

# 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

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

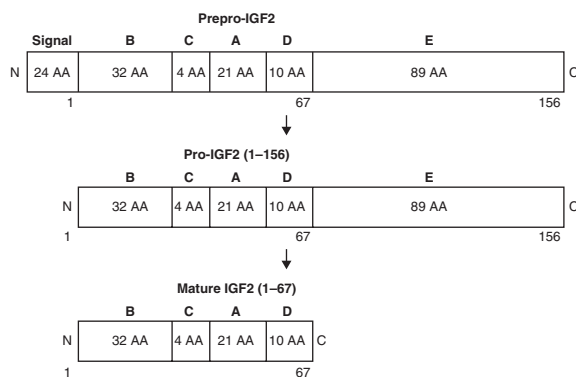
E-domain, known collectively 'big' IGF2. These are secreted into the circulation, normally accounting for 10–20% of total IGF2 (Gowan *et al.* 1987, Daughaday & Trivedi 1992a). The glycosylation on big IGF2 may promote ternary complex formation in serum. Big IGF2 also forms binary complexes with IGFBP2, IGFBP3 and IGFBP5 (Qiu *et al.* 2010, Greenall *et al.* 2013).

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

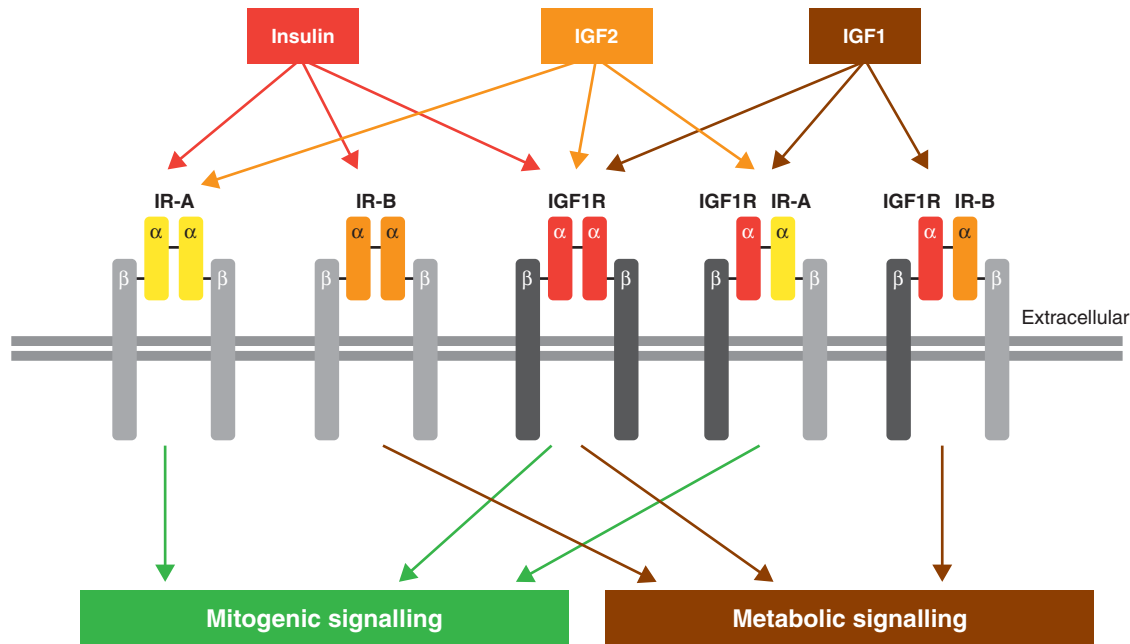
IGF2 signals via three receptor complexes namely the IGF1 receptor (IGF1R), insulin receptor isoform A (IR-A) and the IGF1R–IR-A hybrid receptor (Fig. 2). IGF1R binds both IGFs with comparable and high affinity (Pandini *et al.* 2002). It is thought to mediate most of the biological effects of IGF2. 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  $\alpha$  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  $\beta$ -subunit leading to an intracellular response (Belfiore & Malaguarnera 2011, Braun *et al.* 2011, LeRoith *et al.* 2011). Autophosphorylation of the  $\beta$ -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



**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.

**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  $\alpha$ -subunit results in activation of a receptor tyrosine kinase located in the transmembrane  $\beta$ -subunit. This activates the intracellular signalling pathway leading to a biological response, which can be mitogenic or

(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

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)

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.* 2003a). 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.

