The insulin-like growth factor (IGF) is a complex system of peptide hormones (insulin-like growth factors of type 1 and 2, IGF-1 and IGF-2), cell surface receptors (insulin receptor, IR; insulin-like growth factor receptors of type 1 and 2, IGF-R1, IGF-R2) and circulating binding proteins (insulinlike growth factor binding proteins, IGF-BP 1-6). IGF-1 and -2 are mitogens that play a role in regulating cell proliferation, differentiation and apoptosis. Their effects are mediated through the IGF-R1 which initiates signaling cascades that result in regulation of a number of biological responses. IGF-R2, together with IGF-BPs is involved in binding, internalization and degradation of IGF-2. IGF proteins regulate cell proliferation in an interconnected action via autocrine, paracrine and endocrine regulatory mechanisms. Consequently, any perturbation in each level of the IGF signaling proteins has been shown to be implicated in development and progression of numerous cancer types. The most important single components in this processes are IGF ligands as well as IGF-R1 - when disturbed they act as oncogenes. It has been shown that: (i) high serum concentrations of IGF-1 and IGF-2 are associated with an increased risk of breast, prostate, colorectal and lung cancers; and (ii) IGF-R1 is commonly disturbed in many tumours (like gastric, lung, endometrial cancer) leading to a phenotype of anchorage-independent tumour growth. In contrast, IGF-R2 is considered to act as a tumour suppressor gene; it protects the cells from neoplastic impulses. Consistent with the IGFs autocrine/paracrine regulation of tumour growth, cancer treatment strategies interfering with IGF-R1 signaling have been developed, that may be useful in future diagnostic and therapeutic strategies.

Key words Cancer treatment strategies - insulin-like - growth factors - oncogenes - tumours
Three critical early findings have been substantial for subsequent research on IGF 1/2 signaling. First, in 1957, Salmon et al found a growth hormone dependent serum factor that stimulated S-sulphate incorporation into rat cartilage. It was designated as «sulphation factor activity» (subsequently also termed somatomedin C). Second, in early 1970s, a polypeptide fraction with mitogenic activity was described in rat liver conditioned medium. It was designated as multiplication stimulating activity, MSA. Third, several laboratories identified serum components with insulin-like activities which were not neutralized by anti-insulin antibodies, so called non suppressible insulin-like activity, NSILA. Based on amino acid sequencing analyses of these factors it was shown that all three activities could be ascribed to two different peptides. Because of their structural and functional homology with insulin all former names are now replaced by the term insulin-like growth factors, more precisely, insulin-like growth factor 1 (IGF-1) and insulin-like growth factor 2 (IGF-2).

Insulin, insulin-like growth factors, as well as later discovered insulin-like growth factor receptors

![Fig. 1. Schematic illustration of insulin, IGF-1 and IGF-2 molecules processing. Insulin is a small protein derived from prepro-insulin (110 AA), by proteolitic cleavage of the signal peptide, formation of three disulphide bonds and cleavage of 35 AA C peptide. Active form of insulin molecule is composed of two polypeptide chains, A (21 AA) and B (30 AA), joined by two disulphide bonds. Both IGFs are highly homologous small single chain peptides derived from prepro-IGF-1 (130 AA) and prepro-IGF-2 (180 AA), respectively. IGF-1 is 70 AA molecule organized into four peptide domains: A (21 AA), B (29 AA), C (12 AA) and D (8 AA). Domains A and B are similar in structure to the insulin compartments (50% sequence homology). The 12 AA C domains are similar in structure to the C-peptide of pro-insulin. The both, IGF-1 and IGF-2 C-terminal E peptides are cleaved before secretion. IGF-2 (67 AA), like IGF-1, is a single polypeptide composed of A and B domains, homologues with insulin and IGF-1, and D (12AA) domains.](image-url)
(IGF R of type 1 and type 2) and insulin-like growth factor binding proteins (IGF-BP 1-6) (IGF family of peptides), play an important role in the normal control of many metabolic and growth related processes. They have been shown to have mitogenic and distinct apoptotic effects regulating thereby the growth of mammalian organism; they can act in endocrine (like a hormone), and autocrine/paracrine manner.

