factor 2 mRNA binding proteins (IMPs) are expressed during embryogenesis and less so in normal adult tissues; however, they are upregulated in a broad range of cancers where their expression correlates with poor prognosis (reviewed in Degrauwe et al., 2016). Moreover, expression of IGFL1R has been shown to be a prerequisite for transformation by several different oncogenes (Sell et al., 1993; Toretsky et al., 1997). Several members of the IGFL family are potentially dysregulated in TGCT. IGFL1 and IGFBP5 are frequently expressed in the precursor TGCT lesion, germ cell neoplasia in situ (Drescher et al., 1997). Large-scale de novo demethylation takes place in primordial germ cells, relaxing imprinting at most genomic locations. TGCTs frequently retain this loss of imprinting, expressing IGFL2 biallelically (Van Gurp et al., 1994), which has been linked to increased tumour aggressiveness in other cancer types (Damascbe et al., 2017). Increased serum levels of IGFL2 and IGFBP2 have been found in non-seminomatous TGCT, decreasing upon successful therapy and increasing again in cases of recurrence (Fottner et al., 2008). Our groups has recently shown that IGFL1R is expressed in approximately half of non-seminomas and influences survival of non-seminoma cells in vitro (Selfe et al., 2018).

The IGFL axis has been implicated in a wide number of oncogenic processes. Signalling through the IGFL1R receptor primarily activates the PI3K/AKT and MAPK (Ras/Raf/MEK/ERK) pathways. Whereas activation of the MAPK pathway drives cellular proliferation through promoting proteins involved in cell cycle progression, signalling via the AKT pathway both activates anti-apoptotic proteins and inhibits anti-apoptotic proteins to enhance cell survival (Chitnis et al., 2008). Our study in TGCT cell lines suggested that these cells primarily signal through the PI3K/AKT pathway in response to IGFL ligand, perhaps reflecting the activation of the MAPK pathway via other means such as the tyrosine kinase receptor KIT and RAS mutation or overexpression (McIntyre et al., 2004, 2005). IGFL2 can rescue a teratocarcinoma cell line from undergoing apoptosis in the absence of serum (Engstrom, 2010), reinforcing the anti-apoptotic properties of IIR signalling in the context of TGCT.

IGFL1R signalling has also been associated with several cellular processes that contribute to metastasis. Migration and invasion have been linked to IGFL1R activity through co-operation with the integrin pathway leading to Rho-A-dependent motility via FAK and RACK1 (Doerr & Jones, 1996; Brooks et al., 1997; Zhang et al., 2005; Montagnani Marelli et al., 2006). The chemokine receptor CXCR4 (Goddard et al., 2007; Gilbert et al., 2009) is reported to be involved in the survival and migration of TGCTs as well as primordial germ cells (reviewed in Gilbert et al., 2011a). Notably, IGFL1 signalling through IGFL1R has been shown to increase migration and CXCR4 expression in both mesenchymal stem cells and embryonic germine stem cells (Li et al., 2007; Kuo et al., 2018).

Matrix metalloproteinases are induced by IGFL1 (Yoon & Hurta, 2001), conferring an invasive phenotype (Das et al., 2018), and MMP-2 and MMP-9 are frequently expressed in non-seminomas (Gilbert et al., 2011b). IGFL1 can also induce VEGF ligands and upregulate vascular vessel formation, thereby exhibiting pro-angiogenic properties (Kurmasheva et al., 2009; Li et al., 2011). IGFL1R signalling appears to be required for epithelial-to-
IGF IN GERM CELLS AND TGCT

ROLE OF IGF SYSTEM IN CHEMORESISTANCE

IGF1R activation has been implicated in resistance to both chemical and radiation based therapies. Investigations in several different tumour types have revealed increased IGF activity in chemoresistant tumours and shown that IGF1R inhibition acts as a chemosensitizer (Dallas et al., 2009; Eckstein et al., 2009; Juan et al., 2011; Ireland et al., 2016; Cao et al., 2017). Downstream activation of the PI3K/AKT pathway has been shown to be instrumental to the mechanism of chemoresistance in many of these studies.

IGF1R has also been found in the nucleus. Intriguingly, nuclear IGF1R was increased in metastatic colorectal tumours compared to matched primary tumours and correlated with poor overall survival (Codony-Servat et al., 2017). Nuclear translocation of IGF1R requires ligand-based activation of the receptor and can be blocked by IGF1R inhibitors (Alekscis et al., 2010). Following entry into the nucleus, IGF1R has been shown to interact with transcriptionally active regions of DNA including the proto-oncogene JUN (Alekscis et al., 2018). It is currently unknown whether IGF1R is found or plays a role in the nuclei of TGCT cells.

