IGF2 and cancer

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Abstract

Insulin-like growth factor 2 (IGF2) is a 7.5 kDa mitogenic peptide hormone expressed by liver and many other tissues. It is three times more abundant in serum than IGF1, but our understanding of its physiological and pathological roles has lagged behind that of IGF1. Expression of the *IGF2* gene is strictly regulated. Over-expression occurs in many cancers and is associated with a poor prognosis. Elevated serum IGF2 is also associated with increased risk of developing various cancers including colorectal, breast, prostate and lung. There is established clinical utility for IGF2 measurement in the diagnosis of non-islet cell tumour hypoglycaemia, a condition characterised by a molar IGF2:IGF1 ratio >10. Recent advances in understanding of the pathophysiology of IGF2 in cancer have suggested much novel clinical utility for its measurement. Measurement of IGF2 in blood and genetic and epigenetic tests of the *IGF2* gene may help assess cancer risk and prognosis. Further studies will determine whether these tests enter clinical practice. New therapeutic approaches are being developed to target IGF2 action. This review provides a clinical perspective on IGF2 and an update on recent research findings.

Key Words
- IGF2
- cancer
- non-islet cell tumour hypoglycaemia

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Introduction

Research during the 1960s discovered an insulin-like factor that could not be abolished by anti-insulin antibodies (Froesch *et al.* 1963). This was called non-suppressible insulin-like activity (NSILA). NSILA had the same properties as sulphation factor, discovered in 1957, required for incorporation of sulphate into cartilage (Salmon & Daughaday 1957). It was renamed somatomedin (Daughaday *et al.* 1972), and when sequenced, it was found to consist two peptides (Rinderknecht & Humbel 1976). These were named insulin-like growth factor (IGF) 1 and 2 because of their homology with insulin and similar metabolic actions. Our understanding of the pathophysiology of IGF2 lags behind that of IGF1, but there have been significant advances in recent years. The purpose of this review is to provide the reader with a perspective and update on IGF2, with respect to its role in cancer and clinical utility for its measurement.

The IGFs are part of a complex system, the components of which act together to influence growth. The system consists of insulin, both IGFs, their cell surface receptors and IGF binding proteins (IGFBPs). The IGFBPs are a family of six proteins that bind IGFs in serum (Clemmons 1998). They transport and sequester IGFs, regulating availability to receptors. About 75% of circulating IGFs exist as 150 kDa ternary complexes consisting of IGF1 or IGF2, IGFBP3 and acid-labile subunit (ALS), an 85 kDa protein synthesised in liver (Baxter 2001, Firth & Baxter 2002). These complexes are confined to the circulation because they are unable to cross capillary endothelia. Some IGFs exist as binary complexes (40–50 kDa) with IGFBPs that can leave the circulation, possibly functioning as a pericellular store of IGFs (Juul 2003). The remaining 'free' IGF ($<1\%$) is considered bioactive. Given the complexity of the system, the

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function of any individual component, such as IGF2, must be considered in the context of the whole system.

IGF2

Mature IGF2 is a 67 amino acid (7.5 kDa) peptide produced mainly by liver, but it is also secreted by most tissues where it can act in an autocrine or paracrine manner. There is considerable evidence that IGF2 regulates cell growth, differentiation and metabolism (O'Dell & Day 1998). It is particularly important in promoting fetal growth, being highly expressed during embryogenesis (Liu *et al.* 1989). The effects of the IGFs overlap. They are both potent mitogens, their relative potency depending on the cell type (Humbel 1990).

The *IGF2* gene (30 kb) is located next to the insulin gene on 11p15.5. IGF2 is initially synthesised as prepro-IGF2 (20.1 kDa, 180 AAs) consisting of A–E domains and a 24-residue signal peptide (O'Dell & Day 1998) (Fig. 1). Post-translational processing begins with cleavage of the signal peptide to yield pro-IGF2 (1–156). This is followed by *O*-linked glycosylation of the 89-residue E-domain that may promote further processing (Daughaday *et al.* 1993). Pro-IGF2 then undergoes sequential proteolysis to mature IGF2 (1–67) that lacks the E-domain. Prohormone convertase 4 (PC4) is the protease thought to cleave the E-domain. Incomplete processing of pro-IGF2 results in various peptides (10–18 kDa) containing all or part of the E-domain, known collectively 'big' IGF2. These are secreted into the circulation, normally accounting for 10–20% of total IGF2 (Gowan *et al.* 1987, Daughaday & Trivedi 1992*a*). The glycosylation on big IGF2 may promote ternary complex formation in serum. Big IGF2 also forms binary complexes with IGFBP2, IGFBP3 and IGFBP5 (Qiu *et al.* 2010, Greenall *et al.* 2013).

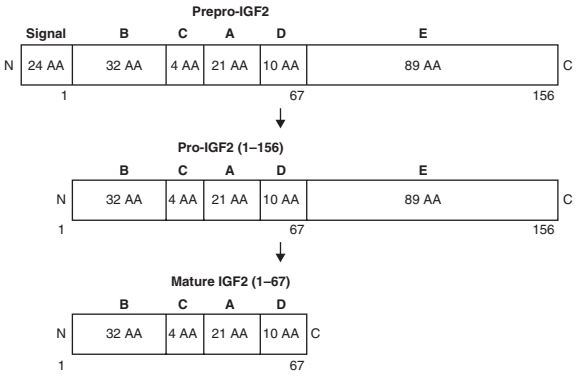

Figure 1
Structure and processing of prepro-IGF2. The *IGF2* gene (30 kb) is located on 11p15.5 comprising nine exons. Exons 7, 8 and 9 are encoding. Translation generates prepro-IGF2 (180 AAs, 20.1 kDa) consisting of five domains (A, B, C, D and E) and a 24-residue N-terminal signal peptide. Proteolysis of the signal peptide by signal peptidase yields pro-IGF2 (1–156). The C-terminal E-domain is glycosylated promoting further processing. Sequential proteolysis removes the E-domain giving mature IGF2 (1–67) that is secreted. Incompletely processed pro-IGF2 peptides (10–18 kDa) containing all or part of the E-domain are also secreted making up 10–20% of total circulating IGF2. These peptides are called 'big' IGF2.

Quantitatively IGF2 is the predominant circulating IGF present in adults at a concentration of ~700 ng/ml (Humbel 1990), three times that of IGF1. Serum IGF2 is low in neonates, climbing in early childhood and then remaining at similar concentrations throughout life, although it may decrease slightly in healthy elderly subjects (Yu *et al.* 1999, Raynaud-Simon 2003). Concentrations are similar in both genders. Free IGF2 circulates at picomolar concentrations, similar to insulin. The portion of IGF2 bound to IGFBPs has a relatively long half-life (10–16 h) compared with that of free IGF2 (a few minutes) (Rajaram *et al.* 1997).

IGF2 signalling

IGF2 signals via three receptor complexes namely the IGF1 receptor (IGF1R), insulin receptor isoform A (IR-A) and the IGF1R–IR-A hybrid receptor (Fig. 2). IGF1R binds both IGFs with comparable and high affinity (Pandini *et al.* 2002). It is thought to mediate most of the biological effects of IGF2. IR-A is an alternatively spliced IR isoform that lacks exon 11 (11-) encoding 12 amino acid residues at the C-terminus of the ligand-binding α subunit. This enables it to bind IGF2 with an affinity 15% of that for insulin, much higher than its affinity for IGF1 (Frasca *et al.* 1999, Nakae *et al.* 2001). While insulin binding stimulates glucose uptake, IGF2 binding is mitogenic (Belfiore *et al.* 2009). Although IR-A is widely expressed throughout life, its physiological role in adults is unclear.

In common with insulin and IGF1, binding of IGF2 to the IGF1R activates a receptor tyrosine kinase (RTK) associated with the β-subunit leading to an intracellular response (Belfiore & Malaguarnera 2011, Braun *et al.* 2011, LeRoith *et al.* 2011). Autophosphorylation of the β-subunit by the RTK recruits insulin receptor substrates (IRS) 1–4. Phosphatidylinositol 3-kinase (PI3-K) then binds to IRS1 via its regulatory subunit and is activated, in turn activating Akt (protein kinase B). This has a number of intracellular effects, which ultimately promote cell survival and mitogenesis. First, it inhibits apoptosis by inactivating BAD (BCL-2 antagonist of cell death). It also phosphorylates tuberous sclerosis complex (TSC1/2) leading to activation of mammalian target of rapamycin

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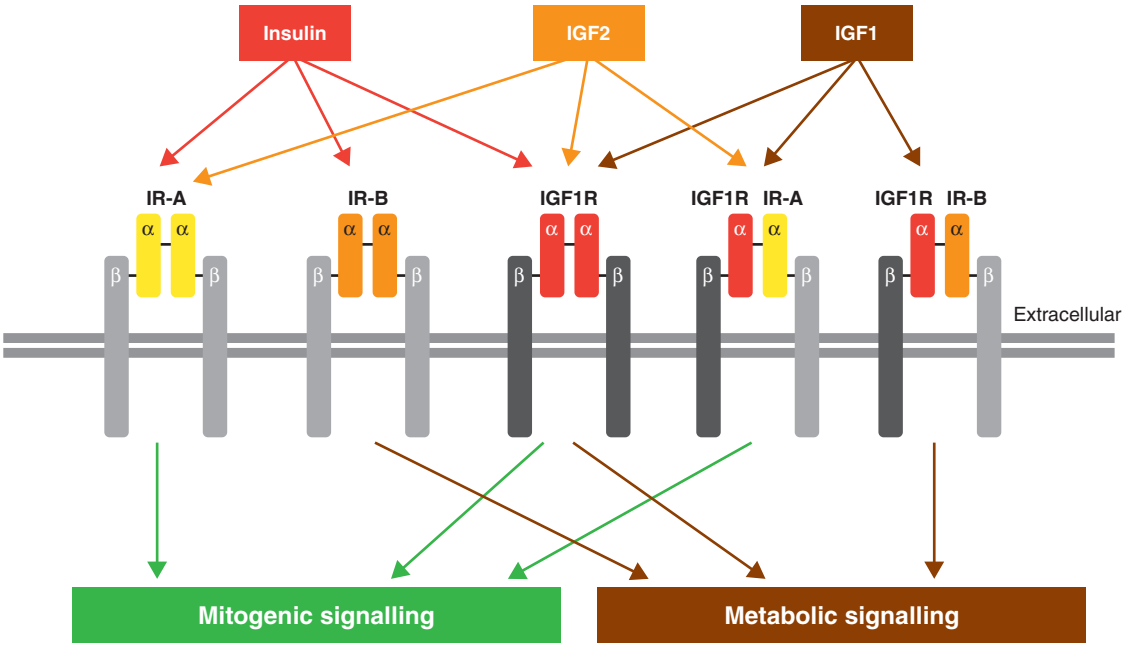

Figure 2

Ligands and receptors of the insulin-like growth factor (IGF) system. The ligands of the IGF system signal by binding to three different receptor complexes, namely the insulin receptor (isoforms A and B), the IGF1 receptor (IGF1R) and hybrids of the IGF1R and insulin receptor. The receptor complexes are $\alpha_2\beta_2$ tetramers. Ligand binding to the extracellular α-subunit results in activation of a receptor tyrosine kinase located in the transmembrane β-subunit. This activates the intracellular signalling pathway leading to a biological response, which can be mitogenic or metabolic in nature. Activation of IR-A and IGF1R initiate mitogenic signalling whereas activation of IR-B causes metabolic responses. Ligand binding to the hybrid receptors can activate either type of signalling depending on the I-R isoform in the complex. Reproduced from LeRoith D, Scheinman EJ & Bitton-Worms K 2011 The role of insulin and insulin-like growth factors in the increased risk of cancer in diabetes. *Rambam Maimonides Medical Journal* **2** e0043. (doi:10.5041/RMMJ.10043)

(mTOR) and subsequent ribosomal protein synthesis that is required for mitogenesis. Akt also has the metabolic action of leading to GLUT4 translocation, which promotes cellular glucose uptake.

By recruiting other adaptor proteins to the receptor complex, ligand activation of IGF1R also leads to activation of the MAPK pathway that transmits the proliferative signals generated at the cell surface to the nucleus. It causes the change in expression of proteins required for cellular proliferation. Phosphorylation of IRS proteins recruits the adaptors Shc and growth factor receptor-bound protein 2 (Grb2), which along with son-of-sevenless form a complex activating the GTP binding protein Ras. There is further phosphorylation and activation of Raf-1 and the kinases (MEK1/2 and ERK1/2) that leads to the activation of transcription factors involved in cell proliferation.

The tumour suppressor phosphatase and tensin analogue (PTEN) dephosphorylates and inhibits PI3-K. It also inhibits Shc and mTOR. It, therefore, inhibits the downstream mitogenic pathways activated by PI3-K, thereby opposing the action of a number of growth factors including IGF2 (Gallagher & LeRoith 2010). In turn, IGF2 signalling appears to increase PTEN expression, which may be a form of feedback loop (Moorehead *et al.* 2003*a*). These pathways are described in detail elsewhere (Belfiore & Malaguarnera 2011).

Physiological regulation of IGF2

Before discussing dysregulation of IGF2 in cancer, it is necessary to cover its physiological regulation. Regulation of both *IGF2* expression and IGF2 action are complex and multifactorial. This complexity appears to permit fine-tuning of responses and to prevent excessive IGF2 action that could lead to disease.

Genetic factors play a significant role in the regulation of IGF2. The proportion of its variance attributable to genetic factors is 66%, compared with 38% for IGF1 (Harrela *et al.* 1996). Transcription is regulated by genomic imprinting, an epigenetic mechanism that restricts expression to the paternal allele in most tissues. Imprinting is achieved by methylation of the differentially methylated region (DMR) on the maternal allele.

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It prevents excessive expression of *IGF2*, which could lead to proliferation and tumours. *IGF2* is transcribed from four promoters (P1–P4) in a tissue-specific manner. During embryogenesis, transcription occurs from P2–P4 resulting in monoallelic expression. In adults, there is also expression from P1 in liver which is biallelic (Vu & Hoffman 1994) accounting for the high circulating IGF2 concentrations in adults. An antisense *IGF2* transcript (IGF2AS) has been described, which is also maternally imprinted (Vu *et al*. 2003). It is expressed at levels similar to *IGF2* but its regulatory role is unknown.

By sequestering both IGFs in the circulation, IGFBP3 inhibits their mitotic effects (Nickerson *et al*. 1997) and insulin-like actions (Boisclair *et al*. 2001). IGFBP2 accounts for most of the remaining IGF2 binding in the circulation (Clemmons *et al*. 1991). IGFBP1 and IGFBP2 are thought to regulate free IGF2 (Clemmons 1997). IGFBPs are themselves subject to regulation by various hormones, including insulin and IGFs (Kelley *et al*. 1996). GH stimulates hepatic IGFBP3 and ALS synthesis that, by increasing ternary complex formation, in turn increases total IGF2 concentrations (Wolf *et al*. 1994). IGF2 is, therefore, in part GH dependent but is less GH dependent than IGF1, which explains the absence of a pubertal increase in its serum concentrations (Zapf *et al*. 1981). IGFBPs have independent effects on cell adhesion and migration (Kelley *et al*. 1996) and effects enhancing the action of IGFs (Clemmons 1997). Mechanisms release IGFs from the complexes enabling receptor interaction (Firth & Baxter 2002). These include IGFBP proteolysis (Muller *et al*. 1994), phosphorylation and binding to the extracellular matrix (LeRoith & Butler 1999). IGFBPs may have biological actions of their own (Jones & Clemmons 1995, Firth & Baxter 2002, Martin & Baxter 2011).

