REVIEW

The role of insulin-like growth factor system in soft tissue sarcomas: from physiopathology to targeted therapeutic approaches

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Abstract

Purpose/Results. Although surgical, chemo- and radiotherapeutic treatment regimens in patients with soft tissue sarcomas have constantly been refined over the past two decades, the survival rate for these patients is rather low.

Discussion. There is a great need to investigate the mechanisms for oncogenesis and to identify the factors involved in malignant transformation in sarcomas. Among these factors, IGFs are thought to play a pivotal role as progression factors in various types of sarcomas. The dysregulation of the IGF-II synthesis, e.g. by loss of imprinting which occurs in most types of sarcomas, is a permissive effect through the suppression of cell death. In addition, cells that overexpress the type I IGF receptors are more susceptible to transformation by oncogenes. As TP53 suppresses the activity of IGF-II P3 and P4, as well as the type I IGF receptor promoter, mutations of TP53 in sarcomas may alternatively lead to the activation of these factors. Finally, the phenomenon of non-islet cell tumour hypoglycaemia that occurs in patients with sarcomas, and which is related to the secretion of IGF-II prohormones, is discussed. Future therapeutic strategies may be based upon the application of antibodies or antisense oligonucleotides directed against the type I IGF receptors, with the common goal of inducing apoptosis in sarcoma cells. Ultimately, these and other therapeutic approaches may lead to a better outcome in patients suffering from sarcoma.

Key words: insulin-like growth factors, IGF-binding proteins, soft tissue sarcoma, Ewing’s sarcoma, rhabdomyosarcoma, tumour hypoglycaemia.

Introduction

Steady progress has been made in the identification of genetic alterations and prognostic factors in sarcomas. However, survival rates of patients with sarcomas remain unsatisfactorily low in spite of aggressive treatment involving highly toxic multi-drug chemotherapeutic as well as radiotherapeutic regimens. However, considerable progress has been made in the past decade in identifying important factors involved in tumour growth. In addition, the factors involved in the induction and modulation of apoptosis, among them IGFs, are of particular interest. IGF-I and IGF-II are mitogenic polypeptides and are synthesized by most normal and malignant tissues, where they are involved in autocrine and/or paracrine types of action. The IGF system is further complicated by the presence of specific IGF-binding proteins (IGFBP-1 to -7) which bind IGFs with high affinity. These IGFBPs are thought to play an important role in modulating IGF responsiveness in normal as well as malignant cells. The biological effects of IGFs are effected through the insulin, type I IGF (IGF-I) and type II (IGF-II) receptors. The type I IGF receptor in particular is involved in growth, differentiation and inhibition of apoptosis depending on activation by IGFs. Rescue from cell death by IGF-I is mediated through this receptor, and antibodies against this receptor can block the rescuing function of the growth factor. Moreover, IGF-I had no effect in preventing etoposide-induced apoptosis in fibroblasts derived from mice embryos that have a targeted disruption of the type I IGF receptor. The autocrine growth hypothesis states that both normal and malignant cells can synthesize and secrete polypeptide growth factors that will bind to their own cell surface receptor and stimulate cell proliferation. IGFs are abnormally expressed in some paediatric solid tumours, and certain tumours are responsive or dependent upon IGFs for proliferation. In addition, many tumour cell lines express IGFs as autocrine factors and IGFBPs, which, in
turn, regulate the bioavailability and bioactivity of IGFs.\textsuperscript{5-7} The imprinted genes IGF-II and H19 are expressed during embryonal life and are downregulated postnatally. IGF-II is upregulated in paediatric tumours and developmental syndromes predisposing to such tumours (e.g. Beckwith–Wiedemann syndrome). These factors represent tumour markers as they display a tissue-specific oncofetal pattern of expression.\textsuperscript{8} Transgenic mice overexpressing IGF-II have a higher risk of developing tumours, including sarcoma, after a long latent period, which suggests that IGF-II functions primarily as a tumour promoter or progression factor in vivo rather than a potent tumour initiator.\textsuperscript{9,10} Understanding the biology of these growth factors and their receptors can lead to new therapeutic approaches.

