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Review

The Effect of Insulin-like Growth Factor II in the Regulation of Tumour Cell Growth *In Vitro* and Tumourigenesis *In Vivo*

MATILDA HALJE, MATILDA NORDIN, DANIEL BERGMAN and WILHELM ENGSTRÖM

Department of Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden

Abstract. *Insulin-like growth factor II (IGF-II) is a protein hormone that has been shown to exert several biological functions in mammals. IGF-II is produced mainly by the liver and to be systemically released to affect both the liver, in an autocrine and paracrine manner, as well as other tissues, through endocrine signaling. Nevertheless, it is also produced locally in various other tissues acting via autocrine and paracrine signalling. Research over the last decades has suggested that IGF-II also has a stimulatory effect on tumour cell growth but there are some notable exceptions to this rule. This conflicting view may have several explanations and hence current research has focused on gaining further insight into the transcriptional regulation and the metabolic pathways of IGF-II, which is hoped will result in greater understanding of the role of IGF-II in tumour development and new cancer therapies. This article aims at reviewing some of the key findings on how IGF-II affects tumour cells in vitro, as well as in vivo.*

Insulin-like growth factors (IGFs) have been of scientific interest since their ability to stimulate cartilage sulfation and replace the sulfation activity of growth hormone was discovered. They were subsequently divided into two groups and named after their homology with insulin – IGF-I and IGF-II (1, 2).

The *IGF-II* gene has since been shown to be expressed by a large variety of normal as well as neoplastic cells when cultured *in vitro* and IGF-II exerts many different biological activities in cultured cells (3). Its transcription is regulated partly by imprinting – the gene is expressed monoallelically from the paternal allele in almost all tissues (4, 5) – but this can also lead to disorders when the imprinting is disrupted (6). Loss of imprinting (LOI) causes biallellic expression and abnormally high IGF-II levels. This has been observed in overgrowth syndromes, such as Beckwith-Wiedemann syndrome (7), which has suggested an impact of IGF-II on growth and development *in vivo*. Transgenic technology has also been developed rapidly and thus has made it possible to examine knockout effects on growth factor genes *in vivo* which has led to further evidence for the involvement of *IGF-II* in growth regulation. Since its impact on normal development and growth has been confirmed and expression has been observed in neoplastic cells *in vitro*, it became of interest to elucidate whether or not IGF-II has an impact on tumour development and growth.

Current scientific research struggles with the issue of whether *IGF-II* and the regulation of its transcription are of clinical significance in the growth of different tumours or not. Previous results have not been entirely consistent regarding as to whether IGF-II gives rise to tumour cell apoptosis or prevents it. Therefore, the impact of IGF-II on tumour growth *in vivo* remains elusive. If more detailed information about the metabolic activity associated with tumour growth could be provided (*e.g.* IGF-II and its regulation), as well as to why tumours arise and what changes in hormone levels they cause, this could lead to new treatment methods or new ways of determining prognosis.

The aim of this review is to summarize current knowledge about and studies of the impact of IGF-II on tumour growth and development. This will include results from experiments on cells cultured *in vitro* and experiments *in vivo*.

IGF-II, its Receptors and Binding Proteins

The IGF family. IGF-II is a single chain polypeptide and a member of a large family of insulin-related peptides. This family also contains IGF-I, relaxin and insulin with a high

Correspondence to: Professor Wilhelm Engström, Department of Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, PO Box 7028, 75007 Uppsala, Sweden. Tel: +46 18671193, Fax: +46 18673532. e-mail: wilhelm.engstrom@slu.se

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degree of structural similarity. In humans, IGF-II consists of 67 amino acids and contains four domains in the following order from the N-terminus: B, C, A and D. The human gene encoding IGF-II is located on chromosome 11p15. The IGF system consists not only of the peptides, but also of their membrane receptors and circulating binding proteins (3, 8). IGF-II binds to three different receptors: the type-1 IGF receptor (IGF1R), type-2 IGF receptor (IGF2R) and the insulin receptor (IR). Ligand activation of each the receptors results in different biological effects (cf Figure 1).

