The Role of the Insulin-like Growth Factors in Cancer

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Insulin-like growth factors 1 and II play critical roles in the growth and differentiation of embryonic tissues [1]. Postnatal IGF-I is important for the peri-pubertal growth spurt in response to pituitary growth hormone release. These growth factors are also essential for many of the differentiated functions of adult tissues including, but not limited to, the reproductive, immune and nervous systems. In the nervous system, IGFs play an important role in preventing apoptosis or programmed cell death that follows ischemic damage or the normal aging process [2].

The IGF-I receptor is ubiquitously expressed and is the major receptor that mediates the signaling of these ligands in the cell [3]. The IGF-11/mannose-6-phosphate receptor has no signaling capabilities but apparently is important in regulating the extracellular concentrations of the IGFs, thereby affecting the biological functions of IGF-I and IGF-II. The interactions of the IGFs with their receptors is regulated by a family of binding proteins (IGFBP1-6) which, in addition to protecting the IGFs in the circulation, may inhibit or enhance the ligand-receptor interactions at the target tissues [4]. For more complete reviews on the above topics we refer the readers to some excellent recent reviews [5,6].

In this brief review we will discuss the role of the IGF system in cancer. We will examine the expression of the various components in cancer cells, their function in promoting cellular proliferation and preventing apoptosis, and possible mechanisms to prevent the IGFs from enhancing tumor growth.

Expression of IGF-I and IGF-II, the IGF-I Receptor and IGFBPs

IGF-I and IGF-II are expressed ubiquitously in adult human tissues. mRNA measurements revealed that liver production of IGF-I was the greatest source and, therefore, the major contributor to circulating or "endocrine" IGF-I [7–9]. In addition, the level of circulating IGF-I is exquisitely sensitive to circulating GH levels, falling after hypophysectomy and rising in cases of GH overproduction. While the liver is probably the major source of circulating IGF-II, IGF-II levels do not fluctuate with changes in GH. IGF-I and IGF-II are also produced by a wide variety of tissues and are thought to act locally in an autocrine/paracrine fashion. Control of gene expression is different for IGF-I and IGF-II. In the case of IGF-I, GH exerts the major influence on liver IGF-I gene expression, with a lesser role in certain tissues and no role in others. In reproductive tissues such as the ovary, testes and uterus, the sex steroids and pituitary gonadotropes play a major role in controlling expression of IGF-I. The control of IGF-II gene expression is less well understood. The gene for IGF-II is imprinted on one allele by a process involving methylation of the gene. Under specific circumstances, as documented in certain cancers, this imprinting appears to be released, and increased gene expression of IGF-II is seen as in certain cancers [10].

The IGF-I receptor is also ubiquitously expressed in adult tissues, with the exception of the liver. Control of its expression has been studied in some detail. Growth factors, such as epidermal growth factor, platelet-derived growth factor and fibroblast growth factor, enhance IGF-I receptor gene expression. This effect may explain how these growth factors enhance cell cycle progression and cancer growth [11]. These growth factors affect expression at the level of the IGF-I receptor gene promoter. Conversely, tumor suppressor gene products such as p53 and Wilm's tumor protein WT1 normally suppress IGF-I receptor gene expression. Interestingly, mutations of the *WT1* gene produce mutant proteins that are unable to interact with the promoter of the IGF-I receptor gene and suppress expression of the gene. This observation supports the hypothesis that Wilm's tumors express a mutant WT1 protein that results in the enhanced expression of the IGF-I receptor, and this supports tumor growth. Similarly, mutations of p53 that are commonly found in patients with colon cancer fail to suppress IGF-I receptor gene expression and, rather, are associated with increased gene expression. It is therefore not surprising that, in addition to the presence of oncogenes, elevated IGF-I receptor expression is a common feature of various cancers.

Most cancer cells also express a combination of IGF-binding proteins, which may enhance or inhibit the actions of the IGFs [1,4]. Whether they inhibit or enhance IGFs' interaction with and activation of the IGF-I receptors is

apparently dependent on whether the IGFBP[1] is present in the extracellular matrix — where it often acts in an inhibitory manner by sequestrating the ligand, or on the cell surface — where it demonstrates a lower affinity for the IGFs by allowing them to interact with the receptor. Proteolysis and phosphorylation of the IGFBPs are other post-translational modifications that affect these functions of the IGFBPs. The IGFBPs that have clearly been shown to enhance IGFs' biological effects include IGFBP-1, 3 and 5, with some experimental results also suggesting that IGFBP-2 and 6 may act similarly. IGFBP-4 is purely inhibitory. It is logical to suspect that the IGFBP production by cancer cells, in whatever combination, augments the growth of these cells.