**Discussion**

The evaluation of 29 human sarcoma specimens revealed high levels of expression for IGF-I, IGF-II and the type I IGF receptor mRNA, as determined by RT-PCR and in comparison with control cell lines.\textsuperscript{11} Analysis of soft tissue sarcoma cells revealed high steady-state levels of the type I IGF receptor mRNA transcripts and protein which correlated with receptor-specific tyrosine kinase activity.\textsuperscript{12} Hyophysectomy profoundly inhibits the metastatic behavior of injected RIF-I fibrosarcoma cells in mice, whereas GH administration provokes the occurrence of lung metastasis, and it was concluded that somatostatin analogues, GH or IGF antagonists may suppress metastasis of certain tumours.\textsuperscript{13} The presence of IGF-I mRNA in leiomyomas and leiomyosarcomas,\textsuperscript{14} and the IGF-I immunoreactivity in leiomyosarcomas (7 out of 8), synovial sarcomas (2/3), liposarcomas (3/6), fibrosarcomas (1/3) and in one angiosarcoma,\textsuperscript{15} suggest the potential role for IGF-I in stimulating cell proliferation in these tumours. Contrary to the uniform pattern of IGF-I immunoreactivity seen in other sarcomas, only the spindle cell and not the epithelial component of synovial sarcomas exhibited strong IGF-I immunoreactivity. In malignant fibrous histiocytomas, heterogeneous staining was observed for IGF-I, mostly seen as a diffuse cytoplasmic reaction in the majority of the tumour cells. A separate study in two noninflammatory fibrous histiocytomas showed also significant immunohistochemical staining for IGF-I.\textsuperscript{16} Abundant IGF-II mRNA species have also been detected in histiocytoma tissue.\textsuperscript{17}

**Leiomyomas and leiomyosarcomas**

In normal myometrium and leiomyomas, the IGF-II gene is expressed at low levels but it is activated in leiomyosarcomas, whereas the IGF-I gene appears repressed in leiomyosarcomas.\textsuperscript{18} The expression of a fourth leader exon (hE4) which leads to the formation of a 5.0-kb mRNA is enhanced 10-fold in leiomyosarcoma tissue, but it has yet to be established whether there is a causal relation between the activation of hE4 expression and tumour formation.\textsuperscript{19} In normal smooth muscle and in leiomyomas the IGF-II gene appeared to be methylated, whereas, in leiomyosarcomas, methylation of DNA was low and it was suggested that there is an inverse correlation between the methylation state and expression of the IGF-II gene.\textsuperscript{20} Relaxation of IGF-II genomic imprinting was also observed in uterine leiomyosarcoma.\textsuperscript{21} There was also a correlation between an AvaII restriction fragment length polymorphism in the IGF-II gene and the occurrence of smooth muscle tumours.\textsuperscript{22} More than 90% of liposarcomas exhibit greatly elevated IGF-II mRNA levels, while normal adipose tissue contained very low or undetectable IGF-II levels.\textsuperscript{23}

**Ewing sarcoma/primitive neuroectodermal tumour**

Most neuroectodermal tumour cell lines and tumours with a t(11;22) translocation (primitive neuroectodermal tumour (PNET), Ewing’s sarcoma, esthesioneuroblastoma) expressed IGF-I mRNA, whereas none of the cell lines without the translocation (PNET, neuroblastoma) expressed IGF-I mRNA transcripts.\textsuperscript{24,25} Data indicate that IGF-I may play an important role for the growth of ES/PNET tumour cells.\textsuperscript{26} Loss of imprinting (LOI) of IGF-II occurs in some Ewing’s sarcomas but is not associated with increased expression of IGF-II mRNA, suggesting that LOI may be related to genetic or epigenetic abnormalities in tumours independent of IGF-II expression.\textsuperscript{27}

The IGF-I receptor-mediated loop was found to be constantly present in ES/PNET cells and the addition of a specific type I IGF receptor (αIR-3) antibody suppressed the growth of ES/PNET cells by decreasing the proliferative rate and increasing apoptosis. Furthermore, the αIR-3 antibody significantly inhibited the ability of ES/PNET cells to grow in soft agar and migrate following a chemotactic stimulus.\textsuperscript{28} A decrease in the number of type I IGF receptors causes massive apoptosis in several transplantable tumours, whereas if overexpressed the type I IGF receptor protects cells from apoptosis in vivo.\textsuperscript{29} The IGF-I receptor appears to mediate cellular proliferation and increased transforming ability through its C-terminal domain.\textsuperscript{30} Serum-free growth of Ewing’s sarcoma cell lines was achieved by supplementing a basal medium containing IGF-I. IGF-I and -II increased DNA synthesis and glucose transport in Ewing’s sarcoma cells.\textsuperscript{31} Modulation of the IGF system, which appears to constitute an important modulator of cell growth in neuroectoderm-derived or -related tumours, can be used to enhance the drug sensitivity of the tumour cells in vivo and in vitro therapeutic procedures.\textsuperscript{32}
Desmoplastic small round cell tumour