IGF1R and the IR. IGF1R has a strong homology to IR as they are both transmembrane proteins formed as heterotetramers with tyrosine kinase activity. Furthermore, depending on the studied domain, their structural similarity varies between 41 and 84%. However, their physiological effects differ substantially: while IGF1R primarily mediates mitogenic and developmental messages, IR is mainly involved in metabolic activities. The differences in effect may be explained by their differences in tissue-specific expression. Tissues that are typically referred to as metabolic tissues, *e.g.* fat and liver, have more insulin receptors than IGF-1 receptors, in contrast to fibroblasts that express higher numbers of IGF1Rs than IRs (9).

Nevertheless, studies have shown that IGF-II, but not IGF-I, has a growth-promoting action through the IR. This was confirmed later when IR-A, a differently spliced form of the IR, was found in many foetal and cancer cells, where it bound IGF-II with high affinity, mediating growth-promoting effects. However, IR-A binds insulin with 3 to 10-fold higher affinity. The second of two isoforms of the IR receptor is IR-B, this isoform promotes the metabolic functions of insulin and is predominantly expressed in adult tissues (9). Even though IGF-II binds with high affinity to IR-A, it has a very low capacity to activate autophosphorylation of IR-B (9).

An alternative approach to functional studies of IGF1R is by using blocking and inactivating antibodies. Alpha IR-3 is an antibody that binds to and inactivates IGF1R. This has been used to show that insulin can stimulate DNA synthesis, not only through the IGF1R, but also through the insulin receptor. Similarly, *in vivo* studies in rats have shown that IGF-I is able to stimulate glucose uptake and inhibit breakdown of muscle protein through the IGF1R (9).

IGF2R. IGF-II has an even higher affinity for the IGF2R. IGF2R is homologous to the mannose-6-phosphate (M6P) receptor that transports M6P-tagged acid hydrolases from the Golgi apparatus to the endosomes (10-12). It is up-regulated in foetal tissues where it acts to degrade excess IGF-II by internalisation of the ligand through endocytosis and subsequent transport to the lysosomes. Thus IGF2R is important in the modulation of circulating levels of IGF-II, but it also plays an important role in normal foetal growth and heart development (12).

Studies on knockout mice have shown that mice lacking a functional *IGF-II* gene are born with only 60% of the weight of normal mice and are dwarfed proportionally, although they have a postnatal growth rate comparable to that of normal mice. Other knock-out studies show that mice lacking *IGF2R* generally die before birth and are approximately 30% bigger than their littermates. In contrast, when both genes are non-functional, live offspring are born, which indicates that the IGF2R has a role in regulating the growth-promoting effects of IGF-II. However, the weight of these mutants is reduced and they die soon after birth (12).

The interaction of IGF-II with IGF2R can further promote β-cells to release insulin, in physiologically relevant concentrations, which makes it an interesting subject for regulating levels of insulin. In cancer cells, IR-A is often aberrantly up-regulated which increases the proliferation effects by IGF-II and insulin. This may offer an explanation for the tendency to develop cancer which has been observed in patients with type 2 diabetis and obesity (9).

Soluble IGF2R. There is a soluble form of the IGF2R (sIGF2R) of which the cytoplasmic and transmembrane domains have been deleted through proteolytic cleavage. sIGF2R is present in urine, serum and amniotic fluid of humans and rodents. Experiments *in vivo* suggest that sIGF2R reduces the organ size in transgenic mice (13) whereas *in vitro* studies suggest that sIGF2R decrease IGF-II-induced DNA synthesis and cell division (14).

This soluble form has been a candidate for novel cancer therapies. The size and complexity of the native sIGF2R however makes it difficult for mass production, but experiments have been made to synthesize a novel form of sIGF2R to function as an IGF-II-specific ligand trap (14). Therefore, its use in cancer therapy could, for instance, be to target IGF-II-producing regions of tumours and thereby inhibit tumour recurrence.

Binding proteins. The biological availability of the IGFs are partly regulated by the IGF-binding proteins (IGFBP), which bind IGF peptides and are able to prolong their half-life in the blood. Moreover, they are able not only to potentiate the proliferative actions of the IGFs but also to inhibit their binding to cell-surface receptors. What makes them important is that they obscure the analysis of IGF levels. There are six canonical forms of IGFBP with high affinity to the IGFs, designated IGFBP-1 to -6. In addition, there are four variants with low affinity, that have been designated IGFBP-related proteins 1 to 4 (IGFBP-rP1-4) (15, 16). All high-affinity IGFBPs form binary complexes with the IGFs, but IGFBP-3 is also able to form a ternary complex, together with an acid-labile subunit, and thus prolong the half-life of the IGFs even more. The majority of circulating IGFs are bound to the IGFBP-3 ternary complex, but these complexes are not able to cross the capillary endothelium like the binary complexes (17).