Recent studies have suggested tumour-associated cells such as tumour-associated macrophages (TAMs) and tumour-associated endothelial cells (TECs) may co-operate in IGF-mediated chemoresistance. TAMs and myofibroblasts were found to be the main sources of IGF production in pancreatic cancer (Ireland et al., 2016). TECs were found to keep tumourigenesis in check by secreting IGFBP7/angiomodulin, a direct IGF1R antagonist (binding to IGF1R itself and not IGF ligands) in the presence of IGFI. However, the administration of chemotherapy appears to alter this process and IGFBP7 expression is suppressed while IGFI expression is enhanced, allowing the TECs to be converted to promoters of tumourigenicity and consequently the emergence of chemoresistance (Cao et al., 2017). The induction of chemotherapy itself initiates the conversion of TECs, which perhaps perceive the chemotherapeutic agent in the same way as an injury and switch their transcriptional programme in response.

IGF1R expression is also associated with a radioreistant phenotype (Turner et al., 1997; Yu et al., 2003; Chen et al., 2017), suggesting that it may have a role in DNA damage response and/or repair. Several different mechanisms for the involvement of IGF1R in radioresistance have been proposed. Nuclear IGFIR is known to physically interact with and phosphorylate proliferating cell nuclear antigen (PCNA), a key mediator of the DNA damage response (Waraky et al., 2017). A role for IGF1R has been suggested in both of the major pathways for repairing DNA double-strand breaks, namely homologous recombination and non-homologous end joining (Chitinis et al., 2014). One of the main downstream effectors of IGF1R signalling, insulin receptor substrate 1 (IRS-1), has been shown to interact with RAD51 which localizes to the sites of double-strand breaks and facilitates repair by homologous recombination (Trojanek et al., 2003). Although a link between IGF signalling in TGCT and DNA repair has not been established in TGCT, modulation of DNA repair capacity is associated with cisplatin resistance in TGCT (Kalavská et al., 2018).

TGCT cells are considered the paradigm of a chemosensitive tumour, readily undergoing apoptosis in response to DNA-damaging agents such as cisplatin via a p53-dependent pathway. The p53 response to DNA damage is intact but leads to apoptosis in preference to cell cycle arrest, in part due to very low levels of p21 in TGCT cells (Spierings et al., 2004). Nevertheless, although the majority of TGCT patients respond to treatment initially, a minority relapse with cisplatin refractory disease. Mutations in the TP53 gene and amplification of its regulatory protein MDM2 are over-represented in cisplatin-resistant TGCT but do not explain all cases (Bagrodia et al., 2016). We have recently described increased IGF1R copy number, expression and activation (with increased phospho-AKT levels) in a model of acquired cisplatin resistance (Selfe et al., 2018) which could subsequently be re-sensitized to cisplatin upon reduction of IGF1R. IGF1R hyperactivation has been specifically linked to cisplatin refractory ovarian cancer (Eckstein et al., 2009). There is additional evidence signifying a role for the AKT pathway in platinum-resistant TGCT. Inhibition of AKT can restore sensitivity to cisplatin-resistant TGCT cells by re-localizing p21 from the cytoplasm to the nucleus (Koster et al., 2010), while PIK3CA and AKT1 mutations are exclusively found in cisplatin-resistant tumours (Feldman et al., 2014). Phospho-AKT levels are significantly higher in cisplatin-resistant disease compared to sensitive or untreated tumours (Julichs et al., 2014). Copy number gain and concomitant overexpression of AKT1 is a frequent event in intracranial germ cell tumours, which, although clinically and histologically similar to gonadal germ cell tumours, are more likely to be refractory to treatment (Wang et al., 2014). Figure 2 summarizes the key alterations in IGF signalling that have been observed in cisplatin-resistant TGCT.

THERAPEUTIC TARGETING OF THE IGF SYSTEM IN CANCER

IGF1R appears to represent an ideal therapeutic target in many cancers; it is expressed on the cell surface, possesses enzymatic activity and has a role in many tumourigenic processes. Two principal classes of inhibitor were initially used in a trial setting: monoclonal antibodies against IGF1R (mAbs) and small molecule tyrosine kinase inhibitors of IGF1R (TKIs). These inhibitors to IGF1R were very enthusiastically explored in many clinical trials as single agents two decades ago. Outcomes in these trials were extremely disappointing due to an overall infrequency of objective responses and a lack of any accurate predictive biomarkers of response. The most promising patient subgroup who might benefit from IGF1R-targeted therapy are Ewing’s sarcoma patients, where a minority have sustained durable responses lasting several years without major side effects (Anderson et al., 2016). The inability to select which patients are likely to respond has severely hampered efforts to employ IGF1R-targeted agents in mainstream treatment.

Several explanations have been proposed to explain the lack of efficacy of IGF1R mAbs and TKIs (reviewed in Simpson et al., 2017). There is a large amount of crosstalk between the
signalling pathways of IGF1R and other RTKs such as EGFR, ERBB2 and PDGFR (Browne et al., 2011; Liu et al., 2014). Cancer cells may therefore be able to circumvent IGF1R inhibition by upregulating another or several RTKs. As a corollary to this, it is also true that upregulation of IGF1R signalling can act as an escape mechanism in response to inhibitors of other RTKs (Ma et al., 2016; Almiron Bonnin et al., 2017; Li et al., 2017). The IGF1R mAbs will not prevent signalling via IG2 binding to the INSR-A receptor, which would be another route to evade IGF1R inhibition; indeed, increased phospho-INSR levels have been observed in response to an EGFR inhibitor in colorectal cancer cells (Jones et al., 2006). IGF1R TKIs block activation of both homotetrameric and heterotetrameric INSR and IGF1R due to the similarity of the kinase regions in both proteins, and this raises the potential problem of dose limitation in order to prevent glucose metabolism being adversely affected. A newer generation of therapeutic antibodies against IGF ligands should avoid both of these pitfalls by allowing insulin to signal normally and preventing IG2 from activating the INSR-A receptor. Targeting the ligands instead of the receptors should also prevent resistance through downregulation of inhibitory IGF binding proteins such as IGFBP2 as seen in rhabdomyosarcoma cells (Kang et al., 2014).