Signaling via the IGF-I Receptor

The IGF-I receptor is a member of the insulin receptor family of receptor tyrosine kinases. Overall, the members of this receptor family share a heterotetrameric $\alpha_2\beta_2$ structure. The $\alpha\beta$ heterodimers, resulting from the proteolysis of the precursor peptide, are linked by disulfide bonds; secondary disulfide bonds link the $\alpha\beta$ heterodimers to form the mature $\alpha_2\beta_2$ receptor. The ligand-binding domain is found in the entirely extracellular α-subunit. The β-subunit is largely intracellular, containing a short trans-membrane domain, the tyrosine kinase domain, and associated motifs that are responsible for the protein–protein interactions of the IGF-I receptor with downstream signaling proteins.

The binding of IGF-I to the ligand-binding domain results in activation of the tyrosine kinase domains within the β-subunit. Three tyrosine residues within the tyrosine kinase domain (tyr-1121, 1135 and 1136) are the first to be phosphorylated, with the phosphorylation of these residues markedly increasing the activity of the kinase domain towards downstream substrates [12–14]. Several other tyrosine residues in the β-subunit are also phosphorylated and appear to have a role in IGF-I receptor signal transduction, while phosphorylation of serine residues might also be involved [3]. Three tyrosine residues within the juxtamembrane region (tyr-943, 950 and 957) are situated proximal to the tyrosine kinase domain [15]. Tyr-950 is situated within an NPXY motif, previously shown to be important for the internalization of some receptors and for receptor–substrate interactions. Deletion of the NPXY motif in the IGF-I receptor affects receptor internalization, reduces autophosphorylation and inhibits postreceptor signaling. The adapter proteins SHC and insulin receptor substrate-1 both bind to this site. Three tyrosine residues within the C-terminal domain of the IGF-I receptor β-subunit (tyr-1250, 1251 and 1316) are also involved in the mitogenic signaling of the IGF-I receptor to varying degrees [16].

The phosphorylation of residues in the β-subunit creates high affinity binding sites for downstream signaling proteins. Four domains involved in protein–protein interactions are relevant for this review: the Src-homology (SH2 and SH3) domains, the plextrin homology domain, and the phosphotyrosine-binding domain. The SH2 and PTB[2] domains bind to motifs containing phosphorylated tyrosines, thus reversible tyrosine phosphorylation can act as a molecular "switch" to induce the association of multiprotein complexes [17]. The SH3 domain binds to proline-containing motifs [17]. The PH[3] domain differs from the other modules discussed in that it mediates protein–protein and protein–lipid interactions, and might also be important in mediating interactions with G-proteins. Signaling proteins can contain one or more of the aforementioned domains, resulting in the selective formation of multiprotein complexes. Presumably, the composition of the multiprotein complex dictates the specific downstream signaling pathways that are activated.

A relatively well-characterized example of the roles that SH domains play in signal transduction is the complex containing the SH2-SH3 adapter protein GRB2 and the guanine nucleotide exchange factor Son of sevenless (mSos), which catalyzes the transition of the inactive Ras-GDP to active Ras-GTP form. mSos binds to the SH3 domains of GRB2, with the binding of mSos to GRB2 increasing the affinity of the GRB2 SH2 domain for its relevant binding sites [18]. The activation of membrane-localized receptor tyrosine kinases leads to the phosphorylation of tyrosines either on the receptor itself or on adapter proteins linked to the receptor, such as SHC or members of the IRS[4] family, thereby creating high affinity binding sites for the GRB2 SH2 domain. In this model, the translocation of the GRB2–mSos complex to the membrane results in the co-localization of mSos with and activation of membrane-associated Ras. Activated Ras can then activate downstream components of the cascade, such as Raf-1.