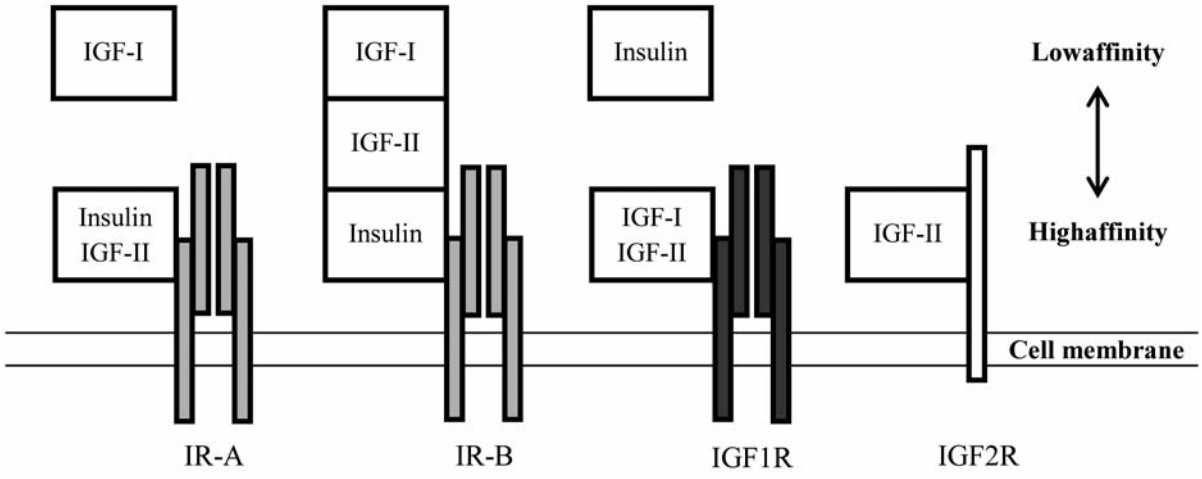

Figure 1. *Simplified scheme of the insulin-IGF-system and the affinity of the ligands to their receptors. IR-A and IR-B are two isoforms of the insulin receptor. IR-A has highest affinity to insulin and IGF-II, however it has low affinity to IGF-I. IR-B has high affinity to insulin, lower to IGF-II and almost none to IGF-I. IGF1R has high affinity to the IGFs but low affinity to insulin. IGF2R has high affinity to IGF-II whereas it does not bind IGF-I or insulin at all. Modified from (7).*

Growth factor synthesis and effects. Four different promoters, P1 to P4, are involved in the transcription of the human *IGF-II* gene (17, 18). These promoters differ between species, and also appear at different levels throughout development. Mouse IGF-II has four promoters, P0 to P3. After weaning, IGF-II transcription ceases in most tissues and in adult mice *IGF-II* gene is only expressed in choroid plexus/leptomeninges.

In humans, the total transcription of IGF-II (mainly from P2, P3 and P4, but also from P1 in the foetal choroid plexus/leptomeninges) peaks shortly after birth and then declines to approximately to one tenth of foetal levels. The amount of *IGF-II* mRNA does not change much from 18 months, onwards, and is then derived primarily from P1, P2 and P4, while there are none, or only low levels of P3, in adults. Thus, postnatally, P1 activity increases and that of P2-P4 decreases; this is particularly the case of P3 (7).

Serum IGF-I and IGF-II are mainly produced by the liver and are released systemically to affect various tissues (endocrine signalling). Furthermore they can be released locally by several different tissues to affect either the same cell they were produced by (autocrine signalling) or adjacent cells (paracrine signalling). They regulate cell proliferation, differentiation and also the survival of the cells, through the different receptors (19).