A major hurdle in the use of anti-IGF1R therapies in the clinic has been the lack of suitable predictive biomarkers. There is conflicting evidence as to whether expression of IGF1R itself, as opposed to activated IGF1R (phospho-IGF1R), identifies patients that would benefit from IGF1R-targeted agents (Cao et al., 2008; Kumasheva et al., 2009; Zha et al., 2009; Cao et al., 2014b). Expression of IGF1R or even presence of activated IGF1R does not always signify cells that are susceptible to IGF1R inhibition. This is seen in the case of the seminoma cell line, TCAM2 (Selfe et al., 2018), which despite comparatively high basal levels of activated IGF1R among TGCT cell lines was among the least responsive to an IGF1R TKI. Expression of other components of the IGF axis such as IRS2 and IGFBP5 has been shown to be important for determining sensitivity to an IGF1R mAb (Pavlicek et al., 2013). Exclusive nuclear IGF1R correlated with a better outcome in sarcoma patients treated with an IGF1R mAb (Amsane et al., 2012), indicating that nuclear staining in the absence of cytoplasmic IGF1R may be a useful biomarker; however, this study had small numbers of patients.

In order to exploit the anti-tumourigenic responses to INSR/IGF1R inhibition seen in preclinical experiments, current investigations are concentrating on combinatorial studies using IGF inhibitors. Combination with other RTK TKIs is used as a means of reducing the emergence of resistance or in addition to either standard chemotherapeutic agents or radiotherapy utilizing their properties as chemo- and radiosensitizers, respectively (McDermott et al., 2017; Schaffrath et al., 2017). Given the importance of the PI3K/AKT pathway in TGCT, simultaneous multiple targeting of this pathway may be effective in TGCT patients by combining IGF1R inhibition with other inhibitors of this pathway. IGF1R inhibition used in conjunction with standard chemotherapy regimens may be effective in some TGCT patients with cisplatin-resistant disease. Initial clinical testing of this hypothesis would likely involve refractory patients for whom existing treatment options were limited or unavailable. Attempting to resensitize patients to cisplatin at an earlier stage would however be potentially more effective than in the heavily pretreated cases where multiple genetic events may have had time to occur and establish resistance by different mechanisms. Careful selection of cases may also be important as if PIK3CA or AKTI mutations are driving resistance, inhibition of the IGF signalling pathway should take place downstream of these. The molecular pathways that allow IGF1R inhibitors to act as chemoresistors are not yet fully understood. Identifying these mechanisms and studying their interaction with the deficiencies in DNA repair in TGCT cells will be necessary in order to exploit the full benefit of targeting the IGF axis. The mTOR inhibitor, everolimus, has shown limited efficacy in two phase-II studies of unselected TGCT patients with refractory disease (Mego et al., 2016; Fenner et al., 2019). This may be due to the pro-oncogenic effects of INSR/IGF1R being at least in part independent of the PI3K/AKT pathway downstream of mTOR or that IGF-targeted therapies must be combined with DNA damaging agents to achieve clinical utility in TGCT.

CONCLUDING COMMENTS
Primordial germ cells, the likely precursor of TGCT, require IGF1R signalling for correct migration to the genital ridge, and the IGF system has many roles in establishing and maintaining testicular function including spermatogenesis and maintaining pluripotency in spermatogonial stem cells. The IGF axis is dysregulated in many tumour types and can contribute to oncogenesis via multiple disparate mechanisms, making it an attractive therapeutic target. The lack of mutations found in IGF proteins in cancer may hint that INSR/IGF1R signalling is not a key driver in many tumours, and together with cross talk between pathways, this could explain the lack of efficacy seen in clinical trials using several different types of IGF1R-targeted agent. However,
there are multiple lines of evidence to suggest that cancers can use the INSR/IGF1R pathway as a resistance mechanism to other treatments and that IGF1R inhibition can augment responses to standard chemo- and radiotherapy. The clinical utility of blocking this pathway may therefore lie in combining newly designed IGF ligand-targeted therapies with existing or new treatments. TGCT cells commonly exhibit aberrant IGF axis activation through elevated IGF1R activity (Selfe et al., 2018) and/or increased IGF2 expression through loss of imprinting (Van Gurp et al., 1994). We have shown that cells with high levels of IGF1R activation are vulnerable to IGF1R inhibition. Cisplatin resistance, the major cause of mortality in TGCT, may be impacted by including IGF1R inhibition.