Cellular responsiveness to IGF2 is influenced by changes in receptor expression. Increased IR-A expression during embryogenesis (Belfiore *et al*. 2009) and increased IR-A:IR-B ratio during de-differentiation (Entingh *et al*. 2003) promote its action. IGF2 also binds to the widely expressed IGF2R, a 250 kDa monomeric, cell surface protein. It is thought to promote endocytosis and lysozomal degradation of IGF2, thereby antagonising its action and acting as a tumour suppressor (Brown *et al*. 2009). IGF2R also binds lysozomal enzymes intracellularly, transporting them from the Golgi to lysozomes (Kornfeld 1992). The *IGF2R* gene, like *IGF2*, is imprinted but expressed from the maternal allele. This reciprocal imprinting may regulate the relative abundance of the two proteins. A soluble form of IGF2R cleaved from the cell surface binds IGF2 in serum and is thought to reduce IGF2 bioactivity *in vivo* (Ellis *et al*. 1996, Scott & Weiss 2000).

Because the IGFs promote growth, it is logical that they are both nutritionally regulated, their concentrations indicating the availability or otherwise of substrate from the diet. Down-regulation of IGF2 during starvation may protect the individual from hypoglycaemia, which would occur if its concentration did not decrease in parallel with ternary complexes. Specific nutrients also influence IGF2, notably down-regulation by vitamin C (Lee *et al*. 2008) and vitamin D (Huynh *et al*. 1998).

Cancer development

Sustained IGF action promotes carcinogenesis (Renehan *et al*. 2006). IGF1 and IGF1R are the components of the system best studied in this process (Larsson *et al*. 2005), but growing evidence from *in vitro* and *in vivo* studies has shown that IGF2 also promotes cancer development and progression (Yu & Rohan 2000).

Over-expression of IGF2

As cells age, dysregulation of the DMR on the maternal chromosome causes loss of imprinting (LOI) of *IGF2* with over-expression and increased sensitivity to IGF2 signalling (Fu *et al*. 2004, Kaneda *et al*. 2007). This exposes cells to excessive IGF2 and the more potent pro-IGF2 (Kalla Singh *et al*. 2008) that promote growth and anti-apoptosis in an autocrine manner (Gallagher & LeRoith 2010). Studies in animals have demonstrated that sustained IGF2 action increases the risk of transformation. Transgenic animals over-expressing *IGF2* were at increased risk of developing mammary gland adenocarcinoma (Bates *et al*. 1995) and lung cancer (Moorehead *et al*. 2003*b*). IGF2 caused earlier and more aggressive cancers (Rogler *et al*. 1994, Pravtcheva & Wise 1998). Conversely, animals with low IGF2 concentrations lived longer, with a lower incidence of tumours (Bartke *et al*. 2002). Other mechanisms of *IGF2* over-expression have been described. For example, the morphogen sonic hedgehog (Shh) is inappropriately activated in some tumours, increasing expression of *IGF2* and other genes involved in regulation of cell growth (Ingram *et al*. 2002, Ruiz *et al*. 2002). *IGF2* expression is also promoted by defective expression of the transcriptional repressor WT1 (Ward 1997). It should be noted that LOI of *IGF2* is not exclusive to cancer cells but is also commonly observed in normal neonates (Rancourt *et al*. 2013) and adult humans (Belharazem *et al*. 2012).

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In humans, there is extensive evidence for *IGF2* dysregulation in tumours. LOI is a common epigenetic abnormality in breast (Hartmann *et al*. 2005), oesophageal (Zhao *et al*. 2009) and ovarian cancer (Murphy *et al*. 2006) and acute myeloid leukaemia (Wu *et al*. 1997). In Wilms' tumours, there is increased *IGF2* expression in 50% of cases, usually due to LOI (Reeve 1996). Increased *IGF2* expression is particularly common in mesenchymal tumours (Steigen *et al*. 2009). The frequency of increased expression was greatest in Ewing's sarcoma and tenosynovial giant cell tumours whereas levels of expression, as assessed by immunoreactivity, were greatest in solitary fibrous tumours. *IGF2* was one of 31 genes up-regulated in hepatitis B virus-associated hepatocellular carcinoma (HCC; Couvert *et al*. 2008), suggesting a role in hepatocarcinogenesis. More recently, work on peripheral blood mononuclear cells found that *IGF2* methylation decreased during progression from cirrhosis to HCC (Couvert *et al*. 2012). LOI of *IGF2* is a common finding in colon cancer (Cui *et al*. 2003, Cui 2007). It is significantly associated with family history of the disease and appears to be a heritable risk factor rather than being acquired because of environmental exposure (Cruz-Correa *et al*. 2004).

Study of the Beckwith–Wiedemann syndrome (BWS), in which LOI of *IGF2* was first described, has provided further evidence for a link between IGF2 dysregulation and cancer development. This is a rare congenital syndrome characterised by placental and postnatal overgrowth. There may be gigantism, macroglossia, organomegaly and a predisposition to tumours, in particular Wilms' tumour (Shapiro *et al*. 1982). Most patients have gene deletions resulting in *IGF2* LOI. This causes biallelic expression with increased IGF2 concentrations (Sparago *et al*. 2004). In 20% of patients, *IGF2* over-expression results from uniparental disomy in which two paternal *IGF2* gene copies are inherited (Biliya & Bulla 2010). Excessive IGF2 is thought to be responsible for the clinical features of BWS because similar features were observed in a mouse transgenic model over-expressing *IGF2* (Sun *et al*. 1997).

In some tumours, over-expression of *IGF2* is insufficient for tumorigenesis (Hahn *et al*. 2000). Additional defects may be required such as loss of repressor function, changes in promoters (Yu & Rohan 2000) or receptor dysregulation (Algire *et al*. 2011). IGF2R defects are also implicated in cancer development. Imprinting makes *IGF2R* susceptible to mutations because a lethal mutation affecting the single active gene copy results in an absence of functional protein. *IGF2R* mutations causing loss of function of the protein have been described in numerous cancers (De Souza *et al*. 1997). They often occur early in carcinogenesis, predisposing to cancer presumably via loss of restraint on IGF2 (Biliya & Bulla 2010). Polymorphisms of *IGF2R* are also linked to increased risk of oral cancer (Yoon *et al*. 2012), colonic cancer (Probst *et al*. 2009) and HCC (Morcavallo *et al*. 2012) possibly because of impaired IGF2 clearance.

Epidemiological studies have linked elevated IGF1 and decreased IGFBP3 to common epithelial cancers (Maki 2010), but few such studies have measured IGF2. In one large follow-up study on breast cancer in postmenopausal women, serum IGF2 and IGFBP3 but not IGF1 were positively associated with oestrogen receptor-positive breast cancer risk (Gronbaek *et al*. 2004), although there was no overall association between IGF2 and breast cancer risk. In the prostate cancer prevention study, neither IGF1 nor IGF2 was associated with prostate cancer (Neuhouser *et al*. 2013) but elevated serum IGFBP2 was a risk factor for low-grade disease. A small study on early-stage breast cancer reported elevated free IGF1 and IGF2 but total IGF2 was reduced (Espelund *et al*. 2008). IGF2 demands further assessment in epidemiological studies of cancer.

IGF2 signalling in cancer development

The signalling mechanisms whereby IGF2, IGF1 and insulin may promote cancer development have been extensively studied. Pathways are now known whereby IGF2-mediated activation of IGF1R or hybrid receptors may promote tumorigenesis (Alvino *et al*. 2011, Pierre-Eugene *et al*. 2012). The MAPK pathway appears to be the main pathway whereby IGF2 and other ligands of the IGF1R activate genes concerned with cell proliferation causing mitogenesis. The PI3-K/Akt pathway is also activated, leading to reduced apoptosis and increased cell survival. It appears to play a supportive role in the process. Sustained IGF2-mediated autocrine IGF1R signalling has also been suggested as the mechanism of sarcoma development in BWS (Ratajczak 2012).

IR-A activation has mitogenic effects and is another mechanism whereby IGF2 may promote tumorigenesis. IGF2 action through IR-A appears to differentially influence gene expression compared with insulin acting through the same receptor (Pandini *et al*. 2004). In addition, quantitative proteomic studies have shown that IGF2 binding to IR-A recruits a different but overlapping set of substrates from insulin (Morcavallo *et al*. 2011). It has been suggested that the differences in IR-A phosphorylation following IGF2 binding compared with insulin may protect IRS proteins from down-regulation, enabling the signal to be sustained (Belfiore & Malaguarnera 2011). Such prolonged

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signalling could be damaging. The elevated insulin concentrations observed in patients with type 2 diabetes could therefore act through IR-A to promote cancer development, accounting for the excess of cancers observed in this condition. However, this mechanism is unproven. IGF2 action appears to be favoured by the increased IR-A:IR-B ratio that occurs with ageing (Serrana *et al*. 2005) and may increase the risk of tumorigenesis. Increased IGF2 action through IR-A is also linked to reduced vertebrate lifespan (Belfiore *et al*. 2009).

Recent work on the transcription factor *E2F3* has suggested that it has a role in causing increased *IGF2* expression in human cancers (Lui & Baron 2013). In mice, *E2f3* is thought to be responsible for the decline in *IGF2* expression, which occurs postnatally. *E2f3* expression declined postnatally whereas restoration of its expression restored *IGF2* expression. Microarray analysis in humans observed E2F3 and *IGF2* expression to decline with age but bladder and prostate cancers that over-expressed the transcription factor also over-expressed *IGF2* (Lui & Baron 2013). This work suggests that E2f3 is a contributing factor in age-related decline in *IGF2* expression and is also a factor driving its pathological over-expression in cancers.

Tumour suppressors

The tumour suppressor PTEN is commonly mutated in human cancers. Absence of its action permits increased mitogenic signalling in the pathways downstream of PI3-K. The sustained mitogenic action will tend to promote carcinogenesis. In studies of breast cancer cells, loss of PTEN action appears to increase IGF2 signalling through IGF1R and IR-A (Perks *et al*. 2007). There is increasing evidence for interaction between IGF2 and p53 in cancer development. Normally, *IGF2* transcription is repressed by the tumour suppressor p53 (Zhang *et al*. 1996, 1998), which also increases IGFBP3 (Buckbinder *et al*. 1995) and suppresses IGF1R expression (Werner *et al*. 1996). Decreased activity of p53 in tumours, therefore, increases both IGF2 expression and action. Recent data suggest that increased IGF2 signalling favours tumour development by suppressing activity of the p53 pathway (Clermont *et al*. 2012). Because the p53 pathway is inactive in most cancers, these findings suggest potential therapeutic benefit in a wide range of cancers from targeting IGF2 signalling.

Obesity

It is well recognised that obesity and diabetes predispose to cancer and the IGF system is believed to have a causal role in the link (Renehan *et al*. 2006, Byers & Sedjo 2011). Although the role of IGF2 is unclear, studies have observed elevated concentrations in obese subjects, presumably an appropriate response to excessive energy provision (Frystyk *et al*. 1999, Espelund *et al*. 2005, Fowke *et al*. 2010). IGF2 concentrations correlated with BMI. Free IGF2 concentrations paralleled these changes, suggesting that total IGF2 can be considered a surrogate for bioactive IGF2 in obesity (Frystyk *et al*. 1999). Subjects with type 2 diabetes, as well as obesity, had even higher IGF2 concentrations (Jeyaratnaganthan *et al*. 2010). Clearly, the increased IGF2 bioactivity could be detrimental by causing sustained mitogenic signalling. Weight reduction resulted in decreases in serum total IGF2 and pro-IGF2, independent of the type of diet (Belobrajdic *et al*. 2010). These decreases may reflect first reduced synthesis in response to reduced dietary energy provision and secondly the increased insulin sensitivity, which occurs upon weight reduction. A recent meta-analysis of epidemiological studies reported that intentional weight loss could reduce cancer incidence (Byers & Sedjo 2011). Use of the insulin sensitizer metformin has also been linked to reduced incidence of cancer (Libby *et al*. 2009) and better treatment response in breast cancer (Gallagher & LeRoith 2010). While the mechanisms are unknown, these findings have important therapeutic implications. The role of IGF2, if any, in the increased cancer incidence of obesity needs to be clarified by future studies.

Parental obesity may influence fetal health and ultimately cancer risk, through epigenetic changes in *IGF2*. Reduced *IGF2* DMR methylation in umbilical cord blood was associated with increased IGF2 concentrations (Hoyo *et al*. 2012*a*). This association was stronger in infants of obese mothers. Increased IGF2 concentrations were significantly associated with high birth weight. In another study, placental *IGF2* methylation was associated with fetal weight (St-Pierre *et al*. 2012). The *IGF2* genotype and epigenotype was estimated to account for 31% of the variation in neonatal weight. These transgenerational effects are not confined to maternal nutritional status. Recently, paternal obesity was associated with hypomethylation of the *IGF2* DMR in offspring (Soubry *et al*. 2013). Periconceptual parental weight therefore appears to influence epigenetic regulation of *IGF2*, which in turn regulates fetal IGF2 concentrations and weight. This may enable parental obesity to predispose to cancer later in the life of the infant. In animals, periconceptual dietary restriction of obese mothers can influence the epigenetic state of the *IGF2* gene (Zhang *et al*. 2011). It is not known whether a similar effect occurs in humans. Exposure to

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environmental compounds during pregnancy can also potentially damage fetal health by causing epigenetic changes. Recent studies have observed that maternal exposure to bisphenol A (Susiarjo *et al*. 2013) and antibiotics (Vidal *et al*. 2013) altered *IGF2* DMR methylation. The former was associated with abnormal placental development and the latter with lower infant birth weight.

Cancer progression

There is extensive evidence that increased *IGF2* expression by tumours is associated with a poorer prognosis, for example greater mortality in breast cancer (Kalla Singh *et al*. 2010*a*), shorter time to disease recurrence in oesophageal cancer (Zhao *et al*. 2009) and more rapid disease progression in chronic myeloid leukaemia (Randhawa *et al*. 1998). Significant progress has been made in understanding the molecular mechanisms whereby IGF2 promotes tumour growth, leading to a poorer prognosis.

Neovascularization

IGF2 promotes neovascularization of tumours, without which growth would be inhibited by hypoxia (De Leon *et al*. 1992). Its expression appears to be hypoxia driven as part of a progression of angiogenic growth factors. Hypoxia-inducible factors up-regulate IGF2 which in turn up-regulates vascular endothelial growth factor (VEGF) leading to angiogenesis (Kim *et al*. 1998, Bae *et al*. 1999). IGF2 also promotes angiogenesis by stimulating differentiation of embryonic stem cells into endothelial cells (Piecewicz *et al*. 2012). Even when not expressed by the tumour itself, IGF2 may be detected in surrounding tissues, suggesting that it can act in a paracrine manner to promote growth (El-Badry *et al*. 1991). Increased IGF2 has also been observed in the transition zone between normal epithelium and preinvasive lesions originating from stromal cells and leucocytes (Heffelfinger *et al*. 1999). Recently, blockage of *IGF2* expression caused down-regulation of VEGF and inhibited growth (Yao *et al*. 2012). These findings make IGF2 a key therapeutic target.