At least five adapter proteins have been shown to bind to the cytoplasmic region of the IGF-I receptor. These include members of the IRS family [19], SHC, 14-3-3e, the p85 subunit of PI3-kinase, the tyrosine phosphatase PTP1D (Syp) [20], and mGRB10. As discussed below, the binding of each of these docking proteins to the IGF-I receptor has the potential to activate distinct signaling systems, although there may be some overlap.

Of the docking proteins that potentially bind to the IGF-I receptor, the IRS proteins represent the largest (60–160 kDa) and most diverse, in terms of the number of bound downstream effector molecules. Four members of the IRS family have been cloned to date [21,22]. The highest degree of homology within the IRS family occurs within the N-terminal domains responsible for binding to the cytoplasmic domain of the IGF-I and insulin receptors. The remainder of the protein coding sequence exhibits a high degree of variability and contains motifs responsible for the binding of signaling proteins such as the adapter proteins GRB2 and Nck, the p85 regulatory subunit of PI-3' kinase [23], the tyrosine phosphatase SH-PTP2, the Src-like kinase Fyn, and the Ca^{2+}-ATPases SERCA1 and SERCA2. The variation in the C-terminal region of the IRS proteins might result in functional heterogeneity. Experimental observations support such a conclusion, with the overexpression of IRS-2 in 3T3 cells derived