REFERENCES

Aksela L, Skakkebaek NE & Juul A. (2008) Abnormal sex chromosome constitution and longitudinal growth: serum levels of insulin-like growth factor (IGF)-I, IGF binding protein-3, luteinizing hormone, and testosterone in 109 males with 47, XXY, 47, XY, or sex-determining region of the Y chromosome (SRY)-positive 46, XX karyotypes. J Clin Endocrinol Metab 93, 169–176.

Aleksic T, Chitnis MM, Perestenko OV, Gao S, Thomas PH, Turner GD, Protheroe AS, Howarth M & Macaulay VM. (2010b) Type 1 insulin-like growth factor receptor translocates to the nucleus of human tumor cells. Cancer Res 70, 6412–6419.

Aleksic T, Gray NE, Wu X, Rieunier G, Oshe R, Mills J, Verrill C, Bryant RJ, Han C, Hutchinson K, Lambert A, Kumar R, Hamdy FC, Weyer-Czernilofsky U, Sanderson M, Bognenrieder T, Taylor S & Macaulay VM. (2018) Nuclear IGF-1R interacts with regulatory regions of chromatin to promote RNA polymerase II recruitment and gene expression associated with advanced tumor stage. Cancer Res 78, 3498.

Amirnov Bonnin DA, Ran C, Havrda MC, Liu H, Hitoshi Y, Zhang Z, Cheng C, Ung M & Israel MA. (2017) Insulin-Mediated Signaling Facilitates Resistance to PDGFIR Inhibition in Proveneic PDGFBR-Driven Gliomas. Mol Cancer Ther 16, 705–716.

Al-Zahrani A, Sandhu MS, Luben RN, Thompson D, Baynes C, Pooley KA, Lucarrini C, Munday H, Perkins B, Smith P, Pharoah D, Wareham NJ, Easton DF, Ponder BAJ & Dunning AM. (2006) IGF1 and IGFBP3 tagging polymorphisms are associated with circulating levels of IGF1, IGFBP3 and risk of breast cancer. Hum Mol Genet 15, 1–10.

Anderson PM, Bielack SS, Gorlick RG, Skubitz K, Daw NC, Herzog CE, Monge OR, Lassaletta A, Boldrini E, Papai Z, Rubino J, Pathiraja K, Hille DA, Ayers M, Yao S-L, Nebzhy M, Lu B & Mauro D. (2016a) A phase II study of clinical activity of SCH 717454 (robatumab) in osteosarcoma. Pediatr Blood Cancer 63, 1761–1770.

Antich M, Fabian E, Sarquella J & Bassas L. (1995) Effect of testicular damage induced by cryptorchidism on insulin-like growth factor I receptors in rat Sertoli cells. J Reprod Fertil 104, 267–275.

Asmone I, Watkin E, Alberti L, Duc A, Montigny G, Rinaldi S, et al. (2006) Polymorphisms of genes coding for insulin-like growth factor 1 and its major binding proteins, circulating levels of IGF-I and IGFBP-3 and breast cancer risk: results from the EPIC study. Br J Cancer 94, 299–307.

Bao C, Yu Y, Darko I, Currier D, Mayeenuddin LH, Wan X, Khanna C & Helman LJ. (2008) Addiction to elevated insulin-like growth factor 1 receptor and initial modulation of the AKT pathway define the responsiveness of rhabdomyosarcoma to the targeting antibody. Cancer Res 68, 8039–8048.

Bao Y, Lindstrom S, Schumacher F, Stevens VL, Albanes D, Berndt S, et al. (2014a) Insulin-like growth factor pathway genetic polymorphisms, circulating IGF1 and IGFBP3, and prostate cancer survival. J Natl Cancer Inst 106, dju065.

Bao Y, Roth M, Piperdi S, Montoya K, Sowers R, Rao P, Geller D, Houghton P, Kolb EA, Gill J & Gorlick R. (2014b) Insulin-like growth factor 1 receptor and response to anti-IGF-1R antibody therapy in osteosarcoma. PLoS ONE 9, e016249.

Bao Z, Scandura JM, Inghirami GG, Shido K, Ding B-S & Rafii S. (2007) Molecular checkpoint decision made by subverted vascular niche transform indolent Tumor cells into chemotherapy resistant cancer stem cells. Cancer Cell 13, 110–126.

Castilla-Cortazar I, Garcia M, Quiroga J, Diez N, Diz-Caballero F, Calvo A, Diaz M & Prieto I. (2000) Insulin-like growth factor-I reverts testicular atrophy in rats with advanced cirrhosis. Hepatol 31, 592–600.

Chen L, Zhu Z, Gao W, Jiang Q, Yu J & Fu C. (2017) Systemic analysis of colorectal cancer cell lines and TCGA datasets identified IGF-1R/EGFR-PPAR-CASPASE axis as an important indicator for radiotherapay sensitivity. Gene 627, 484–490.

Chia VM, Sakoda LC, Graubard BI, Rubetone MV, Chanock SJ, Erickson RL & McGlynn KA. (2008) Risk of testicular germ cell tumors and polymorphisms in the insulin-like growth factor genes. Cancer Epidemiol Biomarkers Prev 17, 721–726.