Receptors and signalling

IGF2 promotes cancer cell growth in part via the IGF1R. Signalling through this receptor has been reviewed in detail elsewhere (Kim *et al*. 2009, Djiogue *et al*. 2013) and is mentioned only briefly here. IGF1R has an established role in tumour progression, its copy number negatively associated with survival time (Natarajan *et al*. 2006). Its increased expression by cancer cells is associated with over-expression of *IGF2* and tendency to metastasize (Guerra *et al*. 1996). Mutations causing constitutive activation of IGFIR are rare. This emphasises the importance of its activation by ligands, including IGF2, in cancer progression (Kim *et al*. 2009).

IR-A is the predominant isoform expressed in cancer cells, its expression highest in de-differentiated malignancies. A high IR-A:IR-B ratio favours IGF2 action, impairing differentiation (Vella *et al*. 2002, Entingh *et al*. 2003, Belfiore *et al*. 2009) and is associated with disease progression (Frasca *et al*. 1999, Belfiore *et al*. 2009). The cause of this aberrant expression is unknown but insulin resistance up-regulates IR-A in cancer cells (Algire *et al*. 2011). Increased IGF2/IR-A signalling can occur following therapeutic blockage of IGF1R causing resistance to treatment (Garofalo *et al*. 2011). In cancer cells, IGF2 can exert all its effects through IR-A. For example, in the leiomyosarcoma cell line SKUT-1, which expresses no IGF1R, IGF2 signals exclusively through IR-A (Sciacca *et al*. 2002). IGF2 appears to be more damaging than insulin when signalling through IR-A (Morcavallo *et al*. 2012). Unlike insulin, sustained IGF2 exposure failed to cause down-regulation of the intracellular signalling protein IRS1. Moreover, IGF2 was less effective than insulin at promoting internalisation of IR-A. These differences may enable its mitogenic stimulus to be sustained. It has, therefore, been hypothesised that IGF2 acts in an autocrine loop with IR-A expression enhancing cancer cell growth (Fig. 3). This has been observed in trophoblastic malignancies (Altieri *et al*. 2003), and thyroid cancer (Vella *et al*. 2002) and may contribute to chemoresistance (Gualberto & Pollak 2009).

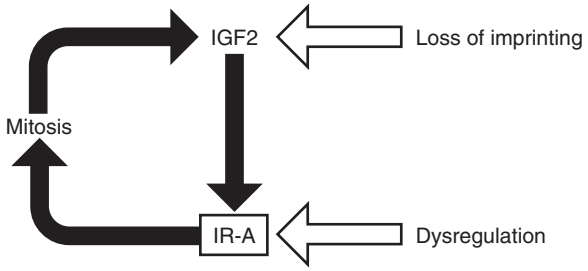

Figure 3
Autocrine loop of IGF2 action in cancer progression. IGF2 can be produced in excess by tumour cells because of loss of imprinting (LOI) of the *IGF2* gene. IGF2 produced by tumour cells can act in an autocrine manner by binding to isoform A of the insulin receptor (IR-A). This results in stimulation of mitosis and continued production of IGF2. Sustained intracellular signalling and impaired IR-A internalisation potentially enable a vicious cycle of increasing growth and IGF2 production.

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Loss of IGF2R is associated with a poor prognosis (Jamieson *et al*. 2003, Pavelic *et al*. 2003). The aggressive phenotype may be caused both by excessive IGF2 and over secretion of lysozomal proteases that promote tumour invasion (Probst *et al*. 2009). Conversely, increased *IGF2R* expression is associated with a better outcome from disease, possibly because of enhanced clearance of IGF2 (Kalla Singh *et al*. 2010*b*). Recent evidence suggests that IGF1R/IR-A hybrid receptors have proliferative effects in cancer cells (Cheng *et al*. 2009). IGF2 may, therefore, also promote tumour growth by signalling through these receptors.

IGF binding proteins

IGFBPs secreted by cancer cells may either enhance or inhibit growth by modulating IGF2 action. Over-expression of IGFBP2 and IGFBP5 has been associated with increased IGF action and poorer cancer prognosis (Pollak 2008). For example, increased IGFBP2 secretion by leukaemic T cells in response to IGF2 promoted growth (Elmlinger *et al*. 1998). Increased proteolysis of IGFBP3 by breast cancer cells stimulated tumour growth by increasing local IGF availability. Protease activity was highest in patients with the most invasive tumours (Helle *et al*. 2001). Conversely, growth was inhibited by increased IGFBP3 expression in response to transforming growth factor β (TGFβ; Oh *et al*. 1995). This anti-proliferative effect was abolished by IGF2, which blocked TGFβ-induced binding of IGFBP3 to the cell surface. Increased IGFBP3 expression has also been reported as a mechanism whereby vitamin D opposes IGF2 action (Huynh *et al*. 1998).

Steroidogenesis

There is recent evidence that both IGF2 and insulin contribute to prostate cancer progression by increasing *de novo* steroidogenesis. IGF2 treatment of androgen receptor-expressing prostate cancer cell lines caused increased steroidogenesis leading to androgen receptor activation and prostate-specific antigen expression (Lubik *et al*. 2013). In the same study, increased *IGF2* expression in prostate cancer tissue from patients was observed to accompany resistance to androgen deprivation therapy (ADT). In a previous study by the same group, insulin treatment of prostate cancer cell lines increased steroidogenic enzyme expression and testosterone secretion (Lubik *et al*. 2011). The authors hypothesised that increased steroidogenesis is a mechanism whereby elevated insulin concentrations that occur during ADT promote prostate cancer progression.

Non-islet cell tumour hypoglycaemia

Non-islet cell tumour hypoglycaemia (NICTH) is a rare paraneoplastic syndrome occurring in association with large or metastatic tumours, usually over 0.5 kg in size (Marks & Teale 1998). It has been reported in almost every type of tumour (de Groot *et al*. 2007). This diagnosis should always be considered when hypoglycaemia occurs in patients with advanced malignancy. The discussion here will be confined to IGF2-related hypoglycaemia, but it should be stated that NICTH can be caused by other factors, namely IR autoantibodies, cytokines and malignant invasion of the liver.

Mechanism of hypoglycaemia

Over-expression of *IGF2* is the central event in NICTH. The serum concentrations of mature IGF2 and big IGF2 are increased in 30 and 70% of patients respectively (Hizuka *et al*. 1998). Big IGF2 contributes up to 60% of the total IGF2 present in NICTH. It retains 21 residues of the E-peptide (IGF2E (68–88)), which is non-glycosylated (Daughaday *et al*. 1993). The reason for impaired proteolytic processing of pro-IGF2 is unclear but recent studies have suggested mechanisms. First, processing may fail because of the absence of glycosylation (Daughaday *et al*. 1993). Secondly, the quantity of pro-IGF2 produced may overwhelm the proteolytic capacity of the tumour cells (Zapf 1993). In support of this, defective PC4 expression has been reported in a tumour causing NICTH (Tani *et al*. 2008). Increased serum concentrations of the E-domain have also been observed in patients with NICTH (Daughaday & Trivedi 1992*b*).

Various factors increase bioavailable IGF2 in NICTH. Big IGF2 binds to IGFBPs with the same affinity as does mature IGF2, but forms ternary complexes less readily, possibly because of reduced affinity for ALS (Daughaday 1996). Binary complexes with IGFBPs (40–50 kDa) are favoured, which can traverse the capillary endothelium (Bond *et al*. 2000). In addition, much big IGF2 remains unbound. Free IGF2 is increased up to 20-fold even when the total IGF2 concentration is normal (Frystyk *et al*. 1998). This is probably because of impaired ternary complex formation or displacement by big IGF2. Both bioavailable big IGF2 and mature IGF2 mimic the action of insulin (Daughaday *et al*. 1988). These increase glucose uptake into peripheral insulin target tissues and suppress hepatic glucose output, leading to hypoglycaemia. Free IGF2 also suppresses ketogenesis, which reduces the body's ability to compensate for hypoglycaemia.

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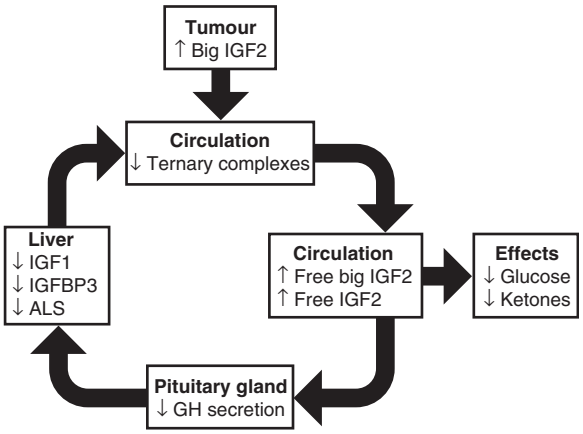

Figure 4
Mechanism of non-islet cell tumour hypoglycaemia (NICTH). Big IGF2 present in NICTH does not readily form ternary complexes with IGFBP3 and ALS. It favours binary complexes with IGFBPs, which traverse the capillary endothelium. Much of the big IGF2 is unbound and bioactive. Free IGF2 is increased up to 20-fold probably because of impaired ternary complex formation or displacement by big IGF2. Both free IGF2 and free big IGF2 are bioactive and can cause hypoglycaemia and other biological effects including suppression of ketogenesis. Free IGF2 also suppresses pituitary GH output. The resulting low GH reduces hepatic synthesis of ternary complex components (IGFBP3, ALS and IGFI). Potentially, big IGF2 can initiate a vicious cycle of impaired ternary complex formation and increased free IGF2. ALS, acid-labile subunit; IGF1, insulin-like growth factor 1; IGFBP3, IGF binding protein 3.

The serum β-hydroxybutyrate (BOHB) concentration therefore tends to be low.

Under normal circumstances, hypoglycaemia stimulates pituitary GH release in order to oppose insulin action. In NICTH, however, this effect is overridden by the suppressive effect of free IGF2 on the GH axis, causing low GH concentrations (LeRoith & Butler 1999). This may render the individual more susceptible to hypoglycaemia, although catecholamine secretion occurs normally (Eastman *et al.* 1992). Low GH also reduces hepatic synthesis of all ternary complex components, potentially resulting in a further increase in free IGF2. It has therefore been hypothesised that there is a vicious circle of increased production of big IGF2, impaired ternary complex formation and suppressed GH, leading to further reduction of ternary complex components (de Groot *et al.* 2007; Fig. 4).

Recent studies have provided new insights into the pathogenesis of NICTH. An *in vitro* study of IGF2 complex formation suggested that bioavailability of big IGF2 depends on the ratio with mature IGF2 (Qiu *et al.* 2010). Under physiological circumstances, with a big IGF2:mature IGF2 ratio of 0.24, big IGF2 preferentially formed complexes with IGFBP3 whereas mature IGF2 complexed with both IGFBP2 and IGFBP3. However, when the ratio was above 1, big IGF2 preferentially formed binary complexes with IGFBP2. The increased binary complex formation that occurs in the presence of excessive big IGF2 probably increases its bioavailability. Big IGF2 isoforms bind less readily to IGF2R, which may impair their clearance further increasing bioavailability (Greenall *et al.* 2013). Interestingly, NICTH and glucose intolerance were recently reported in the same patient (Thabit *et al.* 2011). The mechanism is unclear but prolonged IR stimulation by big IGF2 may cause post-receptor insulin resistance manifesting itself as impaired glucose tolerance. It remains unclear why IGF2-related hypoglycaemia is so rare given that IGF2 secretion by tumours is common.

Other clinical features A variety of other clinical features have been reported attributable to big IGF2 and mature IGF2. Acromegaloid skin changes (Trivedi *et al.* 1995) and goitre (Thabit *et al.* 2011) are trophic effects possibly caused by prolonged IGF1R activation. Hypokalaemia may be caused by insulin-like action of IGF2 causing cellular uptake of potassium (Fukuda *et al.* 2006). Another characteristic feature of NICTH is elevation of IGFBP2, the mechanism of which is not understood but may be caused by the tumour itself (Elmlinger *et al.* 1998, Hoogwerf *et al.* 2013). Increased production of IGFBP6 has also been reported (Hoekman *et al.* 1999). Reported biochemical findings in NICTH are summarised in Table 1.

Treatment The treatment of choice in NICTH is surgery or debulking of the tumour to remove the underlying cause, namely IGF2 or big IGF2 production.

Table 1 Reported serum biochemical findings in NICTH

Analyte	NICTH	References
Glucose	↓	Marks & Teale (1998)
NEFA	↓	Zapf (1993)
Insulin	↓	Marks & Teale (1998)
C-peptide	↓	Marks & Teale (1998)
GH	↓	de Groot *et al.* (2007)
IGF1	↓	Marks & Teale (1998)
IGF2	↑/N/↓	de Groot *et al.* (2007)
IGF2:IGF1	↑	Marks & Teale (1998)
Big IGF2	↑/N	de Groot *et al.* (2007)
Free IGF1	↑	Frystyk *et al.* (1998)
Free IGF2	↑	Frystyk *et al.* (1998)
IGFBP2	↑	Baxter *et al.* (1995)
IGFBP3	↓	de Groot *et al.* (2007)
IGFBP6	↑	Hoekman *et al.* (1999)
ALS	↓	de Groot *et al.* (2007)
Potassium	↓	Fukuda *et al.* (2006)

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Medical treatments include chemotherapy, glucocorticoids (Baxter *et al*. 1995, Teale & Marks 1998), diazoxide (Mitchell *et al*. 1968), recombinant GH (Drake *et al*. 1998), glucagon (Phillips & Robertson 1993), somatostatin analogues (Perros *et al*. 1996) or combinations thereof. Of these, high-dose glucocorticoids are the most successful reducing both big IGF2 production and tumour size. Surgical removal of the tumour can return the IGF2:IGFI ratio to normal, abolish the hypoglycaemia and restore normal GH and IGFBP3 concentrations (Zapf 1993, Perros *et al*. 1996). In addition, mature IGF2 climbs following treatment, suggesting that big IGF2 has a suppressive effect on its concentrations (Zapf *et al*. 1992).

Clinical utility for IGF2 and related tests in cancer

In order for IGF2 measurement to be worthwhile in any clinical context, it must guide the management of the patient. The high cost of IGF2 testing emphasises the importance of its utility being clear. For the purpose of discussion, the utility of IGF2 and related tests is divided into established and potential. These are summarised in Table 2.

Established utility

The only established indications for IGF2 measurement are in diagnosis and monitoring of NICTH. Because of the rarity of NICTH, IGF2 measurement is infrequently requested, the analysis being confined to specialist centres or research laboratories. The biochemical findings in NICTH are hypoglycaemia in the presence of appropriately suppressed insulin and C-peptide concentrations but inappropriately low GH and IGF1. While serum IGF2 concentrations are normal in about 70% of cases, IGF1 is invariably low (Hizuka *et al*. 1998). This causes the IGF2:IGF1 ratio to be >10 (normal <3) which is the key diagnostic finding (Marks & Teale 1998). Besides NICTH, the only possible cause of an elevated IGF2:IGF1 ratio is hypoglycaemia associated with sepsis. This should be straightforward to distinguish from NICTH because sepsis reduces hepatic synthesis of IGFs causing both their concentrations to decrease.