Desmoplastic small round cell tumour (DSRCT) is an abdominal malignancy in children characterized by a recurrent chromosomal translocation, t(11;22) (p13;q12). A genomic DNA fragment containing a Ewing’s sarcoma (EWS) and a Wilms’ tumour (WT1) fusion gene has been isolated from these tumours. The aberrant EWS/WT1 transcription factor activated the type 1 IGF receptor promoter by approximately 340%, whereas expression vectors encoding either EWS or WT1 reduced the activity of the promoter to 46 and 58% of control values, respectively. Since the EWS WT1 chimeric protein obtains the three C-terminal zinc fingers of the DNA binding domain of WT1, it is possible that this fusion protein may modulate transcription of target genes containing WT1 binding motifs, such as the type 1 IGF receptor gene. Thus, activation of the IGF-I receptor promoter by the EWS/WT1 fusion protein may constitute a potential mechanism for the etiology of this particular malignancy. In addition, Ewing’s sarcoma cells expressing antisense EWS fusion transcripts showed loss of anchorage-independent growth and tumorigenicity in nude mice, which emphasizes the importance of targeting the EWS fusion products as a therapy for the Ewing family of tumours. Interestingly, in the case of the EWS/FLI-1 fusion protein, the IGF-I receptor is required for the transformation of cells.

Rhabdoid tumour of the kidney

Malignant rhabdoid tumour of the kidney (MRTK) showed strong and specific IGF-II mRNA expression by tumour cells. Clear cell sarcoma of the kidney expresses IGF-II but not WT1 transcripts, and in situ hybridization patterns for IGF-II are similar to primitive metanephrogenic blastemal cells and early stromagenic cells. Slot blot hybridization revealed that IGF-II mRNA was only slightly increased in a clear cell sarcoma of the kidney, but was not elevated in two malignant rhabdoid tumours of the kidney.

Rhabdomyosarcoma

Recent evidence links abnormal development of the skeletal muscle pathway with rhabdomyosarcoma. The shh/ptc/pax signaling pathway is involved in the induction of myogenic differentiation in somites and in neural tube tissue. A consistant feature of alveolar rhabdomyosarcoma is a translocation involving either a fusion of Pax-3 or, to a lesser extent, Pax-7 with another transcription factor fkhrl. Binding of shh to ptc activates the zinc finger transcription factor gli-1, which is frequently amplified in human sarcomas and brain tumours. Mice heterozygous for ptc inactivation show a high incidence of rhabdomyosarcoma with overexpression of ptc, gli-1 and IGF-II in the tumour, but not in surrounding normal skeletal tissue. This suggests a cross-talk between the ptc and IGF-II signalling pathways in the pathogenesis of rhabdomyosarcoma.

Most rhabdomyosarcomas possess two or more copies of active IGF-II alleles, arising either by relaxation of imprinting or duplication of the active allele, whereas in normal muscle monoallelic expression of the IGF-II gene is conserved. Of the four RMS heterozygotes, 50% had biallelic expression of IGF-II. Furthermore, the imprinting of all IGF-II promoters is relaxed in RMS, indicating that loss of imprinting of IGF-II gene promoters may be regulated in a coordinated manner by a common mechanism in these tumours. In embryonal RMS, a tumour-suppressor locus has been implicated at chromosome band 11p15.5. Furthermore, there is evidence that this tumour suppressor is imprinted in a manner opposite to that of IGF-II. Matsumoto et al. concluded that loss of imprinting of IGF-II itself might not induce tumour occurrence in tissues where the control of tissue-specific expression of IGF-II is maintained, and that increased expression of IGF-II due to maternal loss of a putative controller gene for IGF-II at 11p15 might predispose to sustaining tumorigenic mutations and tumour progression, and that loss of a putative onco-suppressor gene at 11p15 might induce RMS occurrence.