Insulin-like growth factors

Both IGFs have been isolated and characterized in the year 1970⁴. They are highly homologous small single chain peptides of 70 (IGF-1) and 67 (IGF-2) amino acids respectively, and approximate 7.5 kD size. The protein molecules of IGF-1 and IGF-2 can be divided to B- (receptor binding domain), C- (determines preferential binding to IGF-R1), A- (in part, receptor binding domain), D- (determines the affinity of IGF-2, but not IGF-1, to receptor binding) and E-domains (Fig. 1). The proteins are 70 per cent identical to one another, and 50 per cent to proinsulin, mostly due to their B (residues 3-29 in IGF-1 and residues 3-32 in IGF-2) domain. The C domains are analogous in location to the C-domain of proinsulin, but are not spliced out from the mature molecule, as it happens during proinsulin processing. There is no C-domain sequence homology either with each other or with proinsulin. Then follows the A-domain, which is homologous to the A-chain of insulin. Short C-terminal D-domain, although homologous to each other, is not found in insulin. The mature IGF-1 and IGF-2 do not possess the most C-terminal E-domain which is cleaved from the promolecules post-translationally⁵.

The gene for IGF-1 is located on chromosome 12q22-q24.1. The gene for IGF-2 is located close to the insulin one, on a chromosome 11p15.5⁶.

IGF-1 is a trophic factor that circulates at high levels in the blood stream. Although the main source of IGF-1 in the serum is liver, many other tissues synthesize it and are sensitive to its action, especially during postnatal development⁷. Regulation of hepatic IGF-1 production is mostly mediated by growth hormone and insulin (Fig. 2). In turn, IGF-1 feeds back to suppress growth hormone and insulin release. In addition to growth hormone, developmental factors as well as nutrition status all modify IGF-1 production⁷.

![Fig. 2. Interactions between growth hormone (GH), insulin-like growth factor I (IGF-I) and insulin-like growth factor binding proteins (IGF-BPs). Arrows indicate activity while thin black lines indicate inhibition. Growth hormone (GH) binds to GH receptor (GH-R) which leads to IGF-1 production. IGF-1 binds to IGF-R1 and causes enhanced growth and cell proliferation, as well as anti-apoptotic and other effects. Interactions of IGF-BPs with IGF-1 reduce the affinity of IGF-1 for IGF-R1. On the other hand, associations of IGF-BPs with ECM decrease the affinity of IGFBPs for IGFs and therefore increasing the level of free IGFs. In addition, proteases cleave IGF-BPs rendering IGF-BPs in fragments which have low affinity for IGFs. IGF-1, insulin-like growth factor 1; IGF-R1, insulin-like growth factor 1 receptor; IGF-BP-3, insulin-like growth factor binding protein 3; IGF-BP-1, insulin-like growth factor binding protein 1; IGF-BP-2, insulin-like growth factor binding protein 2; GH, growth hormone; GH-R, growth hormone receptor; ECM, extracellular matrix.](image-url)
The synthesis of IGF-2 is relatively growth hormone independent. Its expression is much higher during foetal development than in postnatal life. It acts as a regulatory peptide; it is mitogenic for a number of cell types. The IGF-2 gene is transcribed from four different promoters (P1-P4). P2-P4 contains CpG islands, and transcription from these promoters is subject to imprinting. Monoallelic expression from these promoters occurs mostly in foetal and young tissues. The P1 promoter, utilized primarily in adult liver tissue, is regulated differentially; it escapes imprinting and is expressed biallelically. The IGF-2 gene can be expressed to produce proteins of various molecular weights. The most active form, with regard to binding to IGF receptors, is 7.5 kDa. Larger forms lack post-transcriptional cleavage and have been implicated in hypoglycaemia, which can accompany a variety of tumours.

**Insulin-like growth factor receptors**

The biological effects of IGF-1 and IGF-2 on a target cell are mediated by two types of cell surface receptors: IGF receptor of type 1 (IGF-R1) and IGF receptor of type 2 (IGF-R2), as well as through binding to receptors for insulin (Fig. 3). IGF-1 binds to the type 1 receptor, and with a lower affinity to insulin receptor. IGF-2 binds with high affinity to the type 2 receptor and with low affinity to the type 1 receptor. It has no affinity for the insulin receptor. The type 2 receptor is identical to the cation-independent mannose-6-phosphate receptor. In general, most of the action of IGFs is mediated via IGF-R1. As well as insulin receptor, it is a member of tyrosine-kinase class of growth factor receptors. It is a heterotetramer, consisting of two α- (ligand binding) subunits, and two transmembrane β-subunits which contain a tyrosine kinase domain, that activates receptor by autophosphorylation. Binding of ligand (IGF-1 and GF-2) to the extracellular part of IGF-R1 initiates a cytoplasmic signal cascade that includes receptor conformational change which enables them to bind ATP and become autophosphorylated on tyrosine residues within the β-subunits, resulting in activation of the intrinsic tyrosine kinase activity of the IGF-R1 and subsequent tyrosine phosphorylation of several substrates, including IRS and Shc. This stimulates downstream signaling through intracellular networks that regulate cell proliferation and survival. Key downstream molecules include the phosphatidyl inositol-3 kinase (PI3K)-serine/threonine protein kinase B (PKB or AKT) - target of rapamycin (TOR) system and serine/threonine kinase (RAF)-mitogen activated protein kinase (MAPK) system. Activation of these pathways results in variety of responses, such as cell proliferation, differentiation, migration and protection from apoptosis.