The Effect of IGF II on Tumour Cells *In Vitro*

It has long been known that a variety of human cell lines, either virally- or chemically-transformed, or established from primary tumours, overexpress the *IGF-II* gene. It is generally accepted that the tumour-promoting effect is mediated *via* membrane receptors. To investigate whether a substance mediates effects through a receptor, there are two commonly used experimental approaches: either by blocking the receptors with specific antibodies, such as αIR-3, or by adding binding proteins which bind to the ligand and thereby making it non-functional. In the early 1980s, three monoclonal antibodies, αIR-1, αIR-2 and αIR-3, were characterized by their ability to bind to either IR or IGF1R (20). It was found that the IR reacts predominantly with αIR-1 while IGF1R reacts with αIR-2 and αIR-3. Thus, these antibodies block the IR and IGF1R respectively and thereby prevent ligand binding. This knowledge has subsequently been used in studies to determine whether a specific ligand acts through these receptors or not. It is performed by using cultured cells with the receptor of interest and a ligand that binds to this receptor. The receptor is later exposed to the antibody and through the results that are obtained, conclusions can be drawn. Whether the antibody inhibits the effect mediated by the ligand or not will indicate if the ligand binds to the receptor. Moreover, monoclonal antibodies can be used to show the existence of a particular component. For example, αIR-3 and 2C2 are commonly used antibodies against IGF1R and IGF2R, respectively, and can thus be used to detect IGF1R and IGF2R by immunohistochemistry.

Embryonic tumours. One of the first tumour cell lines to be thoroughly investigated with respect to the IGF-II signalling system was the human embryonal carcinoma cell line Tera 2 (21). Tera 2 cells express receptors for IGF-II and it was shown that IGF-II is a crucial factor, required for the maintenance of their proliferation (22). A study reported that apoptosis of Tera 2 cells, resulting from serum deprivation, could be counteracted through the addition of a physiological concentration of IGF-II

(23). Tera-2 cells do not produce enough IGF-II themselves to maintain an autocrine loop, but IGF-II synthesis is sufficiently high to allow a portion of the cells to survive, indicating that IGF-II can act as a survival factor in teratocarcinoma.

In the mid-1980s, it was discovered that the levels of IGF-II transcripts in Wilms' tumours and embryonal cell lines are elevated (24, 25). Much later it was shown that apoptosis and necrosis of Wilms' tumour cells (WCCS-1) could be induced by IGF-I and IGF-II (26). This finding counteracts the common theory that IGF-II promotes growth of Wilms' tumour and instead gives it a new role as a tumour suppressor.

Mammary carcinoma. Early experiments on the action of IGF-II in breast cancer cells *in vitro* revealed that IGF-II acts *via* IGF1R to induce cell proliferation in those cell lines (27). The results obtained in that study supported the hypothesis that IGF-II would act in an autocrine/paracrine manner. Further studies on breast cancer cell lines have been carried out to investigate the autocrine signalling of IGF-II *via* the IR. Sciacca *et al*. performed tests on cancer cell lines as well as normal breast cell lines, showing the growth promoting activity of IGF-II through the IR (28). IGF-II was found to have strong potency in the cancer cell lines (63% of the potency of insulin), but the potency in the normal cell lines was only 1% of that of insulin. To evaluate the autocrine signalling of IGF-II in cancer cell lines the researchers first detected the release of IGF-II in the culture medium, and then measured the cell growth in absence and presence of an IGF-II-blocking antibody. Subsequently, the same experiments were performed on tissue samples. In all of these samples, except one, IGF-II was more potent in cancer tissues than in normal breast tissues. In 3 out of 25 cases IGF-II was almost as potent as insulin. IR-A was found to be predominantly expressed in cancer cell lines and cancer tissue specimens, but expressed to a low degree in normal cell lines and normal breast tissue (28). These results imply that IGF-II has a role in the proliferation of breast cancer cells, through the IR-A receptor. Recent data have pointed to the effect of IGF-II on breast cancer cells being mainly to stimulate antiapoptotic mechanisms (29).

Lung cancer. In 1990, the presence of IGFs in lung cancer was investigated and *IGF-II* mRNA was found to be expressed in cell lines from small cell lung cancer (SCLC) as well as non-small cell lung cancer (NSCLC) (30). Three years later, studies of the expression and localization of IGF1R and IGF2R in SCLC, NSCLC and in normal human lung showed that IGF1R was expressed in almost all NSCLC and, through electron microscopy, a stringently membrane-bound localization of IGF1R was seen in SCLC (31). It was shown that all promoters of *IGF-II* were biallelically expressed in lung cancer (32). Elevated levels of *IGF-II* mRNA were detected in 8 out of 10 SCLC and 11 out of 12 NSCLC cell lines (33). This was achieved by isolation of RNA from human lung cancer cell lines, by reverse transcription of mRNA and PCR, followed by detection analysis.