H19 is another possible tumour-suppressor gene for embryonal rhabdomyosarcoma as it is also located at chromosome 11p15.5 and is paternally imprinted. Expression of H19 was observed in four out of six embryonal rhabdomyosarcomas. The expression of the H19 gene is significantly suppressed as compared to normal muscle tissue in 13 out of 15 rhabdomyosarcomas with embryonal histology, and in three out of 11 rhabdomyosarcomas classified as alveolar subtype. It is evident that the genetic and epigenetic alterations affecting chromosome 11p15 in a high number of RMSs cause deregulation of several imprinted genes, including the extinction of H19 and an increase in the number of active IGF-II alleles, thus eventually leading to tumour growth. On the contrary, cellular growth rates are reduced in H19 transfected embryonal rhabdomyosarcoma cell line.
the prepropeptide on membrane-bound polysomes, whereas a major 6.0-kb mRNA was present in a cytoplasmatic particle, suggesting that translational discrimination between the mRNAs is dictated by their different 5'-untranslated regions. High levels of IGF-II mRNA are found in both alveolar and embryonal RMS. IGF-II mRNA expression is limited to tumour cells and is not found in the surrounding stroma, suggesting that an autocrine loop for IGF-II may be functional in vivo in RMS. Poorly differentiated RMS showed the highest level of IGF-II mRNA expression, whereas well-differentiated RMS showed low expression, albeit still significantly higher than in normal differentiated skeletal muscle fibers. Thus, IGF-II has potential as a marker for rhabdomyosarcomas and other soft tissue sarcomas that could be especially useful in differential diagnosis of these tumours. Further-more, IGF-II overexpression in myoblasts resulted in an increased proliferative rate, impairment of the ability to differentiate into myoblasts and acquisition of the capability of anchorage-independent growth. It was concluded that IGF-II overexpression in muscle myoblasts leads to morphological and biological changes typical of the malignant phenotype, and represents a pivotal event in the pathogenesis of RMS.

The capacity of growth factors to induce a motility response in cells has important implications for the invasive and metastatic potential of tumour cells in particular. IGFs, in particular, have the capacity to stimulate cellular motility in tumour cell lines via different receptors and probably different signal transduction pathways. As a consequence, enhanced motility confers upon the cell an increased metastatic potential. Exogenous IGF-II stimulates cellular motility of rhabdomyosarcoma cell lines via an IGF-I receptor-independent pathway. IGF-II elicits a mitogenic response through the type I IGF receptor and a motility response through the type II IGF receptor.

Suramin is a polysulfonated naphthyl-urea with antineoplastic activity which interferes non-selectively with the binding of growth factors to their cellular receptors. In particular, it displaces \( {\text{I}} \) from the type I IGF receptor, indicating that suramin exerts its effect on RMS cell growth by interrupting the IGF-II autocrine loop in these cells. In the alveolar RMZ-RC2 and the embryonal CCA RMS cells, suramin induces a significant increase in the proportion of myosin-positive cells over control cultures. Thus, suramin both inhibits growth and induces myogenic differentiation.

In RD and HTB114 RMS cell lines which express mutant TP53 protein, transfection with wild-type TP53 expression vectors led to a reduction in IGF-II P3 promoter expression in these cells. Furthermore, TP53 binds to the P4 proximal promoter element which results in the inhibition of P4 activity together with a 5-fold reduction of IGF-II in mRNA derived from the P4 promoter. In RMS cells, tumour-derived forms of TP53 stimulated the activity of the type I IGF receptor, suggesting that wild-type TP53 has the potential to suppress the type I IGF receptor in the differentiated cell, thus resulting in low levels of receptor gene expression in adult tissues.

Alveolar rhabdomyosarcoma cell lines are very sensitive to the growth-inhibiting effects of the immunosuppressive agent, rapamycin, which inhibits the type I IGF receptor-mediated signalling. A specific type I IGF receptor-blocking antibody (aIR-3) suppresses RMS growth in vivo. The decrease in tumour growth was associated with a decrease of p34\(^{\text{bb}} \), which is involved in cell cycle regulation suggesting that treatment results in the arrest of cellular proliferation. Transfection of a human alveolar RMS cell line with an amplifiable type I IGF receptor antisense expression vector was associated with markedly reduced growth rates in vitro, impaired colony formation in soft agar and a failure of tumour formation in immunodeficient mice. The Rh30 alveolar RMS cells, which are stably transfected with antisense type I IGF receptor, showed significant reduction in growth rate, an increased expression of MyoD, myosin heavy chain, and an increased number of multinucleated cells in comparison to the parental line. The expression of a type I IGF receptor that carries a mutation in the intracellular \( \beta \)-subunit markedly decreased the response of RMS cells to stimulation with IGF-I and, in addition, resulted in a decrease of RMS growth in vivo suggesting that a prospective gene therapy may use this novel strategy to inhibit RMS growth. In addition, recombinant human \( \alpha_2 \)-interferon induced growth arrest in these cells, associated with down-regulation of the type I IGF receptor. Over-expression of type I IGF receptors and/or IGF ligands may thus confer a proliferative advantage on sarcomas over normal adjacent tissues. Treatment of both embryonal and alveolar human RMS cell lines with all-trans-retinoic acid resulted in a dose-dependent inhibition of cell growth which is not reversed by addition of exogenous IGF-II. Molecular therapies may also target the signal transduction pathway, in particular the SHC-GRB2 protein complexes and MAP kinases which have been characterized in RMS tumours and cell lines.