In addition to be stimulated by IGF-2, IGF-R1 is overexpressed in some tumours. Overexpression of these receptors, independent of exogenous peptides transforms cells to a phenotype of anchorage-independent growth.

The type 2 IGF receptor, also known as cation-independent mannose-6-phosphate receptor (IGF-R2/ M-6-P), is structurally and functionally different from the IGF-R1. The receptor is monomeric membrane spanning glycoprotein of 250 kDa, with a large extracellular domain, which binds M-6-P, lysosomal enzymes, and IGF-2. The extracellular part contains one binding site for IGF-2 and two sites for M-6-P containing ligands. This suggests that IGF-R2/M-6-P may be involved in the clearance of IGF-2 from the circulation (on the cell surface the IGF-R2 is constitutively endocytosed, where its main role is the binding and internalization of IGF-2) and in the modulation of trafficking of lysosomal enzymes. IGFR2 does not have intracytoplasmatic signaling domain and is thought to be recycled between the plasma and different cellular compartments. The gene for IGF-R2 is located on chromosome 6q. In opposite to the gene for IGF-2 it is paternally imprinted (Fig. 3).
IGF binding proteins and proteases

The IGF binding proteins represent a family of six conserved proteins (IGF-BP 1-6) with the common property of binding IGF-1 and IGF-2 present in serum and other biological fluids\textsuperscript{12} (Table; Fig. 4). The genes for human IGF-BP are located on different chromosomes: genes for IGF-BPs 1 and 3 on chromosome 7, genes for IGF-BPs 2 and 5 on chromosome 2, the gene for IGF-BP 4 on chromosome 17 and the gene for IGF-BP 6 on chromosome 6\textsuperscript{13}. IGF binding to IGF-BP may be modulated by IGF-BP modifications, such as glycosylation, phosphorylation and proteolysis, and by IGF-BP association with cell surface or components of the extracellular matrix. All IGF-BPs inhibit IGF action by sequestering IGFs, and prolong their half-life

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<th>Table. Some structural and functional properties of insulin-like growth factor binding proteins (IGF-BP 1-6)</th>
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<tr>
<td>Gene location (chromosome)\textsuperscript{a}</td>
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<tr>
<td>Amino acids</td>
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<tr>
<td>Cysteine rich N-region</td>
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<tr>
<td>Cysteine rich C-region</td>
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<td>Glycosilation</td>
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\textsuperscript{a}all genes are structurally similar; possess 4 conserved exons; \textsuperscript{b}exact position not known; \textsuperscript{c}not determined; \textsuperscript{d}ND, exact identity not established; \textsuperscript{e}PAPP-A, pregnancy associated plasma protein (cation dependent serine protease); \textsuperscript{f}MMPs, matrix metalloproteases1 and 2; \textsuperscript{g}PSA, prostate specific antigen; \textsuperscript{h}RGD motif, Arg-Gly-Asp - integrin-binding motif; \textsuperscript{i}ECM, extracellular matrix
in the plasma, whereas some of them, like IGF-BP 1, -3 and -5 also potentiate IGFs action. In addition, IGF-BP 3 and -5 also have IGFs independent action, via signaling through BPs cell surface receptors. (Fig. 4).

Proteins that bind IGF-BPs can be classified into three families. The first includes components of the extracellular matrix where binding of the IGF-BPs regulates IGF bioavailability. The second includes transmembrane proteins that are plasma membrane components and can induce intracellular signals (L5β1 integrin, type V transforming growth factor TGFβ receptor) while the third includes intracellular proteins localized in nucleus or cytosol (retinoid X receptor-α, retinoic acid receptor-α, importin-β, Four-and-a-half LIM protein 2)14.