Chitnis MM, Yuen JSP, Protheroe AS, Pollack M & Macaulay VM. (2008) The type 1 insulin-like growth factor receptor pathway. Clin Cancer Res 14, 6364–6370.

Chitnis MM, Lodhia KA, Aleksic T, Gao S, Protheroe AS & Macaulay VM. (2014) IGF-1R inhibition enhances radiosensitivity and delays double-stand break repair by both non-homologous end-joining and homologous recombination. Oncogene 33, 5262–5273.

Codony-Servat J, Cuatrecasas M, Asensio E, Montiorni C, Martinez-Cardus A, Marin-Aguilera M, et al. (2017) Nuclear IGF-1R predicts chemotherapy and targeted therapy resistance in metastatic colorectal cancer. Br J Cancer 117, 1777–1786.

Crowe FL, Key TJ, Allen NE, Appleby PN, Overvad K, Gronbaek H, et al. (2011) A cross-sectional analysis of the associations between adult height, BMI and serum concentrations of IGF-I and IGFBP-1 -2 and -3.
in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Ann Hum Biol* 38, 194–202.

Dallas NA, Xia L, Fan F, Gray MJ, Gaur P, Van Buren G, Samuel S, Kim MP, Lim SJ & Ellis LM. (2009) Chemoresistant colorectal cancer cells, the cancer stem cell phenotype, and increased sensitivity to insulin-like growth factor-I receptor inhibition. *Cancer Res* 69, 1951–1957.

Damaschke NA, Yang B, Bhusari S, Avilla M, Zhong W, Blute ML, Huang W & Jarrard DF. (2017) Loss of Igf2 Gene Imprinting in Murine Prostate Promotes Widespread Neoplastic Growth. *Cancer Res* 77, 5236–5247.

Das SK, Pradhan AK, Bhoopathi P, Talukdar S, Shen X-N, Sarkar D, Emadl D & Fisher PB. (2018) The MDA-9/Syntenin/IGFIR/STAT3 Axis Directs Prostate Cancer Invasion. *Cancer Res* 78, 2852–2863.

Degrawe U, Savia ML, Janiszewska M, Riggi N & Stamenkovic I. (2016) IMPs: an RNA-binding protein family that provides a link between stem cell maintenance in normal development and cancer. *Genes Dev* 30, 2459–2474.

Doerr ME & Jones JI. (1996) The roles of integrins and extracellular matrix proteins in the insulin-like growth factor I-stimulated chemotaxis of human breast cancer cells. *J Biol Chem* 271, 2443–2447.

Drescher B, Lauke H, Hartmann M, Davidoff MS & Zumkeller W. (1997) The roles of integrins and extracellular matrix proteins in the insulin-like growth factor receptor signaling pathway is an essential event for cisplatin resistance of ovarian cancer cells. *Cancer Res* 69, 2996–3003.

El-Shewy HM, Lee M-H, Obeid LM, Jaffa AA & Luttrell LM. (2007) The insulin-like growth factor type 1 and insulin-like growth factor type 2/mannose-6-phosphate receptors independently regulate ERK1/2 activity in HEK293 cells. *J Biol Chem* 282, 26150–26157.

Engstrom W. (2010) Effects of insulin-like growth factor binding protein 7 on apoptosis in human teratocarcinoma cells in vitro. *Anticancer Res* 30, 911–914.

Feldman DR, Iyer G, Van Alstine L, Patil S, Al-Ahmadie H, Reuter VE, Bosl MJ, Dietel M, Royer-Pokora B, Denkert C & Royer H-D. (2009) Hyperactivation of the insulin-like growth factor I receptor signaling pathway is an essential event for cisplatin resistance of ovarian cancer cells. *Cancer Res* 69, 2996–3003.

Fottner C, Sattarova S, Hoffmann K, Sportl G & Weber MM. (2008) Elevated serum levels of IGF-binding protein 2 in patients with non-seminomatous germ cell cancer: correlation with tumor markers alpha-fetoprotein and human chorionic gonadotropin. *Eur J Endocrinol* 159, 317–327.

Froment P, Staub C, Hembert S, Pisselet C, Magistrani M, Delaleu B, Seurin D, Levine JE, Johnson L, Binoux M & Monget P. (2004) Reproductive Abnormalities in Human Insulin-Like Growth Factor-Binding Protein-1 Transgenic Male Mice. *Endocrinology* 145, 2080–2091.

Fu P, Ibusuki M, Yamamoto Y, Hayashi M, Murakami K, Zheng S & Iwase H. (2011) Insulin-like growth factor receptor gene expression is associated with survival in breast cancer: a comprehensive analysis of gene copy number, mRNA and protein expression. *Breast Cancer Res Treat* 130, 307–317.

Gahele MD, Córdoba-Chacón J, Lantvit DD, Ortega-Salas R, Sanchez-Sanchez R, Perez-Jiménez F, López-Miranda J, Swanson SM, Castano JP, Luque RM & Kineman RD. (2014) Elevated GH/IGF-I promotes mammary tumors in high-fat, but not low-fat, fed mice. *Carcinogenesis* 35, 2467–2473.