Increased circulating levels of IGFBP-2 were found in various neoplastic conditions, including Wilm's tumours and it was concluded that IGFBP-2 measurements might be of value as a marker for monitoring tumour patients during therapy. Serum IGFBP-2 levels were increased in patients with solid peripheral tumours, whereas patients in complete remission had normal IGFBP-2 levels. In culture, the A 673 RMS cell line has been shown to secrete a specific IGFBP found also in the spinal fluid. In media conditioned by RMS cell lines A 673 and RD, leiomyosarcoma cell line
SK-LMS, as well as leiomyoblastoma cell line G 402, IGFBP-2 has been found in large amounts. Whereas IGFBP-3 are synthesized at high levels by the leiomyoblastoma cell line G 402, levels were decreased in leiomyosarcoma sections which may confer a growth advantage upon malignant smooth muscle cells.

**Non-islet cell tumour hypoglycaemia**

Sarcomas are occasionally associated with the occurrence of hypoglycaemia. Non-islet cell tumours which induce hypoglycaemia are rare. They are usually intra-abdominal or thoracic. The underlying mechanism for the hypoglycaemia is the production of IGF-II, predominantly as a high-molecular weight form (‘big’ IGF-II), by these tumours. The IGF-II gene is overexpressed in many mesenchymal tumours, and the levels of ‘big’ IGF-II are increased in serum from patients with non-islet cell tumour hypoglycaemia (NICTH). In patients with haemangiopericytoma, hypoglycaemia was associated with increased serum levels of ‘big’ IGF-II. The serum of a patient with a large intra-abdominal haemangiopericytoma contained mainly a large-molecular weight precursor IGF-II (mol. wt. 15–20 kDa), which disappeared from the serum after operation. In another patient with a meningal haemangiopericytoma and a large metastatic liver, hypoglycaemia was associated with low insulin, distinctly decreased IGF-I and normal IGF-II, but high ‘big’ IGF-II levels. High levels of IGF-II mRNA and IGF-II peptide were detected in both primary meningal haemangiopericytoma and metastatic foci in the liver. In a patient with pelvic clear cell sarcoma, severe hypoglycaemia linked to elevated production of ‘big’ IGF-II with acromegaly swelling returned to normal after tumour resection. A patient with a huge fibrosarcoma in the right liver lobe, associated with hypoglycaemia, became euglycaemic after transcatheter arterial embolization. Interestingly, IGF-II intensely stained in the Golgi area of the tumour cells. In a leiomyosarcoma, high concentrations of IGF-II mRNA and elevated IGF-II immunoreactivity were detected with a 77% fraction of high-molecular weight IGF-II and, in a histiocytoma, a 100-fold elevated IGF-II mRNA level was found as compared to normal liver. Thus, low or normal IGF-II levels are found in serum, despite demonstrable overexpression of IGF-II mRNA by the tumour. It was suggested that abnormal IGF-II binding to the 150-kDa IGFBP may play a role in tumour-associated hypoglycaemia. The pro-IGF-II is, to a large extent, bound to low-molecular weight IGFBPs which are able to freely exit the vascular compartment and reach target tissues, where the IGF-II may exert its insulin-like activity. Serum IGFBP-3 was also expressed in forms of about 60 kDa instead of the expected size of about 140 kDa. Furthermore, not only suppression of IGFBP-3 but also a 10-fold increase of IGFBP-2 levels has been described.

**Conclusion**

There is formidable evidence which supports the notion that IGFs play a pivotal role in human cancer. Relaxation of IGF-II genomic imprinting occurs in childhood as well as adult-onset tumours, and may thus represent a novel epigenetic mechanism for oncogenesis throughout life. The overexpression of the type I IGF receptor renders the cells susceptible to transformation by oncogenes. Thus, attempts are currently being made to inhibit cell proliferation by targeting the IGF-I receptor, by means of anti-receptor antibodies, IGF analogues or antisense strategies, with the common goal of inducing apoptosis of neoplastic cells. These therapeutic possibilities do offer an intriguing scenario to be developed further in an effort to provide patients affected with different kinds of soft tissue sarcomas with a better outcome.

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