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 lysosomal degradation of IGF2, thereby antagonising its action and acting as a tumour suppressor (Brown *et al.* 2009). IGF2R also binds lysosomal enzymes intracellularly, transporting them from the Golgi to lysosomes (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.* 2003b). 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).



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

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 (Jeyaratnaganathan *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.* 2012a). 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

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.* 2010a), 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

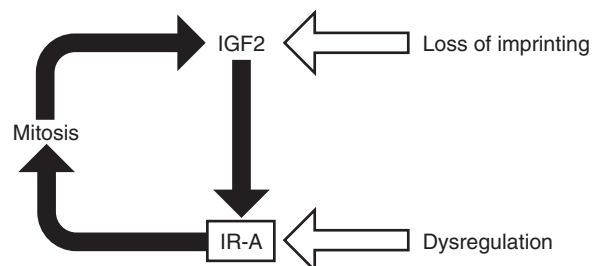
*IGF2* promotes neovascularization of tumours, without which growth would be inhibited by hypoxia (De Leon *et al.* 1992). Its expression appears to be hypoxia driven as part of a progression of angiogenic growth factors. Hypoxia-inducible factors up-regulate *IGF2* which in turn up-regulates vascular 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 (Pieciewicz *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 *IGF1R* 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 (Sciaccia *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.

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 lysosomal 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.* 2010b). 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  $\beta$  (TGF $\beta$ ; Oh *et al.* 1995). This anti-proliferative effect was abolished by IGF2, which blocked TGF $\beta$ -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

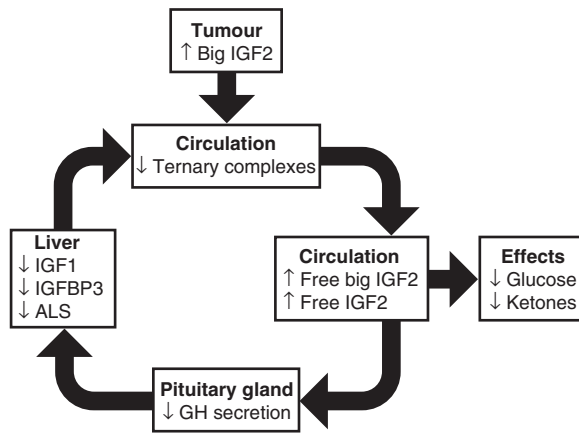
Non-islet cell tumour hypoglycaemia (NICTH) is a rare paraneoplastic syndrome occurring in association with large or metastatic tumours, usually over 0.5 kg in size (Marks & Teale 1998). It has been reported in almost every type of tumour (de Groot *et al.* 2007). This diagnosis should always be considered 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 1992b).

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.



**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 IGF1). 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  $\beta$ -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. Acromegalooid 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 <i>et al.</i> (2007)
IGF1	↓	Marks & Teale (1998)
IGF2	↑/N/↓	de Groot <i>et al.</i> (2007)
IGF2:IGF1	↑	Marks & Teale (1998)
Big IGF2	↑/N	de Groot <i>et al.</i> (2007)
Free IGF1	↑	Frystyk <i>et al.</i> (1998)
Free IGF2	↑	Frystyk <i>et al.</i> (1998)
IGFBP2	↑	Baxter <i>et al.</i> (1995)
IGFBP3	↓	de Groot <i>et al.</i> (2007)
IGFBP6	↑	Hoekman <i>et al.</i> (1999)
ALS	↓	de Groot <i>et al.</i> (2007)
Potassium	↓	Fukuda <i>et al.</i> (2006)

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

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 prognostically 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.

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.* 2012b, 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](http://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, IGF2 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



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.

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