The BOHB concentration is typically below 600 μmol/l (Marks & Teale 1998). Levels of non-esterified fatty acids (NEFA) are also low. In a hypoglycaemic adult, the finding of low BOHB in the presence of suppressed insulin and C-peptide levels suggests the presence of an agent mimicking insulin and indicates measurement of the IGF2:IGF1 ratio. Measurement of IGFBP2 is a useful part

Table 2 Clinical utility for IGF2 and related tests in cancer

Test	Utility	References
Established utility		
IGF2:IGF1 ratio	Diagnosis and monitoring of NICTH	Marks & Teale (1998)
Potential utility		
IGF2	Early detection of colonic cancer	Renehan *et al*. (2000)
IGF2	Tumour surveillance	Pavelic *et al*. (1999)
IGF2	Prognosis of HCC	El-Tayebi *et al*. (2011)
IGF2	Prediction of HCC in HCV-associated cirrhosis	Couvert *et al*. (2012)
IGF2	Prediction of colonic cancer in women	Hunt *et al*. (2002)
IGF2	Prediction of colorectal cancer	Gao *et al*. (2012)
IGF2	Prognosis of prostate cancer	Rowlands *et al*. (2012)
IGF2	Prognosis of head and neck cancer	Alajez *et al*. (2012)
IGF2 immunohistochemistry	Prognosis in GIST	Steigen *et al*. (2009)
Big IGF2 histochemistry	Prognosis of GIST	Rikhof *et al*. (2012)
IGF2 mRNA in tumour	Prediction of recurrence of adrenocortical tumours	Gicquel *et al*. (2001)
IGF2 LOI in lymphocytes	Prediction of colorectal cancer	Cui *et al*. (2003)
IGF2 LOI in lymphocytes	Prediction of prostate cancer	Belharazem *et al*. (2012)
IGF2 polymorphisms	Prediction of carcinogenesis in HBV infection	Kim *et al*. (2006)
IGF2 SNP	Prediction of post-operative recurrence of HCC	Lee *et al*. (2012)
IGF2R polymorphisms	Prediction of cancer risk	Morcavallo *et al*. (2012) and Yoon *et al*. (2012)
Gene analysis	Prediction of carcinogenesis	Hoshida (2011)
Gene expression signature	Early diagnosis of epithelial ovarian cancer	Pils *et al*. (2013)
Single cell gene analysis	Prognosis of prostate cancer	Chen *et al*. (2013)

GIST, gastrointestinal stromal tumour; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IGF2, insulin-like growth factor 2; *IGF2R*, type 2 IGF receptor gene; LOI, loss of imprinting; NICTH, non-islet cell tumour hypoglycaemia.

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of the diagnostic process. Ideally big IGF2 and free IGF2 should be measured but assays for these are not widely available in clinical laboratories. In making the diagnosis, all biochemical findings should be interpreted in clinical context. The diagnosis can be further confirmed by the response of the biochemical parameters to treatment.

Potential utility

Recent research findings suggest novel uses for IGF2 measurement and related tests in patients with cancer. These are not yet established in clinical practice, but future studies will clarify their utility. It is likely that the need for such tests will increase in the near future once treatments targeting IGF2 are established. Tests may be needed firstly to determine which patients will benefit from treatment and secondly to monitor the response.

Early diagnosis and monitoring The observation that some tumours over-express *IGF2* has prompted investigation into utility of IGF2 measurement in early diagnosis. Serum IGF2 has been suggested as a marker of colonic cancer because it was elevated early in the disease (Renehan *et al*. 2000). In HCC, however, it performed poorly as a tumour marker compared with methylation analysis and alphafetoprotein (Morace *et al*. 2010). IGF2 concentrations decrease following surgery, which suggests utility in monitoring tumour burden (Pavelic *et al*. 1999, Fukuda *et al*. 2006). However, its use could be limited in tumours, which do not secrete IGF2 throughout the disease course. In addition, circulating IGF2 arises in part from liver, its concentration having been reported to reflect hepatic integrity (Nikolic *et al*. 2000, Weber *et al*. 2002). Liver disease could, therefore, confound interpretation of the concentration. Gene expression and plasma protein signatures may enable early diagnosis of cancer in the future. A recently described blood-based signature including 13 genes and six plasma proteins, including IGF2, increased the sensitivity and specificity of diagnosis of epithelial ovarian cancer (Pils *et al*. 2013).

Assessment of prognosis IGF2 production by tumours is associated with more aggressive disease. This suggests prognostic utility for its measurement, which could guide decisions on treatment (Avnet *et al*. 2009). Over-expression of *IGF2* is a common feature of hepatoblastoma, a tumour with a tendency to vascular invasion. Down-regulation of IGFBP3 in this tumour was strongly associated with increased vascular invasion, possibly because of lack of restraint on IGF2 (Regel *et al*. 2012). Restoration of IGFBP3 expression was associated with reduced aggression. This suggests prognostic utility for IGF2 and IGFBP3 measurement. Because IGF2 reflects hepatic integrity, it may have utility in assessment of hepatic function and prognosis of liver disease (Nikolic *et al*. 2000). However, it is not clear whether its measurement would offer additional utility over tests already available.

Although circulating total IGF2 is convenient to measure, it may not accurately reflect IGF2 acting locally to promote invasion. Direct immunohistochemical measurement of IGF2 in the tumour would be anticipated to be more closely linked to outcome. A recent study of GISTs showed that big IGF2 measured immunohistochemically was associated with aggressive disease (Rikhof *et al*. 2012). Similarly, mature IGF2 measured immunohistochemically in GIST tumours has also been linked to a poorer prognosis (Steigen *et al*. 2009). Further studies will be necessary to determine whether their serum concentrations reflect their activity in the tumour. Over-expression of *IGF2* as assessed by tumour IGF2 mRNA content has been strongly linked to reduced disease-free survival in adrenocortical tumours (Gicquel *et al*. 2001). Loss of *IGF2R* expression in some cancers (Ellis *et al*. 1996, Jamieson *et al*. 2003) suggests prognostic utility for measurement of IGF2R in the circulation or immunohistochemically. Detection of *IGF2R* mutations may also be of value in managing cancer (Pavelic *et al*. 2003). One *IGF2* polymorphism was recently observed to independently predict tumour recurrence following surgery for HCC (Lee *et al*. 2012).

As the bioactive component, free IGF2 is potentially more relevant progonostically than total IGF2 (Frystyk *et al*. 1998). It is technically difficult to measure but has been measured using ultrafiltration by centrifugation to isolate the free fraction followed by time-resolved fluoroimmunoassay (Frystyk *et al*. 1994) and has also been measured using a neutral C-18 Sep-Pak extraction procedure (Daughaday *et al*. 1995). Rather than measuring free IGF2 directly, an alternative approach may be to measure IGF2 as a ratio with IGFBP1 or IGFBP2, which indirectly reflects bioactivity. The prognostic value of these ratios has not been studied but the IGF1:IGFBP1 ratio has been found to predict therapeutic benefit of an IGF1R MAB (Gualberto *et al*. 2011). Although free IGF2 has been measured in patients with cancer (Daughaday *et al*. 1995, Frystyk *et al*. 1998), measurement of total IGF2 currently appears to suffice for clinical utility. In time, utility will likely emerge for free IGF2 measurement, which will drive the development of new assays.

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A new approach to assessment of prognosis may be to examine tumour cells isolated from peripheral blood. This technique was used in patients with prostate cancer to examine expression of multiple epithelial–mesenchymal transition genes including *IGF2* (Chen *et al.* 2013). The expression of these genes was associated with metastatic, treatment-resistant cancer.

Prediction of cancer risk Studies suggest that IGF2 measurement and genetic tests may have a role in prevention of cancer. Increased IGF2 concentrations in blood appear to predict development of colonic cancer in women (Hunt *et al.* 2002). More recently, a study investigated the ability of a number of IGF system components to predict colorectal cancer risk (Gao *et al.* 2012). IGF2 was the most effective predictor. IGF2 does not act in isolation during carcinogenesis but is modulated positively and negatively by other components of the system, which may themselves have prognostic value. For example, over-expression of *IGF1R* is associated with more aggressive tumours (Hakam *et al.* 1999) and IGFBP3 may have a role both in early diagnosis (Darago *et al.* 2011) and prediction of death (Rowlands *et al.* 2012) in prostate cancer and in prediction of risk of colorectal cancer (Wu *et al.* 2011). In view of this, a more accurate assessment of an individual's cancer risk or prognosis may be to combine a panel of measurements as an index.

IGF2 LOI is an early event in cancer development, the detection of which may enable assessment of cancer risk. A pilot study reported that colorectal cancer risk could be predicted by *IGF2* LOI in peripheral blood lymphocytes (Cui *et al.* 2003). This requires further investigation by prospective studies to determine outcome and lead time in making the diagnosis but it raises the exciting possibility of being able to assess cancer risk using a non-invasive test (Cui 2007). Individuals testing positive could be targeted for regular colonoscopy. More recently, genetic studies of *IGF2* were carried out in lymphocytes from patients with a history of prostate cancer (Belharazem *et al.* 2012). The study observed that uncoupling of IGF2 concentrations from imprinting status, rather than LOI alone, had the potential to identify individuals at risk of developing prostate cancer. In the same study, the *IGF2* 820 G/A genotype predicted prostate cancer diagnosis at a younger age. In view of the links between *IGF2R* polymorphisms and cancer (Hoyo *et al.* 2012*b*, Yoon *et al.* 2012), *IGF2R* testing may have a predictive role and demands further investigation. Recent research findings suggest much potential clinical utility for IGF2 testing in the context of liver cancer. The possibility of predicting hepatocarcinogenesis by genetic testing is perhaps the most exciting (Couvert *et al.* 2012).

Genomic assays that provide molecular signatures for multiple genes, including *IGF2*, may also predict cancer risk (Hoshida 2011). The Collaborative Oncological Gene-environment study has already detected more than 80 gene variants associated with increased risk of breast, prostate and ovarian cancers. This is a rational approach to prediction because *IGF2* is only one of many genes working together to determine risk. These techniques could potentially be combined with traditional screening approaches to increase efficacy in disease detection. It is increasingly recognised that methylation patterns can be used as biomarkers for disease or predisposition to disease (Biliya & Bulla 2010). The Human Epigenome Project is underway to identify methylation patterns throughout the genome (www.epigenome.org, 2013).

Therapeutic approaches

The evidence discussed above has stimulated interest in cancer treatments targeting IGF2 action. IGF2 is an attractive therapeutic target because its apparently minor physiological role in adults suggests that ablation of its action carries little potential for disrupting normal processes. There have been exciting recent developments in therapies targeting IGF2, a full account of which is beyond the scope of this review. Interested readers are directed elsewhere (Gualberto & Pollak 2009, Heidegger *et al.* 2011).

Possible therapeutic targets are IGF2 itself, IGFBPs, receptors or intracellular signalling. To date, most work has focussed on blocking IGF action at the IGF1R. Antibody blockade inhibits mitogenic signalling by blocking ligand binding and enhancing receptor endocytosis. This can reduce growth of IGF2-secreting cancer cells (Lahm *et al.* 1994). However, IGF1R blockade may result in a compensatory increase in IR-A signalling, enabling cells to respond to IGF2 and become resistant to therapy (Belfiore *et al.* 2009, Gualberto & Pollak 2009, Garofalo *et al.* 2011). Prevention of autocrine IGF2 action, therefore, requires blockade of both receptors (Vella *et al.* 2002). Such simultaneous targeting of both IGF2 signalling routes has proven effective in osteosarcoma (Avnet *et al.* 2009). Recent work on GIST cell lines, expressing big IGF2 and IR-A, but not IGF1R, showed that cell survival was reduced when signalling was disrupted by down-regulation of big IGF2 or IR-A. Big IGF2/IR-A signalling is, therefore, a potential therapeutic target (Rikhof *et al.* 2012).

Targeting IGF2 itself could prevent its action through either receptor. First, antisense oligonucleotides could

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potentially reduce *IGF2* expression. Alternatively, IGF2 could be inactivated following its secretion. MABs that bind IGF2 inhibit IGF1R phosphorylation and growth of cancer cells (Feng *et al*. 2006, Gao *et al*. 2011) but have not yet been trialled in humans. Tumour gene therapies targeted at cells expressing IGF2 are another exciting recent development (Amit & Hochberg 2010, Pan *et al*. 2010). Vitamins C and D reduce IGF2 production and IGF1R signalling (Oh *et al*. 2001, Galbiati *et al*. 2003, Lee *et al*. 2008). There may be a place for combining these vitamins, or analogues thereof with other treatments. Releasing hormones have potential as therapeutic agents. GNRH reduced production of IGF2 in ovarian cancer and endometrial cancer cells resulting in reduced growth (Kleinman *et al*. 1993, Ho *et al*. 1997). Antagonists of GHRH also block IGF2 production by cancer cell lines inhibiting their growth (Csernus *et al*. 1999). In principle, IGF2 concentrations could be lowered by enhancing IGF2R-mediated clearance or by reducing *IGF2* expression by epigenetic modification, although it is not yet known how to achieve this.

Given that components of the IGF system interact to determine IGF bioactivity, it is likely that approaches targeting multiple sites in the system will prove more effective than single-site approaches. Clinical trials will clarify whether inhibition of IGF2 signalling can prevent tumour growth *in vivo* in humans.

Future considerations

This is an exciting time in our understanding of IGF2. Knowledge of its role in disease is starting to suggest new diagnostic tests and therapeutic approaches. However, despite much research, the autocrine action of IGF2 *in vivo* is poorly understood, mainly because this cannot easily be measured. A challenge for the future will be to understand how IGF2 interacts with other components of the system at tissue level to influence cancer development and progression. Similarly, genetic and epigenetic changes affecting *IGF2* need to be considered in the context of the whole genome.

While there has been abundant research into the disease association of IGF system components, future work needs to place a greater emphasis on the clinical value of measurement of these components, including IGF2, as diagnostic tests. In order to be adopted into the clinical repertoire, such tests will require to have demonstrable clinical utility and preferably also to be non-invasive and low cost. The expense and lack of availability of IGF2 assays in clinical laboratories has hindered clinical studies in the past, but this may change with the advent of its measurement by liquid chromatography mass spectrometry (Bystrom *et al*. 2012).

Cancer prevention is a promising area for future IGF2 research. The suggestion that *in utero* exposures predispose to cancer in adult life, in part through changes in *IGF2* expression, raises the question of whether these exposures can be reduced, for example by periconceptual optimisation of parental body weight. This demands investigation by prospective studies. Studies also need to establish whether IGF2 predisposes to cancer in obesity and whether lowering its concentration reduces risk. If so, there may be a case for targeting individuals for risk reduction therapies.