Hepatocellular carcinoma. Hepatocellular carcinoma cell lines often overexpress the *IGF-II* gene. (34). This initial finding was followed by the demonstration of a reversiblility of the transcriptional up-regulation of this gene. Interferon gamma readily reduced the endogenous IGF-II expression. Moreover, RNA interference resulted in a specific reduction in IGF-II in hepatocellular carcinoma cells *in vitro* (35). The effect of IGF-II on tumour cell proliferation, as well as migration was almost exclusively due to the IGF1R whereas the IR played a very minor role in this context (36). Interestingly it has since been shown that the CCAAT/enhancer binding protein alpha gene was specifically up-regulated by IGF-II in hepatoblastoma cells, which gives further insights into the intricacy of this growth factor and its effects (37).

Colorectal carcinoma. Twelve colorectal carcinoma cell lines were used to further investigate the role of IGFs in proliferation of this cancer type (38). IGF1R was expressed in all of the cell lines and IGF-II transcripts were reported to be abundantly expressed. In seven of those cell lines, proliferation was inhibited by αIR-3, but these effects were neutralized when cells were exposed to an excess of IGFs. This study proposed an autocrine-stimulating regulation of the IGF-II *via* IGF1R on colorectal carcinoma *in vitro*. This issue was further investigated and it was recently demonstrated that luteolin, a flavone found in fruits and vegetables, down-regulates the Phosphoinositide 3-kinase(PI3K)/AKT and Extracellular signal regulated kinase (ERK) 1/2 pathways *via* a reduction in IGF1R signalling in colon cancer cells (39).

LOI in the *IGF-II* gene mainly refers to the activation of the usually silenced maternal allele, which results in a biallelic expression of IGF-II. Near the promoter of IGF-II, there is a differently methylated region (DMR), and biallelic expression has been reported to have strong correlation with hypomethylation of the DMR. Moreover, the relationship between overexpression of IGF-II, IGF-II LOI and DMR suggests that two forms of abnormal *IGF-II* gene expression are associated with colorectal cancer (40).

The Effects of IGF-II on Tumour Growth and Development *In Vivo*

In vivo experiments are crucial in medical research since results *in vitro* are, as mentioned above, not always applicable *in vivo*. Two forms of such studies are animal studies and clinical trials. Examples of animal studies are the production of transgenic mice, where different genes are inserted, or construction of knockout mice, with subsequent experiments and analysis.

In vivo studies on IGF-II and its impact on tumor growth have been limited in comparison with studies *in vitro*. This is understandable since *in vitro* studies offer a simplified model of the living organism and make experimenting easier. Transgenic animals were not commonly used in the early days of these studies, but the use of this method has increased in recent years and has made fundamental progress.

Embryonic tumours. When different testicular neoplasms were examined for the expression of four different growth-regulatory genes, including that for *IGF-II,* a rather inconsistent pattern was revealed. It was found that one seminoma, one lymphoma and one teratoma were negative for *IGF-II,* whereas one embryonal carcinoma (teratocarcinoma) and one Leydig cell tumour expressed high levels of *IGF-II* transcripts (41). At that time it was not clear what the main function of IGF-II in humans was, but it was thought to function as a foetal growth factor in rodents (42). It was furthermore discovered that the amount of *IGF-II* mRNA was substantially increased (10 to 100-fold) in primary Wilms' tumours compared to normal kidney, but that the synthesis of IGF-II peptides did not follow that pattern. This appeared to depend on some defect in the translational system, but could have been due to a more rapid degradation or secretion of IGF-II than in normal tissues, or that part of the mRNA was nonfunctional and thus not translated (42). The results obtained from this study did not, however, reveal whether IGF-II had an impact on tumor cell proliferation or if the IGF-II levels resulted from the tumours. In subsequent studies, eight Wilms' tumours were examined and in five of these, IGF-II antisense transcripts were found (43). This finding provided an explanation for the low levels of IGF-II peptides despite high expression of *IGF-II* mRNA. Two isoforms of the *WT1* gene, the gene that encodes Wilms' tumour protein which is an important tumour suppressor, were found to have different effects on the transcription of IGF-II from the P3 promoter (44). One isoform, WT1[–KTS], suppresses the activity of P3, and the other, WT1[+KTS], activates P3. The authors suggested that WT1[–KTS] may act as an oncogene since it up-regulates the expression of IGF-II, and probably also that of other growth factors.