Gilbert DC, Chandler I, McIntyre A, Goddard NC, Gabe R, Huddart RA & Shipley I. (2009) Clinical and biological significance of CXXC12 and CXXC842 expression in adult testes and germ cell tumours of adults and adolescents. *J Pathol* 217, 94–102.

Gilbert D, Rapley E & Shipley J. (2011a) Testicular germ cell tumours: predisposition genes and the male germ cell niche. *Nat Rev Cancer* 11, 278–288.

Gilbert DC, Chandler I, Summersgill B, McIntyre A, Missiaglia E, Goddard NC, Huddart RA & Shipley I. (2011b) Genomic gain and over expression of CCL2 correlate with vascular invasion in stage I non-seminomatous testicular germ-cell tumours. *Int J Androl* 34, e114–e121.

Goddard NC, McIntyre A, Summersgill B, Gilbert D, Kitazawa S & Shipley J. (2007) KIT and RAS signalling pathways in testicular germ cell tumours: new data and a review of the literature. *Int J Androl* 30, 337–348; discussion 349.

Graham TB, Zhai HE, Odero-Marah VA, Osunkoya AO, Kimbro KS, Tighiouart M, Liu T, Simon JW & O’Regan RM. (2008) Insulin-like growth factor-I-dependent up-regulation of ZEB1 drives epithelial-to-mesenchymal transition in human prostate cancer cells. *Cancer Res* 68, 2479–2488.

Griffith RJ, Carretero J & Burks DJ. (2013) Insulin receptor substrate 2 is required for testicular development. *PLoS ONE* 8, e62103.

Huang Y-H, Chin C-C, Ho H-N, Chou C-K, Chen C-N, Kuo H-C, Wu T-J, Wu Y-C, Hung C-C & Ling T-Y. (2009) Pluripotency of mouse spermatogonial stem cells maintained by IGF-1-dependent pathway. *FASEB J* 23, 2076–2087.

Ireland L, Santos A, Ahmed MS, Rainer C, Nielsen SR, Quaranta V, Weyer-Czernilofsky U, Engle DD, Perez-Mancera PA, Coupland SE, Takakia A, Bogemirider T, Tuveson DA, Campbell F, Schmid MC & Mielgo A. (2016) Chemoresistance in pancreatic cancer is driven by stroma-derived insulin-like growth factors. *Cancer Res* 76, 6851–6863.

Jones HE, Gee JMW, Barrow D, Tonge D, Holloway B & Nicholson RI. (2006) Inhibition of insulin receptor isoform-A signalling restores sensitivity to gefitinib in previously de novo resistant colon cancer cells. *Br J Cancer* 95, 172–180.

Jian H-C, Tsai H-T, Chang P-H, Huang C-YF, Hu C-P & Wong F-H. (2011) Insulin-like growth factor 1 mediates 5-fluorouracil chemoresistance in esophageal carcinoma cells through increasing survivin stability. *Apoptosis* 16, 174–183.

Juliaich M, Muñoz C, Moutinho CA, Vidal A, Condom E, Esteller M, Graupera M, Casanovas O, Germà JR, Villanueva A & Víñals F. (2014) The PDGFRβ-AKT pathway contributes to CDDP-acquired resistance in testicular germ cell tumors. *Clin Cancer Res* 20, 658–667.

Jung SY, Ho G, Rohan T, Strickler H, Beal J, Papp J, Sobel E, Zhang Z-F & Chandall C. (2017) Interaction of insulin-like growth factor-I and insulin resistance-related genetic variants with lifestyle factors on postmenopausal breast cancer risk. *Breast Cancer Res Treat* 164, 475–495.

Kalavská K, Coneduca V, De Giorgi U & Megó M. (2018) Molecular Mechanisms of Resistance in Testicular Germ Cell Tumours – clinical implications. *Curr Cancer Drug Targets* 18, 967–978.

Kang Z, Yu Y, Zhu YJ, Davis S, Walker R, Meltzer PS, Helman LJ & Cao L. (2014) Downregulation of IGFBP2 is associated with resistance to IGFIR therapy in rhabdomyosarcoma. *Oncoogene* 33, 5697–5705.

Kawamoto K, Onodera H, Kondo S, Kan S, Ikeuchi D, Maetani S & Imamura M. (1998) Expression of insulin-like growth factor-2 can predict the prognosis of human colorectal cancer patients: correlation with tumor progression, proliferative activity and survival. *Oncology* 55, 242–248.

Koster R, Di Pietro A, Timmer-Bosscha H, Gibcus JH, Van Den Berg A, Suurmeijer AJ, Bischoff R, Gietema JA & De Jong S. (2010) Cytoplasmic
Kuo Y-C, Au H-K, Hsu J-L, Wang H-F, Lee C-J, Peng S-W, Lai S-C, Wu Y-C, Ho H-N & Huang Y-H. (2007) Insulin-like growth factor I receptor family function in mice. *J Clin Invest* 120, 3594–3605.