IGF-BP degrading proteases act as growth stimulators by increasing local IGF availability. They fall into three major categories15. Kallikrein-like serine proteases, which cleave IGF-BP 3, include prostate specific antigen (PSA), gamma nerve growth factor and plasmin. Thrombin, another serine protease, cleaves IGF-BP 5. The second major category is cathepsins, intracellular proteinases, and the third category involves matrix metalloproteinases, a family of peptide hydrolases that function in tissue remodeling by degradation of extracellular matrix components. Proteolytic activity by proteases may play a role in normal and abnormal tissue proliferation by cleaving IGF-BP into fragments with lower affinity for IGFs, thereby increasing the levels of free IGFs to activate IGF-R1.

IGFs and cancer

During the past two decades, the joint efforts of several laboratories have firmly established the important role of IGF family of peptides (ligands, receptors, binding proteins and proteases) as mitogens for variety of tumour types. As their action is strongly interrelated, any deregulation of interactions among them may lead to the pathological condition, mostly cancer formation and progression. In this respect, very often, IGF-1 and IGF-2 overproduced by tumour cells act as autocrine stimulators of malignant cells division, through binding and stimulating the activity of IGF-R1. These have been shown first in vitro16,17, and later on in vivo16-21 as well as in clinical studies22. Based on a cell culture experiments conducted on prostate, breast, lung, colon, stomach, endometrial, liver, brain, and many other cell lines it was presumed that growth hormone-IGF-1 axis plays a role in neoplastic pathology16,17. In vivo, for instance, it was possible to induce tumour growth by the molecules isolated from the tumour stromal cells or tumour host serum18-21. Moreover, tumour cells with nonfunctional IGF receptors could induce their own proliferation by the synthesis of endogenous IGF molecules. This process of autocrine stimulation contributed in part to the autonomous and faster tumour growth22.

More than 20 yr ago we have also described the involvement of IGF in cancer16,21. Increased glucose level in the blood of diabetic but normoinsulinaemic mice was accompanied by suppressed growth of mammary carcinoma23. The same tumours, as well as some other murine tumour cell types maintained in hypoinsulinaemic mice grew faster after each subsequent transplantation into diabetic mice. The observed proliferation enhancement of tumours was caused by de novo synthesis of insulin-like protein, by the tumour cells themselves18-20. Later we showed that IGFs appear in many experimental and human tumours21,24-27. As it was shown for human hemangiopericytomas, increase in tumour size was paralleled by an elevation of IGFs in the serum. Removal of tumours (from the patients) eliminated most of the IGF-2 serum activity. Moreover, when IGF-2 was added to the cell cultures of hemangiopericytomas, cell proliferation was significantly increased. It appeared that IGF-2 produced by tumour cells stimulated tumour cell proliferation by autocrine mechanism21,24.
Maybe the most important proof for the involvement of IGFs to tumour growth and progression comes from the clinical studies. For instance, several studies have shown the link between serum concentration of (i) IGF-1 and IGF-BP 3 with increased risk of breast, prostate, colorectal and lung cancer; (ii) IGF-2 with increased risk of colorectal cancer. Overexpression of IGF-2, as measured at
the level of mRNA and protein, is also found in variety of cancers, including lung, gastric cancer and hemangiopericytomas\textsuperscript{21,25-27}.

However, it seems today that the predominating single factor among IGFs implicated in cancer, is IGF-R1 whose overexpression (therefore augmented signaling) could promote tumour formation and maintenance even at the normal levels of IGF-1 and IGF-2, by an autocrine mechanism. It has also been shown to be crucial for anchorage-independent growth, the property unique to tumour cells. Several studies, both experimental and clinical, have demonstrated that the IGF-R1 is overexpressed in tumour cells\textsuperscript{30} contributing to tumour formation, maintenance and progression. The results of our studies also indicate the importance of IGF-R1, together with IGF1 and IGF-2, in pathogenesis of lung cancer, hemangiopericytomas, and gastric cancer. For instance, 12 of 22 tested human hemangiopericytomas overexpressed IGF-2, and even

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**Fig. 4.** Structure of IGF-BP 1-6. IGF-BPs are approximately 30 kDa proteins that share a common domain organization consisting of cysteine-rich N and C terminal domains connected by a flexible linker region (L). IGF binding sites are located on both the N and C domains. The only identified function of N-domains of IGF-BPs is IGF binding, but C-domains are implicated in wide range of function by interacting with numerous molecules that can modulate IGF-dependent or IGF-independent action. The L domains of IGF-BPs 1-6 are not conserved and are not directly involved in high-affinity IGF binding. The L domains are also sites of post-translation modification (glycosylation and phosphorylation).
90 per cent of them IGF-R1, as recorded at the level of mRNA. Similarly, we have also shown that majority of human lung cancers tested (all together 69) overexpress simultaneously IGF-1, IGF-2, IGF-R1 and IGF-BP 4. Seventeen tumours were concomitantly positive for all four IGFs, whereas 34 were positive for IGF-2, IGF-R1 and IGF-BP 4. At the same time, these tumours were negative for IGF-R2. IGF-2 is also secreted by human gastric cancer cells, of both, diffuse and intestinal type, as we have shown by culturing these tumours in vitro. At the same time the mRNA for IGF-2 and IGF-R1 was also overexpressed in both gastric cancer types. In contrast, the synthesis of IGFR-2 was diminished in these cancers.