Declaration of interest

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References

Alajez NM, Shi W, Wong D, Lenarduzzi M, Waldron J, Weinreb I & Liu FF 2012 Lin28b promotes head and neck cancer progression via modulation of the insulin-like growth factor survival pathway. *Oncotarget* **3** 1641–1652.

Algire C, Amrein L, Bazile M, David S, Zakikhani M & Pollak M 2011 Diet and tumour LKB1 expression interact to determine sensitivity to anti-neoplastic effects of metformin *in vivo*. *Oncogene* **30** 1174–1182. (doi:10.1038/onc.2010.483)

Altieri A, Franceschi S, Ferlay J, Smith J & La Vecchia C 2003 Epidemiology and aetiology of gestational trophoblastic diseases. *Lancet Oncology* **13** 670–678. (doi:10.1016/S1470-2045(03)01245-2)

Alvino CL, Ong SC, McNeill KA, Delaine C, Booker GW, Wallace JC & Forbes BE 2011 Understanding the mechanism of insulin and insulin-like growth factor (IGF) receptor activation by IGF-II. *PLoS ONE* **6** e27488. (doi:10.1371/journal.pone.0027488)

Amit D & Hochberg A 2010 Development of targeted therapy for bladder cancer mediated by a double promoter plasmid expressing diphtheria toxin under the control of H19 and IGF2-P4 regulatory sequences. *Journal of Translational Medicine* **16** 134. (doi:10.1186/1479-5876-8-134)

Avnet S, Sciacca L, Salerno M, Gancitano G, Cassarino MF, Longhi A, Zakikhani M, Carboni JM, Gottardis M, Giunti A *et al.* 2009 Insulin receptor isoform A and insulin-like growth factor-II as additional treatment targets in human osteosarcoma. *Cancer Research* **69** 2443–2452. (doi:10.1158/0008-5472.CAN-08-2645)

Bae SK, Bae MH, Ahn MY, Son MJ, Lee YM, Bae MK, Lee OH, Park BC & Kim W 1999 Egr-1 mediates transcriptional activation of IGF-II gene in response to hypoxia. *Cancer Research* **59** 5989–5994.

Bartke A, Chandrashekar V, Bailey B, Zaczek D & Turyn D 2002 Consequences of growth hormone (GH) overexpression and GH resistance. *Neuropeptides* **36** 201–208. (doi:10.1054/npep.2002.0889)

Bates P, Fisher R, Ward A, Richardson L, Hill DJ & Graham CF 1995 Mammary cancer in transgenic mice expressing insulin-like growth factor-II (IGF-II). *British Journal of Cancer* **72** 1189–1193. (doi:10.1038/bjc.1995.484)

Published by Bioscientifica Ltd.

Baxter RC 2001 Changes in the IGF–IGFBP axis in critical illness. *Best Practice & Research. Clinical Endocrinology & Metabolism* **15** 421–434. (doi:10.1053/beem.2001.0161)

Baxter RC, Holman SR, Corbould A, Stranks S, Ho PJ & Braund W 1995 Regulation of the insulin-like growth factors and their binding proteins by glucocorticoid and growth hormone in nonislet cell tumor hypoglycaemia. *Journal of Clinical Endocrinology and Metabolism* **80** 2700–2708. (doi:10.1210/jc.80.9.2700)

Belfiore A & Malaguarnera R 2011 Insulin receptor and cancer. *Endocrine-Related Cancer* **18** R125–R147. (doi:10.1530/ERC-11-0074)

Belfiore A, Frasca F, Pandi 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. (doi:10.1210/er.2008-0047)

Belharazem D, Kirchner M, Geissler F, Bugert P, Spahn M, Kneitz B, Riedmiller H, Sauer C, Kuffer S, Trojan L *et al*. 2012 Relaxed imprinting of IGF2 in peripheral blood cells of patients with a history of prostate cancer. *Endocrine Connections* **1** 87–94. (doi:10.1530/EC-12-0054)

Belobrajdic DP, Frystyk J, Jeyaratnagantham N, Espelund U & Flyvbjerg A 2010 Moderate energy restriction-induced weight loss affects circulating IGF levels independent of dietary composition. *European Journal of Endocrinology* **162** 1075–1082. (doi:10.1530/EJE-10-0062)

Biliya S & Bulla LA 2010 Genomic imprinting: the influence of differential methylation in the two sexes. *Experimental Biology and Medicine* **235** 139–147. (doi:10.1258/ebm.2009.009251)

Boisclair YR, Rhoads RP, Ueki I, Wang J & Ooi GT 2001 The acid-labile subunit (ALS) of the 150 kDa IGF-binding protein complex: an important but forgotten component of the circulating IGF system. *Journal of Endocrinology* **170** 63–70. (doi:10.1677/joe.0.1700063)

Bond JJ, Meka S & Baxter RC 2000 Binding characteristics of pro-insulin-like growth factor-II from cancer patients: binary and ternary complex formation with IGF binding proteins-1 to -6. *Journal of Endocrinology* **165** 253–260. (doi:10.1677/joe.0.1650253)

Braun S, Bitton-Worms K & LeRoith D 2011 The link between metabolic syndrome and cancer. *International Journal of Biological Sciences* **7** 1003–1015. (doi:10.7150/ijbs.7.1003)

Brown J, Jones EY & Forbes BE 2009 Keeping IGF-II under control: lessons from the IGF-II–IGF-2R crystal structure. *Trends in Biochemical Sciences* **34** 612–619. (doi:10.1016/j.tibs.2009.07.003)

Buckbinder L, Talbott R, Velasco-Miguel S, Takenaka I, Faha B, Seizinger BR & Kley N 1995 Induction of the growth inhibitor IGF-binding protein 3 by p53. *Nature* **377** 646–649. (doi:10.1038/377646a0)

Byers T & Sedjo RI 2011 Does intentional weight loss reduce cancer risk? *Diabetes, Obesity & Metabolism* **13** 1063–1072. (doi:10.1111/j.1463-1326.2011.01464.x)

Bystrom C, Sheng S, Zhang K, Caulfield M, Clarke NJ & Reitz R 2012 Clinical utility of insulin-like growth factor 1 and 2; determination by high resolution mass spectrometry. *PLoS ONE* **7** e43457. (doi:10.1371/journal.pone.0043457)

Chen CL, Mahalingam D, Osmulski P, Jadhav RR, Wang CM, Leach RJ, Chang TC, Weitman SD, Kumar AP, Sun L *et al*. 2013 Single cell analysis of circulating tumour cells identifies cumulative expression patterns of EMT-related genes in metastatic prostate cancer. *Prostate* **73** 813–826. (doi:10.1002/pros.22625)

Cheng I, Stram DO, Burtt NP, Gianniny L, Garcia RR, Pooler L, Henderson BE, Le ML & Haiman CA 2009 IGF2R missense single-nucleotide polymorphisms and breast cancer risk: the multiethnic cohort study. *Cancer Epidemiology, Biomarkers & Prevention* **18** 1922–1924. (doi:10.1158/1055-9965.EPI-09-0253)

Clemmons DR 1997 Insulin-like growth factor binding proteins and their role in controlling IGF actions. *Cytokine & Growth Factor Reviews* **8** 45–62. (doi:10.1016/S1359-6101(96)00053-6)

Clemmons DR 1998 Role of insulin-like growth factor binding proteins in controlling IGF actions. *Molecular and Cellular Endocrinology* **140** 19–24. (doi:10.1016/S0303-7207(98)00024-0)

Clemmons DR, Snyder DK & Busby-WH J 1991 Variables controlling the secretion of insulin-like growth factor binding protein-2 in normal human subjects. *Journal of Clinical Endocrinology and Metabolism* **73** 727–733. (doi:10.1210/jcem-73-4-727)

Clermont F, Nittner D & Marine JC 2012 IGF2: the Achilles' heel of p53 deficiency? *EMBO Molecular Medicine* **4** 688–690. (doi:10.1002/emmm.201201509)

Couvert P, Carrie A, Paries J, Vaysse J, Miroglio A, Kerjean A, Nahon P, Chelly J, Trinchet JC, Beaugrand M *et al*. 2008 Liver insulin-like growth factor 2 methylation in hepatitis C virus cirrhosis and further occurrence of hepatocellular carcinoma. *World Journal of Gastroenterology* **14** 5419–5427. (doi:10.3748/wjg.14.5419)

Couvert P, Carrie A, Tezenas du Montcel S, Vaysse J, Sutton A, Barget N, Trinchet JC, Beaugrand M, Ganne N, Giral P *et al*. 2012 Insulin-like growth factor 2 gene methylation in peripheral blood mononuclear cells of patients with hepatitis C related cirrhosis or hepatocellular carcinoma. *Clinics and Research in Hepatology and Gastroenterology* **36** 345–351. (doi:10.1016/j.clinre.2012.06.013)

Cruz-Correa M, Cui H, Giardiello FM, Powe NR, Hylind L, Robinson A, Hutcheon DF, Kafonek DR, Brandenburg S, Wu Y *et al*. 2004 Loss of imprinting of insulin growth factor II gene: a potential heritable biomarker for colon neoplasia predisposition. *Gastroenterology* **126** 964–970. (doi:10.1053/j.gastro.2003.12.051)

Csernus VJ, Schally AV, Kiaris H & Armatis P 1999 Inhibition of growth, production of insulin-like growth factor-II (IGF-II) and expression of IGF-II mRNA of human cancer cell lines by antagonistic analogs of growth hormone releasing hormone *in vitro*. *PNAS* **96** 3098–4103. (doi:10.1073/pnas.96.6.3098)

Cui H 2007 Loss of imprinting of IGF2 as an epigenetic marker for the risk of human cancer. *Disease Markers* **23** 105–112. (doi:10.1155/2007/363464)

Cui H, Cruz-Correa M, Giardiello FM, Hutcheon DF, Kafonek DR, Brandenburg S, Wu Y, He X, Powe NR & Feinberg AP 2003 Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. *Science* **299** 1753–1755. (doi:10.1126/science.1080902)

Darago A, Sapota A, Matych J, Nasiadek M, Skrzypinska-Gawrysiak M & Kilanowicz A 2011 The correlation between zinc and insulin-like growth factor 1 (IGF-1), its binding protein (IGFBP-3) and prostate specific antigen (PSA) in prostate cancer. *Clinical Chemistry and Laboratory Medicine* **49** 1699–1705. (doi:10.1515/CCLM.2011.651)

Daughaday WH 1996 Free insulin-like growth factor (IGF) in disorders of IGF binding protein 3 complex formation. *Journal of Clinical Endocrinology and Metabolism* **89** 3–5. (doi:10.1210/jc.2003-030882)

Daughaday WH & Trivedi B 1992*a* Measurement of derivatives of proinsulin-like growth factor-II in serum by a radioimmunoassay directed against the E-domain in normal subjects and patients with non-islet cell hypoglycaemia. *Journal of Clinical Endocrinology and Metabolism* **75** 110–115. (doi:10.1210/jc.75.1.110)

Daughaday WH & Trivedi B 1992*b* Heterogeneity of serum peptides with immunoactivity detected by a radioimmunoassay for proinsulin-like growth factor-II E domain: description of a free E domain peptide in serum. *Journal of Clinical Endocrinology and Metabolism* **75** 641–645. (doi:10.1210/jc.75.2.641)

Daughaday WH, Hall K, Raben MS, Salmon WD, van den Brande JL & Van Wyk JJ 1972 Somatomedin: proposed designation for sulphation factor. *Nature* **235** 107. (doi:10.1038/235107a0)

Daughaday WH, Emanuele MA, Brooks MH, Barbato AL, Kapadia M & Rotwein P 1988 Synthesis and secretion of insulin-like growth factor-II by a leiomyosarcoma with associated hypoglycaemia. *New England Journal of Medicine* **319** 1434–1440. (doi:10.1056/NEJM198812013192202)

Daughaday WH, Trivedi B & Baxter RC 1993 Serum 'big insulin-like growth factor-II' from patients with tumour hypoglycaemia lacks normal E-domain *O*-linked glycosylation, a possible determinant of normal propeptide processing. *PNAS* **90** 5823–5827.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-13-0231

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Daughaday WH, Trivedi B & Baxter RC 1995 Abnormal serum IGF-II transport in non-islet cell tumor hypoglycaemia results from abnormalities of both IGF binding protein-3 and acid labile subunit and leads to elevation of serum free IGF-II. *Endocrinology* **3** 425–428. (doi:10.1007/BF02935648)

De Leon DD, Wilson DM, Powers M & Rosenfeld RG 1992 Effects of insulin-like growth factors and IGF receptor antibody on the proliferation of human breast cancer cells. *Growth Factors* **6** 327–336. (doi:10.3109/08977199209021544)

De Souza AT, Yamada T, Mills JJ & Jirtle RL 1997 Imprinted genes in liver carcinogenesis. *FASEB Journal* **11** 60–67.

Djiogue S, Kamdje AHN, Vecchio L, Kipanyula MJ, Farahna M, Aldebasi Y & Etet PFS 2013 Insulin resistance and cancer: the role of insulin and IGFs. *Endocrine-Related Cancer* **20** R1–R17. (doi:10.1530/ERC-12-0324)

Drake WM, Miraki F, Siddiqi A, Yateman M, Barnes NC, Camacho-Hubner C & Monson JP 1998 Dose-related effects of growth hormone on IGF-I and IGF-binding protein-2 levels in non-islet cell tumour hypoglycaemia. *European Journal of Endocrinology* **139** 532–536. (doi:10.1530/eje.0.1390532)

Eastman RC, Carson RE, Orloff DG, Cochran CS, Perdue JF, Rechler MM, Lanau F, Roberts CT, Shapiro J & Roth J 1992 Glucose utilisation in a patient with hepatoma and hypoglycaemia: assessment by a positron emission tomography. *Journal of Clinical Investigation* **89** 1958–1963. (doi:10.1172/JCI115803)

El-Badry OM, Romanus JA, Helman LJ, Cooper MJ, Rechler MM & Israel MA 1991 Insulin-like growth factor-II mediated proliferation of human neuroblastoma. *Journal of Clinical Investigation* **87** 648–657. (doi:10.1172/JCI115042)

Ellis MJ, Leav BA, Yang Z, Rasmussen A, Pearce A, Zweibel JA, Lippman ME & Cullen KJ 1996 Affinity for the insulin-like growth factor-II (IGF-II) receptor inhibits autocrine IGF-II activity in MCF-7 breast cancer cells. *Molecular Endocrinology* **10** 286–297. (doi:10.1210/me.10.3.286)

Elmlinger MW, Sanatani MS, Bell M, Dannecker GE & Ranke MB 1998 Elevated insulin-like growth factor (IGF) binding protein (IGFBP)-2 and IGFBP-4 expression of leukaemic T cells is affected by autocrine/paracrine IGF-II action but not by IGF type 1 receptor expression. *European Journal of Endocrinology* **138** 337–343. (doi:10.1530/eje.0.1380337)

El-Tayebi HM, Salah W, El Sayed IH, Salam EM, Zekri AR, Zayed N, Salem ES, Esmat G & Abdelaziz AI 2011 Expression of insulin-like growth factor-II, matrix metalloproteinases and their tissue inhibitors as predictive markers in the peripheral blood of HCC patients. *Biomarkers* **16** 346–354. (doi:10.3109/1354750X.2011.573095)

Entingh AJ, Taniguchi CM & Kahn CR 2003 Bi-directional regulation of brown fat adipogenesis by the insulin receptor. *Journal of Biological Chemistry* **278** 33377–33383. (doi:10.1074/jbc.M303056200)

Espelund U, Bruun JM, Richelsen B, Flyvberg A & Frystyk J 2005 Pro- and mature IGF-II during diet-induced weight loss in obese subjects. *European Journal of Endocrinology* **153** 861–869.