Kuo Y-C, Au H-K, Hsu J-L, Wang H-F, Lee C-J, Peng S-W, Lai S-C, Wu Y-C, Ho H-N & Huang Y-H. (2007) Insulin-like growth factor-I receptor-targeting antibody, CP-75,187, suppresses tumor-derived VEGF and synergizes with rapamycin in models of childhood sarcoma. *Cancer Res* 69, 7662–7671.

Li Y, Yu X, Lin S, Li X, Zhang S & Song Y-H. (2007) Insulin-like growth factor I enhances the migratory capacity of mesenchymal stem cells. *Biochem Biophys Res Commun* 356, 780–784.

Li H, Adachi Y, Yamamoto H, Min Y, Ohashi H, Mi M, Arimura Y, Endo T, Lee C-T, Carbone DP, Imai K & Shimomura 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 L, Gu X, Yue J, Zhao Q, Lv D, Chen H & Xu L. (2017) Acquisition of IGF-1R Promotes Symmetric Self-Division in MKN45.

Lin T, Haskell J, Vinson N & Terracio L. (1986) Direct stimulatory effects of insulin and IGF1 receptors are essential for XX and XY gonadal differentiation and adrenal development in mice. *Am J Epidemiol* 123, 355–363.

Montagani Marelli M, Moretti RM, Procacci P, Motta M & Limonta P. (2006) Insulin-like growth factor-I promotes migration in human androgen-independent prostate cancer cells via the alphaVbeta3 integrin and PI3-K/Akt signaling. *Int J Oncol* 28, 725–730.

Mussa C, Molinatto G, Baldassarre G, Riberi E, Russo S, Larizza L, Riccio A & Ferrero G. (2016) Cancer risk in Beckwith-Wiedemann Syndrome: a systematic review and meta-analysis outlining a novel (Epi)genotype specific phenotype targeted screening protocol. *J Pediatr* 176, 142–149.

Nef S, Verma-Kurvari S, Merenmies J, Vassalli J-D, Efstratiadis A, Accili D & Parada LF. (2003) Testis determination requires insulin receptor family function in mice. *Nature* 426, 291–295.

Neinjck J, Calpav K, Kilcoyne KJ, Kuhne F, Steviant I, Griffith RJ, Pitetti J-L, Andric SA, Hu M-C, Pralong F, Smith LB & Nef S. (2018) Insulin and IGF1 receptors are essential for the development and stochastic function of adult Leydig cells. *FASEB J* 32, 3321–3335.

Nichols CR, Heerema NA, Palmer C, Loehrer PJ Sr, Williams SD & Einhorn LH. (1987) Klinefelter’s syndrome associated with mediastinal germ cell neoplasms. *J Clin Oncol* 5, 1290–1294.

Nunney L. (2018) Size matters: height, cell number and a person’s risk of cancer. *Proc R Soc B Biol Sci* 285, 20181743.

Papa V, Gliozzo B, Clark GM, McGuire WL, Moore D, Fujita-Yamaguchi Y, Vigneri R, Goldfine ID & Pezzino V. (1993) Insulin-like growth factor-I receptors are overexpressed and predict a low risk in human breast cancer. *Cancer Res* 53, 3736–3740.

Pavlicek A, Lira ME, Lee NV, Ching KA, Ye J, Cao J, Garza SJ, Hook KE, Ozeck M, Shi ST, Yuan J, Zheng X, Rejto PA, Kan JLC & Christenson JG. (2013) Molecular predictors of sensitivity to the insulin-like growth factor I receptor inhibitor Figitumumab (CP-75,871). *Mol Cancer Ther* 12, 2929–2939.

Pitetti J-L, Calpav P, Romero Y, Conne B, Truong V, Papaioannou MD, Schaad O, Doqueira M, Herrera PL, Wilhelm D & Nef S. (2013a) Insulin and IGF1 receptors are essential for XX and XY gonadal differentiation and adrenal development in mice. *PLoS Genet* 9, e1003160.

Pitetti J-L, Calpav P, Zimmermann C, Conne B, Papaioannou MD, Aubry F, Cederroth CR, Urner F, Fumel B, Crausaz M, Doqucier M, Herrera PL, Pralong F, Germond M, Guilou F, Jegou B & Nef S. (2013b) An essential role for insulin and IGF1 receptors in regulating sertoli cell proliferation, testis size, and FSH action in mice. *Mol Endocrinol* 27, 814–827.

Rasmussen F, Gunnell D, Ekmom A, Hallqvist J & Tynelius P. (2003) Birth weight, adult height, and testicular cancer: cohort study of 337,249 Swedish young men. *Cancer Causes Control* 14, 595–598.

Renehan AG, Zwahlen M, Minden C, D’Oweny ST, Shalet SM & Egger M. (2004) Insulin-like growth factor (IGF)-1, IGF binding protein–3, and cancer risk: systematic review and meta-regression analysis. *Lancet* 363, 1346–1353.

Richardi L, Asling J, Granath F & Arke O. (2003) Body size at birth and adulthood and the risk for germ-cell testicular cancer. *Cancer Epidemial Biomarkers Prev* 12, 669–673.