Regarding to the role of IGF-R2 in cancer formation, it should be emphasized that this receptor has no intrinsic signaling transduction capability and in the context of the IGF system primarily acts to sequester IGF-2 from the circulation. Mutation or loss of expression, resulting in loss of heterozygosity, of the IGF-R2 gene occurs in several cancers and correlates with a poor prognosis, indicating this gene as a tumour suppressor. We have shown, in human lung cancers, that IGF-R2 gene deregulation is connected to the mutations in this tumour suppressor. The mutations were connected with more aggressive tumour types, high proliferation index, as well as autocrine overproduction of IGF-2.

As the defects in IGFs that are commonly seen in cancers are mostly associated with overexpression of IGF-1/IGF-2 and/or IGF-R1 as well as mutations in the IGF-R2 gene, targeting at the level of these four molecules would be a reasonable choice for the treatment of IGF-dependent cancers.

Targeting growth factors and their receptors

In principle, autocrine circuits of IGFs can be interrupted at two points: outside and inside the cell. The most widely investigated outside approach is the use of blocking antibodies directed against the extracellular part of the receptor. However, laboratory studies are also directed to the use of inhibitors of ligand binding, and the use of competitive binding antagonists. Inside the cell antisense strategies and the use of small interfering RNA (siRNA) are the tools of choice to reduce receptor expression. There is also a great interest in therapeutic strategies that target signaling downstream of IGF-R1.

There are only a few studies that describe targeting of IGF-2 production as cancer therapy strategy. For instance, it was shown that an analogue of gonadotropin-releasing hormone (GnRH), tryptorelin, exerts a biphasic growth effect on ovarian cancer cells (that express GnRH binding sites), by a mechanism of diminished autocrine production of IGF-2. Similarly, the GnRH antagonist, SB-75, inhibited growth of gynaecological cancer cells, again by inhibition of IGF-2 secretion.

Reduction of IGF-1 levels by the use of somatostatin analogues, GHRH or GH antagonists, have shown, so far, only a modest success. However, Csernus et al. suggested that antagonistic analogues of GHRH can inhibit the growth of certain tumours not only by inhibiting the GHRH-GH-IGF-2 axis, but also by reducing IGF-2 production and by interfering with the autocrine regulatory pathway. Antagonists of GHRH directly block the expression of IGF-2 mRNA and, as a consequence, the production of IGF-2. GHRH significantly inhibits the rate of proliferation of mammary, prostatic, and pancreatic cancer cell lines.

Blocking of IGF-R1 seems to be promising approach for the development of an IGF-2 dependent cancer therapy. Receptor function can be abrogated by utilizing monoclonal antibodies against receptors such as αIR, use of polyanionic compound suramine, or exploiting antisense oligodeoxynucleotides directed
against the IGF-R1 mRNA. The αIR monoclonal antibody recognizes human IGF-R1 and neutralizes IGF-1 and IGF-2 mediated signal transduction pathway. Its blocking effects on IGF-1 and IGF-2 mediated responses have been shown in vitro and in vivo. This approach was also tested and supported with results of our previous experiments conducted on malignant hemangiopericytomas.

According to Arteaga, blockade of the IGF-R1 may be even more effective when used in conjunction with other manipulations that also target the IGF system. This approach was supported by the results obtained in our previous experiments. IGF-BP and somatostatin analogues are also potentially rational candidates for combined therapy with monoclonal antibodies or antisense oligonucleotides against IGFR1/IGF-2.

Conclusions

Since IGF proteins are involved in cell proliferation regulation, any disturbance of the IGF signaling proteins has been shown to be connected with development and progression of many cancer types. Considering that high serum concentrations of IGF-1 and IGF-2 are associated with an increased risk of breast, prostate, colorectal and lung cancers and that IGF-R1 is commonly overexpressed in many tumours (like gastric, lung, endometrial cancer) leading to a phenotype of anchorage-independent tumour growth, it is reasonable that interfering with IGF-R1 signaling approach is considered as one of the promising cancer treatment strategies.

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