Espelund U, Cold S, Frystyk J, Orskov H & Flyvbjerg A 2008 Elevated free IGF2 levels in localized, early-stage breast cancer in women. *European Journal of Endocrinology* **159** 595–601. (doi:10.1530/EJE-08-0154)

Feng Y, Zhu Z, Xiao X, Choudhry V, Barrett JC & Dimitrov DS 2006 Novel human monoclonal antibodies to insulin-like growth factor (IGF)-II that potently inhibit the IGF receptor type I signal transduction function. *Molecular Cancer Therapeutics* **5** 114–120. (doi:10.1158/1535-7163.MCT-05-0252)

Firth SM & Baxter RC 2002 Cellular actions of the insulin-like growth factor binding proteins. *Endocrine Reviews* **23** 824–854. (doi:10.1210/er.2001-0033)

Fowke JH, Matthews CE, Yu H, Cai Q, Cohen S, Buchowski MS, Zheng W & Blot WJ 2010 Racial differences in the association between body mass index and serum IGF1, IGF2 and IGFBP3. *Endocrine-Related Cancer* **17** 51–60. (doi:10.1677/ERC-09-0023)

Frasca F, Pandini G, Scalia P, Sciacca L, Minco R, Costantino A, Goldfine ID, 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.

Froesch ER, Buergi H, Ramseier EB, Bally B & Labhart A 1963 Antibody-suppressible and non-suppressible insulin-like activities in human serum and their physiologic significance. An insulin assay with adipose tissue of increased precision and specificity. *Journal of Clinical Investigation* **42** 1816–1834. (doi:10.1172/JCI104866)

Frystyk J, Skjaerbaek C, Dinesen B & Orskov H 1994 Free insulin-like growth factors (IGF-I and IGF-II) in human serum. *FEBS Letters* **348** 185–191. (doi:10.1016/0014-5793(94)00602-4)

Frystyk J, Skjaerbaek C, Zapf J & Orskov H 1998 Increased levels of circulating free insulin-like growth factors in patients with non-islet cell tumour hypoglycaemia. *Diabetologia* **41** 589–594. (doi:10.1007/s001250050951)

Frystyk J, Skjaerbaek C, Vestbo E, Fisker S & Orskov H 1999 Circulating levels of free insulin-like growth factors in obese subjects: the impact of type 2 diabetes. *Diabetes/Metabolism Research and Reviews* **15** 314–322. (doi:10.1002/(SICI)1520-7560(199909/10)15:5<314::AID-DMRR56>3.0.CO;2-E)

Fu VX, Schwarze SR, Kenowski ML, Leblanc S, Svaren J & Jarrard DF 2004 A loss of insulin-like growth factor-2 imprinting is modulated by CCCTC-binding factor down-regulation at senescence in human epithelial cells. *Journal of Biological Chemistry* **279** 52218–52226. (doi:10.1074/jbc.M405015200)

Fukuda I, Hizuka N, Ishikawa Y, Yasumoto K, Murakami Y, Sata A, Morita J, Kurimoto M, Okubo Y & Takano K 2006 Clinical features of insulin-like growth factor-II producing non-islet cell tumor hypoglycaemia. *Growth Hormone & IGF Research* **16** 211–216. (doi:10.1016/j.ghir.2006.05.003)

Galbiati F, Polastri L, Thorens B, Dupraz P, Fiorina P, Cavallaro U, Christofori G & Davalli AM 2003 Molecular pathways involved in the antineoplastic effects of calcitriol on insulinoma cells. *Endocrinology* **144** 1832–1841. (doi:10.1210/en.2002-221014)

Gallagher EJ & LeRoith D 2010 The proliferating role of insulin and insulin-like growth factors in cancer. *Trends in Endocrinology and Metabolism* **21** 610–618. (doi:10.1016/j.tem.2010.06.007)

Gao J, Chesebrough JW, Cartlidge SA, Ricketts SA, Incognito L, Veldman-Jones M, Blakey DC, Tabrizi M, Jallal B, Trail PA *et al.* 2011 Dual IGF-I/IGF-II neutralizing antibody MEDI-573 potently inhibits IGF signalling and tumor growth. *Cancer Research* **71** 1029–1040. (doi:10.1158/0008-5472.CAN-10-2274)

Gao Y, Karki H, Graibard B, Pollak M, Martin M, Tao Y, Schoen RE, Church T, Hayes RB, Greene MH *et al.* 2012 Serum IGF1, IGF2 and IGFBP-3 and risk of advanced colorectal adenoma. *International Journal of Cancer* **131** E105–E113. (doi:10.1002/ijc.26438)

Garofalo C, Manara MC, Nicoletti G, Marino MT, Lollini PL, Astolfi A, Pandini G, Lopez-Guerrero JA, Schaefer KL, Belfiore A *et al.* 2011 Efficacy and resistance to anti-IGF-1R therapies in Ewing's sarcoma is dependent on insulin receptor signaling. *Oncogene* **30** 2730–2740. (doi:10.1038/onc.2010.640)

Gicquel C, Bertagna X, Gaston V, Coste J, Louvel A, Baudin E, Bertherat J, Chapuis Y, Duclos JM, Schlumberger M *et al.* 2001 Molecular markers and long-term recurrences in a large cohort of patients with sporadic adrenocortical tumors. *Cancer Research* **61** 6762–6767.

Gowan LK, Hampton B, Hill DJ, Schlueter RJ & Perdue JF 1987 Purification and characterization of a unique high molecular weight form of insulin-like growth factor II. *Endocrinology* **121** 449–458. (doi:10.1210/endo-121-2-449)

Greenall SA, Bentley JD, Pearce LA, Scobie JA, Sparrow LG, Bartone NA, Xiao X, Baxter RC, Corsgrove LJ & Adams TE 2013 Biochemical characterization of individual human glycosylated insulin-like growth factor (IGF)-II and big-IGF-II isoforms associated with cancer. *Journal of Biological Chemistry* **288** 59–68. (doi:10.1074/jbc.M112.432013)

Gronbaek H, Flyvbjerg A, Mellemkjaer L, Tjonneland A, Christensen J, Sorensen HT & Overvad K 2004 Serum insulin-like growth factors, insulin-like growth factor binding proteins and breast cancer risk in

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-13-0231

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postmenopausal women. *Cancer Epidemiology, Biomarkers & Prevention* **13** 1759–1764.
de Groot JWB, Rikhof B, van Doorn J, Bilo HJG, Alleman MA, Honkoop AH & van der Graaf WT 2007 Non-islet cell tumour induced hypoglycaemia: a review of the literature including two new cases. *Endocrine-Related Cancer* **14** 979–993. (doi:10.1677/ERC-07-0161)
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. (doi:10.1038/onc.2009.172)
Gualberto A, Hixon ML, Karp DD, Li D, Green S, Dolled-Filhart M, Paz-Ares LG, Novello S, Blakely J, Langer CJ *et al.* 2011 Pre-treatment levels of circulating free IGF-I identify NSCLC patients who derive clinical benefit from figitumumab. *British Journal of Cancer* **104** 68–74. (doi:10.1038/sj.bjc.6605972)
Guerra FK, Eijan AM, Puricelli L, Alonso DF, Bal de Kier Joffe E, Kornblihgtt AR, Charreau EH & Elizalde PV 1996 Varying patterns of expression of insulin-like growth factors-I and-II and their receptors in murine mammary adenocarcinoma of different metastasizing ability. *International Journal of Cancer* **65** 812–820. (doi:10.1002/(SICI)1097-0215(19960315)65:6<812::AID-IJC18>3.0.CO;2-5)
Hahn H, Wojnowski L, Specht K, Kappler R, Calzada-Wack J, Potter D, Zimmer A, Müller U, Samson E, Quintanilla-Martinez L *et al.* 2000 Patched target Igf2 is indispensable for the formation of medulloblastoma and rhabdomyosarcoma. *Journal of Biological Chemistry* **275** 28341–28344. (doi:10.1074/jbc.C000352200)
Hakam A, Yeatman TJ, Lu L, Mora L, Marcet G, Nicosia SV, Karl RC & Coppola D 1999 Expression of insulin-like growth factor-1 receptor in human colorectal cancer. *Human Pathology* **30** 1128–1133. (doi:10.1016/S0046-8177(99)90027-8)
Harrela M, Koistinen H, Kaprio J, Lehtovirta M, Tumilehto J, Eriksson J, Toivanen L, Koskenvuo M, Leinonen P, Koistinen R *et al.* 1996 Genetic and environmental components of interindividual variation in circulating levels of IGF-I, IGF-II, IGFBP-1 and IGFBP-3. *Journal of Clinical Investigation* **98** 2612–2615. (doi:10.1172/JCI119081)
Hartmann LC, Sellers TA, Frost MH, Lingle WL, Degnim AC, Ghosh K, Vierkant RA, Maloney SD, Pankratz VS, Hillman DW *et al.* 2005 Benign breast disease and the risk of breast cancer. *New England Journal of Medicine* **353** 229–237. (doi:10.1056/NEJMoa044383)
Heffelfinger SC, Miller MA, Yassin R & Gear R 1999 Angiogenic growth factors in preinvasive breast disease. *Clinical Cancer Research* **5** 2867–2876.
Heidegger I, Pircher A, Klocker H & Massoner P 2011 Targeting the insulin-like growth factor network in cancer therapy. *Cancer Biology & Therapy* **11** 701–707. (doi:10.4161/cbt.11.8.14689)
Helle SI, Geisler S, Aas T, Paulsen T, Holly JM & Lonning PE 2001 Plasma insulin-like growth factor binding protein-3 proteolysis is increased in primary breast cancer. *British Journal of Cancer* **85** 74–77. (doi:10.1054/bjoc.2001.1860)
Hizuka N, Fukuda I, Takano K, Okubo Y, Asakawa-Yasumoto K & Demura H 1998 Serum insulin-like growth factor II in 44 patients with non-islet cell tumor hypoglycemia. *Endocrine Journal* **45** (Suppl) S61–S65. (doi:10.1507/endocrj.45.Suppl_S61)
Ho MN, Delgado CH, Owens GA & Steller MA 1997 Insulin-like growth factor-II participates in the biphasic effect of a gonadotropin releasing hormone agonist on ovarian cancer cell growth. *Fertility and Sterility* **67** 870–876. (doi:10.1016/S0015-0282(97)81399-4)
Hoekman K, van Doorn J, Gloudemans T, Maassen JA, Schuller AG & Pinedo HM 1999 Hypoglycaemia associated with the production of insulin-like growth factor-II and insulin-like growth factor binding protein-6 by a haemangiopericytoma. *Clinical Endocrinology* **51** 247–253. (doi:10.1046/j.1365-2265.1999.00833.x)
Hoogwerf D, van Doorn J & Maartense E 2013 The insulin-like growth factor system in a patient with diffuse large B-cell non-Hodgkin's lymphoma and lactic acidosis. *Annals of Clinical Biochemistry* **50** 169–172. (doi:10.1258/acb.2012.012125)
Hoshida Y 2011 Molecular signatures and prognosis of hepatocellular carcinoma. *Minerva Gastroenterologica e Dietologica* **57** 311–322.
Hoyo C, Fortner K, Murtha AP, Schildkraut JM, Soubry A, Demark-Wahnefried W, Jirtle RL, Kurtzberg J, Forman MR, Overcash F *et al.* 2012*a* Association of cord blood methylation fractions at imprinted insulin-like growth factor 2 (IGF2), plasma IGF2 and birth weight. *Cancer Causes & Control* **23** 635–645. (doi:10.1007/s10552-012-9932-y)
Hoyo C, Murphy SK, Schildkraut JM, Vidal AC, Skaar D, Millikan RC, Galanko J, Sandler RS, Jirtle R & Keku T 2012*b* IGF2R genetic variants, circulating IGF2 concentrations and colon cancer risk in African Americans and whites. *Disease Markers* **32** 133–141. (doi:10.1155/2012/492068)
Humbel RE 1990 Insulin-like growth factors I and II. *European Journal of Biochemistry* **190** 445–462. (doi:10.1111/j.1432-1033.1990.tb15595.x)
Hunt KJ, Toniolo P, Akhmedkhanov A, Lukanova A, Dechaud H, Rinaldi S, Zeleniuch-Jacquotte A, Shore RE, Riboli E & Kaaks R 2002 Insulin-like growth factor-II and colorectal cancer risk in women. *Cancer Epidemiology, Biomarkers & Prevention* **11** 901–905.
Huynh H, Pollak M & Zhang JC 1998 Regulation of insulin-like growth factor (IGF)-II and IGF binding protein 3 autocrine loop in human PC-3 prostate cancer cells by vitamin D metabolite 1,25(OH)2D3 and its analog EB1089. *International Journal of Oncology* **13** 137–143.
Ingram WJ, Wicking CJ, Grimmond SM, Forrest AR & Wainwright BJ 2002 Novel genes regulated by sonic hedgehog in pluripotent mesenchymal cells. *Oncogene* **21** 8196–8205. (doi:10.1038/sj.onc.1205975)
Jamieson TA, Brizel DM, Killian JK, Oka Y, Jang HS, Fu X, Clough RW, Vollmer RT, Anscher MS & Jirtle RL 2003 M6P/IGF2R loss of heterozygosity in head and neck cancer associated with poor patients prognosis. *BMC Cancer* **3** 4. (doi:10.1186/1471-2407-3-4)
Jeyaratnaganthan N, Hojlund K, Kroustrup JP, Larsen JF, Bjerre M, Levin K, Beck-Nielsen H, Frago S, Hassan AB, Flyvbjerg A *et al.* 2010 Circulating levels of insulin-like growth factor-II/mannose-6-phosphate receptor in obesity and type 2 diabetes. *Growth Hormone & IGF Research* **20** 185–191. (doi:10.1016/j.ghir.2009.12.005)
Jones JI & Clemmons DR 1995 Insulin-like growth factors and their binding proteins: biological actions. *Endocrine Reviews* **16** 3–34.
Juul A 2003 Serum levels of insulin-like growth factor-I and its binding proteins in health and disease. *Growth Hormone & IGF Research* **13** 113–170. (doi:10.1016/S1096-6374(03)00038-8)
Kalla Singh S, Moretta D, Almaguel F, De Leon M & De Leon D 2008 Precursor IGF-II and mature IGF-II induce Bcl-2 and Bcl-XL expression through different signaling pathways in breast cancer cells. *Growth Factors* **26** 92–103. (doi:10.1080/08977190802057258)
Kalla Singh S, Tan QW, Brito C, De Leon M, Garberoglio C & De Leon D 2010*a* Differential insulin-like growth factor-II (IGF-II) expression: a potential role for breast cancer survival disparity. *Growth Hormone & IGF Research* **20** 162–170. (doi:10.1016/j.ghir.2009.12.002)
Kalla Singh S, Tan QW, Brito C, De Leon M & De Leon D 2010*b* Insulin-like growth factors I and II receptors in the breast cancer survival disparity among African-American women. *Growth Hormone & IGF Research* **20** 245–254. (doi:10.1016/j.ghir.2010.03.001)
Kaneda A, Wang CJ, Cheong R, Timp W, Onyango P, Wen B, Iacobuzio-Donahue CA, Ohlsson R, Andraos R, Pearson MA *et al.* 2007 Enhanced sensitivity to IGF-II signalling links loss of imprinting of IGF2 to increase cell proliferation and tumour risk. *PNAS* **104** 20926–20931. (doi:10.1073/pnas.0710359105)
Kelley KM, Oh Y, Gargosky SE, Gucev Z, Matsumoto T, Hwa V, Ng L, Simpson DM & Rosenfeld RG 1996 Insulin-like growth factor binding proteins (IGFBPs) and their regulatory dynamics. *International Journal of Biochemistry & Cell Biology* **28** 619–637. (doi:10.1016/1357-2725(96)00005-2)
Kim KW, Bae SK, Lee OH, Bae MH, Lee MJ & Park BC 1998 Insulin-like growth factor-II induced by hypoxia may contribute to angiogenesis of human hepatocellular carcinoma. *Cancer Research* **58** 348–351.
Kim YJ, Yoon JH, Kim CY, Park BL, Shin HD & Lee HS 2006 IGF2 polymorphisms are associated with hepatitis B virus clearance and

hepatocellular carcinoma. *Biochemical and Biophysical Research Communications* **346** 38–44. (doi:10.1016/j.bbrc.2006.05.080)