B-Cell tumours. In 1994 transgenic mice expressing the simian virus 40 (SV40) T-antigen (Tag) were first used for organ-specific expression in cancer research (45). Normal pancreatic islets were compared to β-cell tumors with respect to growth factor, receptor and oncogene expression. Through these studies, it was suggested that IGF-II is involved in the proliferation of tumour cells *in vivo*, as the tumour growth and malignancy was reduced and apoptosis was increased in IGF-II-null mice. The major conclusion of this study was however that an additional signal to the oncogene is required for hyperproliferation, and several results point to oncogene expression without a second signal resulting in apoptosis, continued quiescence or less malignant tumours.

Mammary carcinoma. *In vitro* studies indicating an involvement of IGF-II in breast cancer led to the first *in vivo* study in 1995, where enhanced levels of IGF-II in the mammary gland of transgenic mice were investigated and reported to have a causal role in tumour development in this organ (46). Regulatory elements of sheep β-lactoglobulin were attached to the coding regions of the mouse IGF-II gene. Both circulating levels and local expression of *IGF-II* protein were elevated. Since previous *in vivo* studies have reported higher circulating levels which didn't increase the incidence of mammary tumours in the first year of life (47), the endocrine action of IGF-II on mammary cancer was unlikely to be the direct cause of the mammary tumour. Instead, local effects seemed to be of greater importance. When human breast cancer samples were thoroughly analysed, it became clear that IGF-II is important for the progression of the malignancy, and that its actions were mainly ascribable to IGF1R and to a lesser extent to the IR (48).

Hepatocellular carcinoma. The effects of IGF-II overexpression were analysed by using transgenic mice that overexpress human pre-pro-IGF-II cDNA (47). The transgenic mice had up to a 30-fold increase of circulating IGF-II, and were found to develop tumours more frequently, particularly hepatocarcinoma, in comparison with controls. This indicates an autocrine action in the liver but also an endocrine role because tumours also developed in organs that did not express the transgene. Mice homozygous for the transgene displayed the highest tumour frequency. Thus, higher dosage of the transgene led to higher probability of developing tumours. Strong evidence, thus, emphasizes the role of the IGF system and of IGF1R signalling in human tumourigenesis. In this connection: (i) changes in the expression pattern of components of the IGF system (autocrine/paracrine expression of IGF-I and -II), overexpression of IGF1R, reduced expression of IGFBPs and of IGF2R, and (ii) increased serum concentrations of proteases that cleave the IGFBPs (*e.g*. cathepsin D) were observed in patients with hepatocellular carcinomas, as well as in rodent models of hepatocarcinogenesis. Accordingly, studies carried out with animal models do suggest that the IGF system and IGF1R signalling may play a role in hepatocarcinogenesis and in deregulated proliferation and apoptosis of hepatocellular carcinoma cells (49)

Lung cancer. Initial reports suggested that IGFBP-3 was capable of inhibiting tumour growth *in vivo*, probably through its regulatory action on IGF activity (50). Exogenous IGFBP-3 had been shown to steadily inhibit cell proliferation *in vitro* by binding to IGF-I and IGF-II, thus preventing receptor binding. This study provided data proposing IGFBP-3 as an inhibitor of tumourigenicity *in vivo*. Results were obtained by transfecting a NSCLC cell line with human *IGFBP-3* cDNA or with a control vector. Cell proliferation was then found to

be stimulated by IGF-II in cells transfected with the vector, whereas in the IGFBP-3-transfected cells, there was no such stimulatory effect. When inserted into nude mice, the cell lines without IGFBP-3 led to noticeable tumour growth but the IGFBP-transfected cell line did not. Thus, there seems to be an inhibition of tumour growth *in vivo* by IGFBP-3, presumably due to the binding of IGFs.

Pulmonary adenocarcinoma (NSCLC) has been associated with enhanced levels of IGF-II in several *in vitro* studies, although it was not until later that a causal role for IGF-II in neoplastic growth in the lung was reported through *in vivo* studies (51). In that study, transgenic mice overexpressed IGF-II in the epithelium of the lung and in 69% of mice over 18 months old, tumours were induced. Integrin α11 was reported to be an important factor in the proliferation of NSCLC, partly through its regulation of IGF-II expression (47). By use of short-hairpin RNA (shRNA), they down-regulated the expression of *IGF-II* gene in wild-type fibroblasts, resulting in a 70% down-regulation of *IGF-II* mRNA compared to the control wild-type cells and the parental wild-type cells. When these down-regulated cells were implanted in SCID mice together with human lung adenocarcinoma cells, a notable reduction of tumour growth was seen as compared to the tumours without *IGF-II* shRNA.