Sang X, Curran MS & Wood AW. (2008) Paracrine insulin-like growth factor signaling influences primordial germ cell migration: in vivo evidence from the zebrafish model. *Endocrinology* 149, 5035–5042.

Schaffrath I, Schmoll H-J, Voigt W, Müller LP, Müller-Tidow C & Mueller T. (2017) Efficacy of targeted drugs in germ cell cancer cell lines with differential cisplatin sensitivity. *PLoS ONE* 12, e0178930.

Schlueter PJ, Sang X, Duan C & Wood AW. (2007) Insulin-like growth factor receptor 1b is required for zebrafish primordial germ cell migration and survival. *Dev Biol* 305, 377–387.

Seino S & Bell GI. (1989) Alternative splicing of human insulin receptor messenger RNA. *Biochem Biophys Res Commun* 159, 312–316.

Sekyi-Ottu A, Bell RS, Ohashi C, Pollak M & Andrulis IL. (1995) Insulin-like growth factor 1 (IGF-1) receptors, IGF-1, and IGF-2 are expressed in primary human sarcomas. *Cancer Res* 55, 129–134.

Selfe J, Goddard NC, McIntyre A, Taylor KR, Renshaw J, Popov SD, Thway K, Summersgill B, Huddart RA, Gilchrist LB & Shipley JM. (2018) IGF1R signalling in testicular germ cell tumour cells impacts on cell survival and acquired cisplatin resistance. *J Pathol* 244, 242–253.
Sell C, Rubini M, Rubin R, Liu JP, 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. Proc Natl Acad Sci USA 90, 11217–11221.

Simpson A, Petnga W, Macaulay VM, Weyer-Czernilofsky U & Bogenrieder T. (2017) Insulin-like growth factor (IGF) pathway targeting in cancer: role of the IGF axis and opportunities for future combination studies. Target Oncol 12, 571–597.

Spiersings DCJ, Ege DV, Stel AJ, Te Rietstap N, Vellenga E & De Jong S. (2017) Stromal-derived IGF2 promotes colon cancer progression via paracrine and autocrine mechanisms. Oncogene 36, 5341–5355.

Van Gurp RJ, Oosterhuis JW, Kalscheuer V, Mariman EC & Looijenga LH. (1994) Biallelic expression of the H19 and IGF2 genes in human testicular germ cell tumors. J Natl Cancer Inst 86, 1070–1075.

Wang L, Yamaguchi S, Burstein MD, Terashima K, Chang K, Ng H-K, et al. (2014) Novel somatic and germline mutations in intracranial germ cell tumours. Nature 511, 241–245.

Warakry A, Lin Y, Warsito D, Haglund F, Aleem E & Larsson O. (2017) Nuclear insulin-like growth factor I receptor phosphorylates proliferating cell nuclear antigen and rescues stalled replication forks after DNA damage. J Biol Chem 292, 18227–18239.

Weber MM, Fottner C, Bin LS, Jung MC, Engelhardt D & Baretton GB. (2002) Overexpression of the insulin-like growth factor I receptor in human colon carcinomas. Cancer 95, 2086–2095.

Wu Y, Cui K, Miyoshi K, Henninghausen L, Green JE, Setser J, Leroith D & Yakar S. (2003) Reduced circulating insulin-like growth factor I levels delay the onset of chemically and genetically induced mammary tumors. Cancer Res 63, 4384–4388.

Yao J, Zuo H, Gao J, Wang M, Wang D & Li X. (2017) The effects of IGF-1 on mouse spermatogenesis using an organ culture method. Biochem Biophys Res Commun 491, 840–847.

Yi Y, Zeng S, Wang Z, Wu M, Ma Y, Ye X, Zhang B & Liu H. (2018) Cancer-associated fibroblasts promote epithelial-mesenchymal transition and EGFR-TKI resistance of non-small cell lung cancers via HGF/IGF-1/ANXA2 signaling. Biochim Biophys Acta Mol Basis Dis 1864, 793–803.

Yoon A & Hurta RA. (2001) Insulin like growth factor-I selectively regulates the expression of matrix metalloproteinase-2 in malignant H-ras transformed cells. Mol Cell Biochem 233, 1–6.

Yu D, Watanabe H, Shibuya H & Miura M. (2003) Redundancy of radioreistant signaling pathways originating from insulin-like growth factor I receptor. J Biol Chem 278, 6702–6709.

Zha J, O'Brien C, Savage H, Huw L-Y, Zhong F, Berry L, Lewis Phillips GD, Luis E, Cavet G, Hu X, Amerl LC & Lackner MR. (2009) Molecular predictors of response to a humanized anti-insulin-like growth factor-I receptor monoclonal antibody in breast and colorectal cancer. Mol Cancer Ther 8, 2110–2121.

Zhang X, Lin M, Van Golen KL, Yoshiba K, Itho K & Yee D. (2005) Multiple signaling pathways are activated during insulin-like growth factor-I (IGF-I) stimulated breast cancer cell migration. Breast Cancer Res Treat 93, 159–168.