Kim SY, Toretsky JA, Scher D & Helman LJ 2009 The role of IGF-1R in pediatric malignancies. *Oncology* **14** 83–91. (doi:10.1634/theoncologist.2008-0189)

Kleinman D, Roberts CT, LeRoith D, Schally AV, Levy J & Sharoni Y 1993 Regulation of endometrial cancer cell growth by insulin-like growth factors and luteinizing hormone releasing hormone antagonist SB-75. *Regulatory Peptides* **48** 91–98. (doi:10.1016/0167-0115(93)90338-9)

Kornfeld S 1992 Structure and function of the mannose-6-phosphate/insulin-like growth factor-II receptors. *Annual Review of Biochemistry* **61** 307–330. (doi:10.1146/annurev.bi.61.070192.001515)

Lahm H, Amstad P, Wyniger J, Yilmaz A, Fischer JR, Schreyer M & Givel JC 1994 Blockade of the insulin-like growth factor-I receptor inhibits growth of human colorectal cancer cells: evidence of a functional IGF-II mediated autocrine loop. *International Journal of Cancer* **58** 452–459. (doi:10.1002/ijc.2910580325)

Larsson O, Girnita A & Girnita L 2005 Role of insulin-like growth factor I receptor signalling in cancer. *British Journal of Cancer* **92** 2097–2101. (doi:10.1038/sj.bjc.6602627)

Lee SK, Kang JS, Jung da J, Hur DY, Kim JE, Hahm E, Bae S, Kim HW, Cho BJ, Cho D *et al.* 2008 Vitamin C suppresses proliferation of the human melanoma cell SK-MEL-2 through the inhibition of cyclooxygenase-2 (COX-2) expression and the modulation of insulin-like growth factor-II (IGF-II) production. *Journal of Cellular Physiology* **216** 180–188. (doi:10.1002/jcp.21391)

Lee SH, Chung YH, Kim JA, Lee D, Jin YJ, Shim JH, Jang MK, Cho EY, Shin ES, Lee JE *et al.* 2012 Single nucleotide polymorphisms associated with metastatic tumour antigen 1 overexpression in patients with hepatocellular carcinoma. *Liver International* **32** 457–466. (doi:10.1111/j.1478-3231.2011.02648.x)

LeRoith D & Butler AA 1999 Insulin-like growth factors in paediatric health and disease. *Journal of Clinical Endocrinology and Metabolism* **84** 4355–4361. (doi:10.1210/jc.84.12.4355)

LeRoith D, Scheinman EJ & Bitton-Worms K 2011 The role of insulin and insulin-like growth factors in the increased risk of cancer in diabetes. *Rambam Maimonides Medical Journal* **2** e0043. (doi:10.5041/RMMJ.10043)

Libby G, Donnelly LA, Donnan PT, Alessi DR, Morris AD & Evans JM 2009 New users of metformin are at low risk of incident cancer. *Diabetes Care* **32** 1620–1625. (doi:10.2337/dc08-2175)

Liu L, Greenberg S, Russell SM & Nicoll CS 1989 Effect of insulin-like growth factors-I and -II on growth and differentiation of transplanted rat embryos and fetal tissues. *Endocrinology* **124** 3077–3082. (doi:10.1210/endo-124-6-3077)

Lubik AA, Gunter JH, Hendy SC, Locke JA, Adomat HH, Thompson V, Herington A, Gleave ME, Pollak M & Nelson CC 2011 Insulin increases *de novo* steroidogenesis in prostate cancer cells. *Cancer Research* **71** 5754–5764. (doi:10.1158/0008-5472.CAN-10-2470)

Lubik AA, Gunter JH, Hollier BG, Ettinger S, Fazli L, Stylianou N, Hendy SC, Adomat HH, Gleave ME & Pollak M 2013 IGF2 increases *de novo* steroidogenesis in prostate cancer cells. *Endocrine-Related Cancer* **20** 173–186. (doi:10.1530/ERC-12-0250)

Lui JC & Baron J 2013 Evidence that Igf2 down regulation in postnatal tissues and up-regulation in malignancies is driven by transcription factor E2f3. *PNAS* **110** 6181–6186. (doi:10.1073/pnas.1219079110)

Maki RG 2010 Small is beautiful: insulin-like growth factors and their role in growth, development and cancer. *Journal of Clinical Oncology* **28** 4985–4995. (doi:10.1200/JCO.2009.27.5040)

Marks V & Teale JD 1998 Tumours producing hypoglycaemia. *Endocrine-Related Cancer* **5** 111–129. (doi:10.1677/erc.0.0050111)

Martin JL & Baxter RC 2011 Signalling pathways of insulin-like growth factors (IGFs) and IGF binding protein-3. *Growth Factors* **29** 235–244. (doi:10.3109/08977194.2011.614237)

Mitchell ML, Ernesti M, Raben MS & Scaliter H 1968 Control of hypoglycemia with diazoxide and hormones. *Annals of the New York Academy of Sciences* **150** 406–414. (doi:10.1111/j.1749-6632.1968.tb19065.x)

Moorehead RA, Hojilla CV, De Belle I, Wood GA, Fata JE, Adamson ED, Watson KL, Edwards DR & Khokha R 2003*a* Insulin-like growth factor-II regulates PTEN expression in the mammary gland. *Journal of Biological Chemistry* **278** 50422–50427. (doi:10.1074/jbc.M306894200)

Moorehead RA, Sanchez OH, Baldwin RM & Khokha R 2003*b* Transgenic overexpression of IGF-II induces spontaneous lung tumors: a model for human lung adenocarcinoma. *Oncogene* **13** 853–857. (doi:10.1038/sj.onc.1206188)

Morace C, Cucunato M, Bellerone R, De Caro G, Crino S, Fortiguerra A, Spadaro F, Zirilli A, Alibrandi A, Consolo P *et al.* 2010 Insulin-like growth factor-II is a useful marker to detect hepatocellular carcinoma? *European Journal of Internal Medicine* **23** 157–161. (doi:10.1016/j.ejim.2012.04.014)

Morcavallo A, Gaspari M, Pandini G, Palummo A, Cuda G, Larsen MR, 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. (doi:10.1210/me.2010-0484)

Morcavallo A, Genua M, Palummo A, Kletvokova E, Jiracek J, Brzozowski AM, Iozzo RV, Befiore A & Morrione A 2012 Insulin and insulin-like growth factor-II differentially regulate endocytic sorting and stability of insulin receptor isoform A. *Journal of Biological Chemistry* **287** 1456–1468. (doi:10.1074/jbc.M111.252478)

Muller HL, Oh Y, Gargosky SE, Wilson KF, Lehrnbecher T & Rosenfeld G 1994 Insulin-like growth factor binding protein-3 protease activity in the sera of patients with malignant solid tumors or leukaemia. *Pediatric Research* **35** 720–724. (doi:10.1203/00006450-199406000-00019)

Murphy SK, Hung Z, Wen Y, Spillman MA, Whitaker RS, Simel LR, Nichols TD, Marks JR & Berchuck A 2006 Frequent IGF2/H19 domain epigenetic alterations and elevated IGF2 expression in epithelial ovarian cancer. *Molecular Cancer Research* **4** 283–292. (doi:10.1158/1541-7786.MCR-05-0138)

Nakae J, Kido Y & Accili D 2001 Distinct and overlapping functions of insulin and IGF-I receptors. *Endocrine Reviews* **22** 818–835. (doi:10.1210/er.22.6.818)

Natarajan R, Reis-Filho JS, Little SE, Messahel B, Brundler MA, Dome JS, Grundy PE, Vujanic GM, Pritchard-Jones K & Jones C 2006 Blasternal expression of type-1 insulin-like growth factor receptor in Wilms' tumors is driven by increased copy number and correlates with relapse. *Cancer Research* **66** 11148–11155. (doi:10.1158/0008-5472.CAN-06-1931)

Neuhouser ML, Platz EA, Till C, Tangen CM, Goodman PJ, Kristal A, Parnes HL, Tao Y, Figg WD, Lucia MS *et al.* 2013 Insulin-like growth factors and insulin-like growth factor binding proteins and prostate cancer risk: results from the prostate cancer prevention trial. *Cancer Prevention Research* **6** 91–99. (doi:10.1158/1940-6207.CAPR-12-0250)

Nickerson T, Huynh H & Pollak M 1997 Insulin-like growth factor binding-protein-3 induces apoptosis in MCF7 breast cancer cells. *Biochemical and Biophysical Research Communications* **237** 690–693. (doi:10.1006/bbrc.1997.7089)

Nikolic JA, Todorovic V, Bozic M, Tosic L, Bulajic M, Alempijevic T, Nedic O & Masnikosa R 2000 Serum insulin-like growth factor (IGF)-II is more closely associated with liver dysfunction than is IGF-I in patients with cirrhosis. *Clinica Chimica Acta* **294** 169–177. (doi:10.1016/S0009-8981(99)00254-5)

O'Dell SD & Day INM 1998 Molecules in focus: insulin-like growth factor II (IGF-II). *International Journal of Biochemistry & Cell Biology* **30** 767–771. (doi:10.1016/S1357-2725(98)00048-X)

Oh Y, Muller HL, Ng L & Rosenfeld RG 1995 Transforming growth factor β-induced cell growth inhibition in human breast cancer cells is mediated through insulin-like growth factor binding protein-3 action. *Journal of Biological Chemistry* **270** 13589–13592. (doi:10.1074/jbc.270.23.13589)

Oh YS, Kim EJ, Schaffer BS, Kang YH, Binderup L, MacDonal RG & Park JH 2001 Synthetic low-calcemic vitamin D (3) analogues inhibit secretion of insulin-like growth factor II and stimulate production of insulin-like growth factor binding protein-6 in conjunction with growth

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-13-0231

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suppression of HT-29 colon cancer cells. *Molecular and Cellular Endocrinology* **183** 141–149. (doi:10.1016/S0303-7207(01)00598-6)

Pan Y, He B, Li T, Zhu C, Zhang L, Wang B, Xu Y, Qu L, Hoffman AR, Wang S *et al.* 2010 Targeted tumor gene therapy based on loss of IGF2 imprinting. *Cancer Biology & Therapy* **10** 290–298. (doi:10.4161/cbt.10.3.12442)

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. (doi:10.1074/jbc.M202766200)

Pandini G, Conte E, Medico E, Sciacca L, Vigneri R & Belfiore A 2004 IGF-II binding to insulin receptor isoform A induces a partially different gene expression profile from insulin binding. *Annals of the New York Academy of Sciences* **1028** 450–456. (doi:10.1196/annals.1322.053)

Pavelic K, Spaventi S, Gluncic V, Matejcic A, Pavicic D, Karapandza N, Kusic Z, Lukac J, Dohoczky C, Cabrijan T *et al.* 1999 The expression and role of insulin-like growth factor-II in malignant hemangiopericytomas. *Journal of Molecular Medicine* **77** 865–869. (doi:10.1007/s001099900068)

Pavelic K, Kolak T, Kapitanovic S, Radosevic S, Spaventi S, Kruslin B & Pavelic J 2003 Gastric cancer: the role of insulin-like growth factor 2 (IGF-2) and its receptors (IGF-1R and MDP/IGF-2R). *Journal of Pathology* **201** 430–438. (doi:10.1002/path.1465)

Perks CM, Vernon EG, Rosendahl AH, Tonge D & Holly JM 2007 IGF-II and IGFBP-2 differentially regulate PTEN in human breast cancer cells. *Oncogene* **26** 5966–5972. (doi:10.1038/sj.onc.1210397)

Perros P, Simpson J, Innes JA, Teale JD & McKnight JA 1996 Non-islet cell tumour hypoglycaemia: ^{111}In-octreotide imaging and efficacy of octreotide, growth hormone and glucocorticoids. *Clinical Endocrinology* **44** 727–731. (doi:10.1046/j.1365-2265.1996.721542.x)

Phillips LS & Robertson DG 1993 Insulin-like growth factors and non-islet cell tumour hypoglycaemia. *Metabolism* **42** 1093–1101. (doi:10.1016/0026-0495(93)90265-P)

Piecewicz SM, Pandey A, Roy B, Xiang SH, Zetter BR & Sengupta S 2012 Insulin-like growth factors promote vasculogenesis in embryonic stem cells. *PLoS ONE* **7** e32191. (doi:10.1371/journal.pone.0032191)

Pierre-Eugene C, Pagesy P, Nguyen TT, Neuille M, Tschank G, Tennagels N, Hampe C & Issad T 2012 Effect of insulin analogues on insulin/IGF1 hybrid receptors: increased activation by glargine but not by its metabolites M1 and M2. *PLoS ONE* **7** e41922. (doi:10.1371/journal.pone.0041992)

Pils D, Tong D, Hager G, Obermayr E, Aust S, Heinze G, Kohl M, Scuster E, Wolf A, Sehouli J *et al.* 2013 A combined blood based gene expression and plasma protein abundance signature for diagnosis of epithelial ovarian cancer – a study of the OVCAD consortium 2013. *BMC Cancer* **13** 178. (doi:10.1186/1471-2407-13-178)

Pollak M 2008 Insulin and insulin-like growth factor signalling in neoplasia. *Nature Reviews. Cancer* **8** 915–928. (doi:10.1038/nrc2536)

Pravtcheva DD & Wise TL 1998 Metastasizing mammary carcinoma in H19 enhancers–Igf2 transgenic mice. *Journal of Experimental Zoology* **281** 43–57. (doi:10.1002/(SICI)1097-010X(19980501)281:1<43::AID-JEZ7>3.3.CO;2-3)

Probst OC, Puxbaum V, Svoboda B, Leksa V, Stockinger H, Mikula M, Mikulits W & Mach L 2009 The mannose 6 phosphate/insulin-like growth factor II receptor restricts the tumorigenicity and invasiveness of squamous cell carcinoma cells. *International Journal of Cancer* **124** 2559–2567. (doi:10.1002/ijc.24236)

Qiu Q, Yan X, Bell M, Di J, Tsang BK & Gruslin A 2010 Mature IGF-II prevents the formation of 'big' IGF-II/IGFBP-2 complex in the human circulation. *Growth Hormone & IGF Research* **20** 110–117.