Colorectal carcinoma. A case control study was implemented in 2011, where serum levels of IGF-I, IGF-II and IGFBP-3 were investigated in relation to various stages of advanced colorectal adenoma (19). Elevated serum levels of IGF-II had a significant association with clinical stage, but only in the highest quartile and when further adjustment for IGF-I and/or IGFBP-3 levels was made, was the association weakened and no longer significant. However, the molar ratio of IGF-II/IGFBP-3 was still associated with risk despite adjustment for IGF-I/IGFBP-3 or IGF-I. These results points to a relatively weak association between IGF-II and advanced colorectal adenoma. It should be noted however, that this study refers to the advanced form of colorectal adenoma. Recently an interesting link between IGF/IGFBP-3 levels and plasma levels of 25-hydroxyvitamin D was established. It was convincingly demonstrated that vitamin D may significantly counteract the tumour-promoting effect of IGFs in this patient group (52).

Discussion

IGF-II is a small but powerful polypeptide that has been shown to exercise an important role in regulating foetal growth. However, it has also been associated with tumour growth. IGF-II exerts its mitogenic effects *via* the IGF1R and the IR. It also binds to IGF2R, but binding to IGF2R does not seem to result in mitogenic actions but rather in endocytosis and degradation of IGF-II.

IGF-II was discovered more than 50 years ago (53) and its primary structure was determined two decades later (1, 2). Since then, several *in vitro*, *in vivo* and epidemiological studies have been carried out and the association between different types of cancer and the presence of IGF-II has received increased attention among scientists.

In vitro studies have been widely used. They have included examination of gene expression in different tumour tissues, the effect of IGF-II in cell cultures, and inhibition of IGF-II or its receptors with *e.g.* monoclonal antibodies, such as αIR-3. The *in vivo* studies have been mainly based on transgenic mice with either hampered or increased IGF-II expression Through these studies and their findings, it seems quite clear that IGF-II has an impact on tumour growth *via* autocrine, paracrine and endocrine mechanisms. However, some of the results are not consistent with others. In Wilms' tumour, growth was long thought to be enhanced by IGF-II due to results *in vitro* because of the high levels of IGF-II transcripts and presumably also because IGF-II in the meantime was shown to enhance growth in other tumors *in vitro* and subsequently also *in vivo*. When IGF-II was reported to inhibit growth in a Wilms' tumour cell line (26), it also gave it a designated role as a tumour suppressor.

The scaffold protein JNK-interacting protein 1 (JIP-1) is a key regulator in the JNK signalling pathway, which is considered to result in mitogenic activity (54). But the JNKs are also capable of inhibiting, as well as stimulating apoptosis. Recent studies have demonstrated co-regulation between the *IGF-II* gene and the *JIP-1* gene which indicates an interaction between these genes. However, Wilms' tumour cell line exhibited low levels of IGF-II expression but JIP-1 expression levels were comparable with those of normal kidney. When exposed to exogenous IGF-II the JIP-1 expression increased (55). Since Wilms tumour WCCS-1 cells undergo apoptosis when exposed to exogenous IGF-II (26), there is reason to believe that JIP-1 could be a crucial factor in this phenomenon.

The phosphatase and tensin homologue *(PTEN)* gene is a tumour suppressor that inhibits mitogen activated protein kinase (MAPK) signalling and cell cycle progression (56). It is induced by IGF-II and in a feedback mechanism, it inhibits IGF-II signalling *in vivo* (55). IGFBP-2 enhances IGF-II signalling but also suppresses PTEN. Thus IGFBP-2 overexpression could lead to tumor growth (56). The knowledge about the regulation of IGF-II signalling by PTEN and IGFBP-2 could be useful when targetting the IGF axis in cancer therapy.

One pillar of clinical management of cancer patients is based on chemotherapy, an approach that is often hampered by complicating side-effects. Detailed information about the metabolic pathways of IGF-II may give rise to new concepts in cancer therapy. There are some possibilities that the current knowledge of how IGF-II influences tumour development, could lead to new diagnostic methods and even new therapeutic approaches – including gene-targeted therapies.

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