Rajaram S, Baylink DJ & Mohan S 1997 Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. *Endocrine Reviews* **18** 801–831. (doi:10.1210/er.18.6.801)

Rancourt RC, Harris HR, Barault L & Michels KB 2013 The prevalence of loss of imprinting of H19 and IGF2 at birth. *FASEB Journal* **27** 3335–3343. (doi:10.1096/fj.12-225284)

Randhawa GS, Cui H, Barletta JA, Strichman-Almashanu LZ, Talpaz M, Kantarjian H, Deisseroth AB, Champlin RC & Feinberg AP 1998 Loss of imprinting in disease progression in chronic myelogenous leukaemia. *Blood* **91** 3144.

Ratajczak MZ 2012 Igf2-H19, an imprinted tandem gene, is an important regulator of embryonic development, a guardian of proliferation of adult pluripotent stem cells, a regulator of longevity and a passkey to cancerogenesis. *Folia Histochemica et Cytobiologica* **50** 171–179. (doi:10.5603/FHC.2012.0026)

Raynaud-Simon A 2003 Levels of plasma insulin-like growth factor-I (IGF-I), IGF-II, IGF binding proteins, type 1 IGF receptor and growth hormone binding protein in community-dwelling elderly subjects with no malnutrition and no inflammation. *Journal of Nutrition, Health & Aging* **7** 267–273.

Reeve AE 1996 Role of genomic imprinting in Wilms tumour and overgrowth disorders. *Medical and Pediatric Oncology* **27** 470–475. (doi:10.1002/(SICI)1096-911X(199611)27:5<470::AID-MPO14>3.0.CO;2-E)

Regel I, Eichenmuller M, Joppien S, Liebl J, Haberle B, Muller-Hocker J, Vollmar A, von Schweinitz D & Kappler R 2012 IGFBP3 impedes aggressive growth of pediatric liver cancer and is epigenetically silenced in vascular invasive and metastatic tumors. *Molecular Cancer* **11** 9. (doi:10.1186/1476-4598-11-9)

Renehan AG, Jones J, Potten CS, Shalet SM & Dwyer ST 2000 Elevated serum insulin-like growth factor (IGF)-II and IGF binding protein-2 in patients with colorectal cancer. *British Journal of Cancer* **83** 1344–1350. (doi:10.1054/bjoc.2000.1462)

Renehan AG, Frystyk J & Flyvbjerg A 2006 Obesity and cancer risk: the role of the insulin–IGF axis. *Trends in Endocrinology and Metabolism* **13** 33–43.

Rikhof B, van der Graaf WT, Suurmeijer AJ, van Doorn J, Meersma GJ, Groenen PJ, Schuring EM, Meijer C & de Jong S 2012 'Big' insulin-like growth factor-II signalling is an autocrine survival pathway in gastrointestinal stromal tumours. *American Journal of Pathology* **181** 303–312. (doi:10.1016/j.ajpath.2012.03.028)

Rinderknecht E & Humbel RE 1976 Amino terminal sequences of two polypeptides from human serum with non-suppressible insulin-like and cell growth-promoting activities: evidence for structural homology with insulin B chain. *PNAS* **73** 4379–4381. (doi:10.1073/pnas.73.12.4379)

Rogler CE, Yang D, Rossetti L, Donohoe J, Alt E, Chang CJ, Rosenfeld R, Neely K & Hintz R 1994 Altered body composition and increased frequency of diverse malignancies in insulin-like growth factor-II transgenic mice. *Journal of Biological Chemistry* **269** 13779–13784.

Rowlands MA, Holly JM, Hamdy F, Phillips J, Goodwin L, Marsden G, Gunnell D, Donovan J, Neal DE & Martin RM 2012 Serum insulin-like growth factors and mortality in localised and advanced clinically detected prostate cancer. *Cancer Causes & Control* **23** 347–354.

Ruiz I, Altaba A, Sanchez P & Dahmane N 2002 Gli and hedgehog in cancer: tumours, embryos and stem cells. *Nature Reviews. Cancer* **2** 361–372. (doi:10.1038/nrc796)

Salmon WD & Daughaday WH 1957 A hormonally controlled serum factor which stimulates sulphate incorporation by cartilage *in vitro*. *Journal of Laboratory and Clinical Medicine* **49** 825–826.

Sciacca L, Minco R, Pandini G, Murabito A, Vigneri R & Belfiore A 2002 In IGF-I receptor-deficient leiomyosarcoma cells autocrine IGF-II induces cell invasion and protection from apoptosis via the insulin receptor isoform A. *Oncogene* **21** 8240–8250. (doi:10.1038/sj.onc.1206058)

Scott CD & Weiss J 2000 Soluble insulin-like growth factor-II/mannose 6 phosphate receptor inhibits DNA synthesis in insulin-like growth factor-II sensitive cells. *Journal of Cellular Physiology* **182** 62–68. (doi:10.1002/(SICI)1097-4652(200001)182:1<62::AID-JCP7>3.0.CO;2-X)

Serrana R, Villar M, Martinez C, Carracosa JM, Gallardo N & Andres A 2005 Differential gene expression of insulin receptor isoforms A and B and insulin receptor substrates 1, 2 and 3 in rat tissues: modulation by aging and differentiation in rat adipose tissue. *Journal of Molecular Endocrinology* **34** 153–161. (doi:10.1677/jme.1.01635)

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Shapiro LR, Duncan PA, Davidian MM & Singer N 1982 The placenta in familial Beckwith–Wiedemann syndrome. *Birth Defects Original Article Series* **18** 203–206.

Soubry A, Schildkraut JM, Murtha A, Wang F, Huang Z, Bernal A, Kurtzberg J, Jirtle RL, Murphy SK & Hoyo C 2013 Paternal obesity is associated with IGF2 hypomethylation in newborns: results from a Newborn Epigenetics Study (NEST) cohort. *BMC Medicine* **11** 29. (doi:10.1186/1741-7015-11-29)

Sparago A, Cerrato F, Vernucci M, Ferrero GB, Silengo MC & Riccio A 2004 Microdeletions in the human H19 DMR result in loss of IGF2 imprinting and Beckwith–Wiedemann syndrome. *Nature Genetics* **36** 958–960. (doi:10.1038/ng1410)

Steigen SE, Schaeffer DF, West RB & Nielsen TO 2009 Expression of insulin-like growth factor 2 in mesenchymal neoplasms. *Modern Pathology* **22** 914–921. (doi:10.1038/modpathol.2009.48)

St-Pierre J, Hivert MF, Perron P, Poirier P, Guay SP, Brisson D & Bouchard L 2012 IGF2 DNA methylation is a modulator of newborn's fetal growth and development. *Epigenetics* **7** 1125–1132. (doi:10.4161/epi.21855)

Sun FL, Dean WL, Kelsey G, Allen ND & Reik W 1997 Transactivation of Igf2 in a mouse model of Beckwith–Wiedemann syndrome. *Nature* **389** 809–815. (doi:10.1038/39797)

Susiarjo M, Sasson I, Mesaros C & Bartolomei MS 2013 Bisphenol A exposure disrupts genomic imprinting in the mouse. *PLoS Genetics* **9** (4) e1003401. (doi:10.1371/journal.pgen.1003401)

Tani Y, Tateno T, Izumiyama H, Doi M, Yoshimoto T & Hirata Y 2008 Defective expression of prohormone convertase 4 and enhanced expression of insulin-like growth factor-II by pleural solitary fibrous tumor causing hypoglycaemia. *Endocrine Journal* **55** 905–911. (doi:10.1507/endocrj.K08E-062)

Teale JD & Marks V 1998 Glucocorticoid therapy suppresses abnormal secretion of big IGF-II by non-islet cell tumours inducing hypoglycaemia. *Clinical Endocrinology* **49** 491–498. (doi:10.1046/j.1365-2265.1998.00564.x)

Thabit H, Healy ML, Royston D, Broe P, Scarramuzzi N, Walsh TN & Sreenan S 2011 A case of spontaneous hypoglycaemia and impaired glucose tolerance in the same patient. *Annals of Clinical Biochemistry* **48** 183–185. (doi:10.1258/acb.2010.010064)

Trivedi N, Mithal A, Sharma AK, Mishra SK, Pandey R, Trivedi B & Daughaday WH 1995 Non-islet cell tumour induced hypoglycaemia with acromegaloid facial and acral swelling. *Clinical Endocrinology* **42** 433–435. (doi:10.1111/j.1365-2265.1995.tb02654.x)

Vella V, Pandini G, Sciacca L, Minco R, Vigneri R, Pezzino V & Belfiore A 2002 A novel autocrine loop involving IGF-II and the insulin receptor isoform-A stimulates growth of thyroid cancer. *Journal of Clinical Endocrinology and Metabolism* **87** 245–254. (doi:10.1210/jc.87.1.245)

Vidal AC, Murphy SK, Murtha AP, Schildkraut JM, Soubry A, Huang Z, Neelon SE, Fuemmeler B, Iversen E, Wang F *et al.* 2013 Associations between antibiotic exposure during pregnancy, birth weight and aberrant methylation at imprinted genes among offspring. *International Journal of Obesity* **37** 907–913. (doi:10.1038/ijo.2013.47)

Vu TH & Hoffman AR 1994 Promoter-specific imprinting of the human insulin-like growth factor-II gene. *Nature* **371** 714–717. (doi:10.1038/371714a0)

Vu TH, Chuyen NV, Li T & Hoffman AR 2003 Loss of imprinting of IGF2 sense and antisense transcripts in Wilms' tumour. *Cancer Research* **63** 1900–1905.

Ward A 1997 Beckwith–Wiedemann syndrome and Wilms' tumour. *Molecular Human Reproduction* **3** 157–168. (doi:10.1093/molehr/3.2.157)

Weber MM, Auernhammer CJ, Lee PD, Engelhardt D & Zachoval R 2002 Insulin-like growth factors and insulin-like growth factor binding proteins in adult patients with severe liver disease before and after orthotopic liver transplantation. *Hormone Research* **57** 105–112. (doi:10.1159/000057960)

Werner H, Karnieli E, Rauscher FJ & LeRoith D 1996 Wild type and mutant p53 differentially regulate transcription of insulin-like growth factor-I receptor gene. *PNAS* **93** 8318–8323. (doi:10.1073/pnas.93.16.8318)

Wolf E, Kramer R, Blum WF, Foll J & Brem G 1994 Consequences of post-natally elevated insulin-like growth factor-II in transgenic mice: endocrine changes and effects on body and organ growth. *Endocrinology* **135** 1877–1886. (doi:10.1210/en.135.5.1877)

Wu HK, Weksberg R, Minden MD & Squire JA 1997 Loss of imprinting of human insulin-like growth factor-II gene, IGF-2 in acute myeloid leukaemia. *Biochemical and Biophysical Research Communications* **231** 466–472. (doi:10.1006/bbrc.1997.6127)

Wu K, Feskanich D, Fuchs CS, Chan AT, Willett WC, Hollis BW, Pollak MN & Giovannucci E 2011 Interactions between plasma levels of 25-hydroxyvitamin D, insulin-like growth factor (IGF)-1 and C-peptide with risk of colorectal cancer. *PLoS ONE* **6** e28520. (doi:10.1371/journal.pone.0028520)

Yao N, Yao D, Wang L, Dong Z, Wu W, Qiu L, Yan X, Yu D, Chen J, Sai W *et al.* 2012 Inhibition of autocrine IGF-II on effect of human HepG2 cell proliferation and angiogenesis factor expression. *Tumour Biology* **33** 1767–1776. (doi:10.1007/s13277-012-0436-x)

Yoon AJ, Zavras AI, Chen MK, Lin CW & Yang SF 2012 Association between Gly1619ARG polymorphism of IGF2R domain 11 (rs629849) and advanced stage of oral cancer. *Medical Oncology* **29** 682–685. (doi:10.1007/s12032-011-9863-6)

Yu H & Rohan T 2000 Role of the insulin-like growth factor family in cancer development and progression. *Journal of the National Cancer Institute* **92** 1472–1489. (doi:10.1093/jnci/92.18.1472)

Yu H, Mistry J, Nicar MJ, Khosravi MJ, Diamandis A, van Doorn J & Juul A 1999 Insulin-like growth factors (IGF-I, free IGF-I and IGF-II) and IGF binding proteins (IGFBP-2, IGFBP-3, IGFBP-6 and ALS) in blood circulation. *Journal of Clinical Laboratory Analysis* **13** 166–172. (doi:10.1002/(SICI)1098-2825(1999)13:4<166::AID-JCLA5>3.0.CO;2-X)

Zapf J 1993 Role of insulin-like growth factor (IGF) II and IGF binding proteins in extrapancreatic tumour hypoglycaemia. *Journal of Internal Medicine* **234** 543–552. (doi:10.1111/j.1365-2796.1993.tb01012.x)

Zapf J, Walter H & Froesch ER 1981 Radioimmunological determination of insulin-like growth factors I and II in normal subjects and in patients with growth disorders and extrapancreatic tumour hypoglycaemia. *Journal of Clinical Investigation* **68** 1321–1330. (doi:10.1172/JCI110379)

Zapf J, Futo E, Peter M & Froesch ER 1992 Can 'big' insulin-like growth factor II in serum of tumor patients account for the development of extrapancreatic tumor hypoglycaemia? *Journal of Clinical Investigation* **90** 2574–2584. (doi:10.1172/JCI116152)

Zhang L, Kashanchi F, Zhan Q, Brady JN, Fornace AJ, Seth P & Helman LJ 1996 Regulation of insulin-like growth factor- II P3 promoter by p53: a potential mechanism for tumorigenesis. *Cancer Research* **56** 1367–1373.

Zhang L, Zhan Q, Zhan S, Kashanachi F, Fornace AJ, Seth P & Helman LJ 1998 p53 regulates human insulin-like growth factor-II gene expression through active P4 promoter in rhabdomyosarcoma cells. *DNA and Cell Biology* **17** 125–131. (doi:10.1089/dna.1998.17.125)

Zhang S, Rattanatray L, McMillen IC, Suter CM & Morrison JL 2011 Periconceptual nutrition and the early programming of a life of obesity or adversity. *Progress in Biophysics and Molecular Biology* **106** 307–314. (doi:10.1016/j.pbiomolbio.2010.12.004)

Zhao R, DeCoteau JF, Geyer CR, Gao M, Cui H & Casson AG 2009 Loss of imprinting of the insulin-like growth factor-II (IGF2) gene in esophageal normal and adenocarcinoma tissues. *Carcinogenesis* **30** 2117–2122. (doi:10.1093/carcin/bgp254)

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