[1] IGFBP = IGF-binding protein

[2] PTB = phosphotyrosine-binding domain

[3] PH = plextrin homology

[4] IRS = insulin receptor substrate

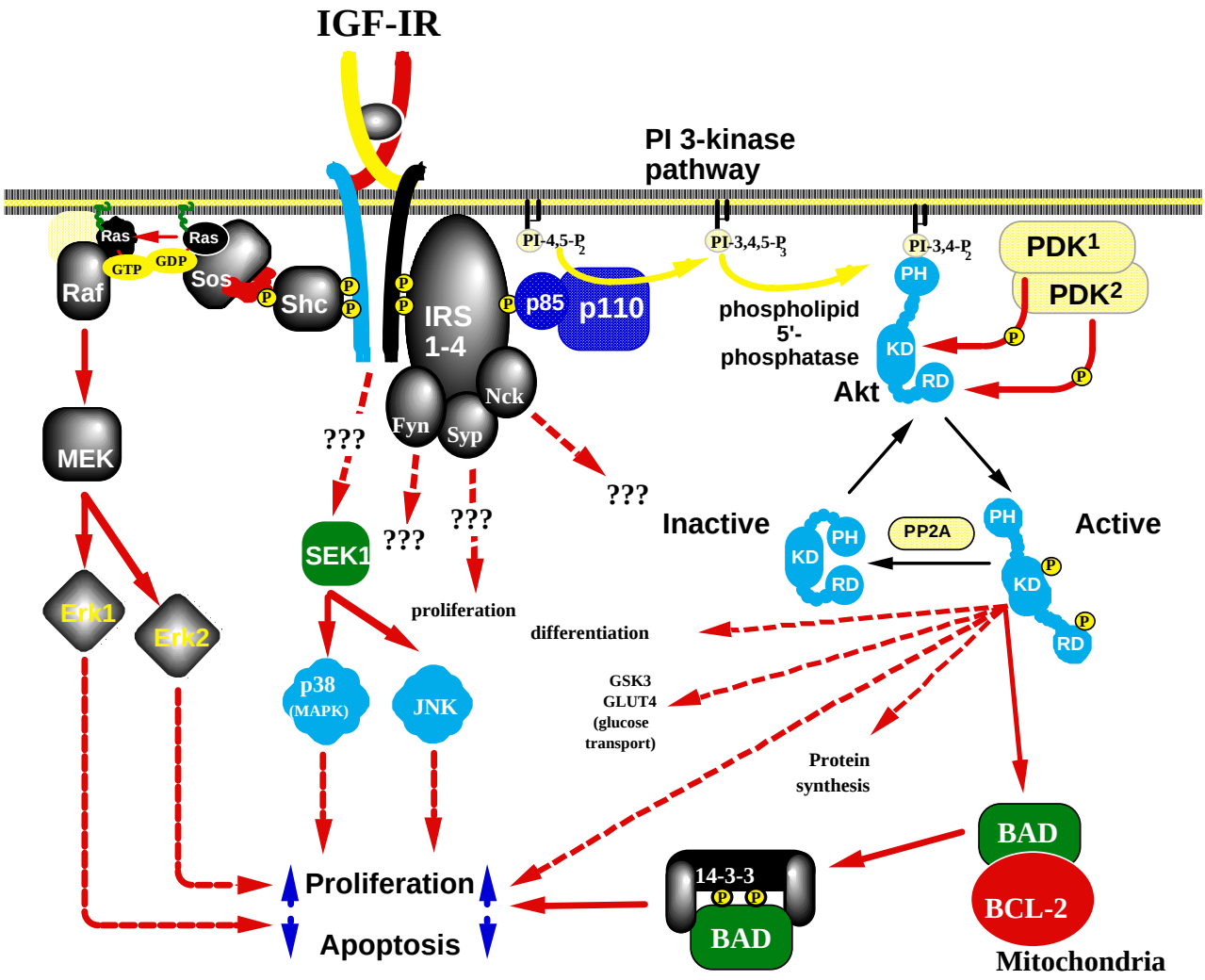

Figure 1. Insulin-like growth factor-I receptor signaling pathways. Once activated, the IGF-I receptor interacts with a number of substrates, which leads to the activation of numerous signaling cascades. The final biological outcomes vary from cell to cell, and include proliferation and an inhibition of apoptosis.

from IRS-1 knockout mice being insufficient to return mitogenic signaling from the IGF-I receptor to normal levels [Figure 1].

The IRS family of proteins is responsible for connecting IGF-I receptor activation with several changes in cellular behavior. A portion of the mitogenic actions of the IGF-I receptor is mediated by IRS-1. In mice nullizygous for the IRS-1 gene, insulin resistance and fetal growth retardation are observed [24]. The important role that IRS-1 has in mediating the mitogenic functions of the IGF-I receptor is also evident from *in vitro* studies in which cell lines derived from IRS1-/- fetuses exhibit a 70–80% reduction in the mitogenic response to IGF-I. Secondly, glucose transport is stimulated in response to the association and subsequent activation of the p85 and p110 subunits of the PI-3' kinases, with insulin-stimulated glucose transport markedly reduced in IRS1-/- cell lines relative to controls [25]. Finally, IRS-1 and IRS-2 have recently been shown to bind with the adult fast-twitch skeletal muscle Ca2+-ATPases SERCA1 and SERCA2, an interaction that might be related to the positive inotropic effects of insulin and IGF-I on cardiac muscle. The binding of the p85 subunit to the IRS proteins and the subsequent activation of the p110 catalystic subunit of the PI-3' kinase link the IGF-I receptor to the phospholipid signal transduction pathways. The IGF-I receptor might also activate PI-3' kinase by binding the p85 subunit directly [19]. It is believed that many cellular processes are regulated by this pathway, including apoptosis, glucose transport and metabolism, protein synthesis, mitosis, and differentiation [26].

An integral component of the phosphoinositide pathway is the serine/threonine kinase Akt, also referred to as PKB (protein kinase B) or RAC-PK (related to A and C protein kinases). The activation of Akt following IGF-I receptor stimulation is a complex process that is poorly understood at present. Two kinases (PDK1, PDK2) have been implicated in the activation of Akt, while it is also possible that the binding of PI(3,4)P2 to the PH domain of Akt is also involved. Akt phosphorylated serine or threonine residues are contained within the consensus sequence RxRyz(S/T)(hy), where x is any amino acid, yz represents small residues other than glycine, and hy is a bulky hydrophobic group [26]. Several substrates of the Akt serine/threonine kinase have been identified. Akt phosphorylate enzymes involved in carbohydrate metabolism include GSK3 and 6-phosphofructo-2-kinase [26]. The α and β forms of GSK3 are both substrates of the Akt kinase, with their phosphorylation resulting in inactivation

and subsequent stimulation of glycogen synthesis. GSK3 also controls several intracellular signaling pathways, including the tumor suppressor APC and the transcription factors CREB and AP1. Akt controls the activity of the ribosomal S6 kinase (p70S6K), which subsequently alters protein synthesis.

The use of pharmacological inhibitors of the PI-3' kinase pathway such as LY294002 and wortmannin, and the use of dominant negative PI-3' kinase mutants have shown that this pathway is an important mediator of the anti-apoptotic effects of IGF-I [27]. The activation of Akt is an important step in the inhibition of apoptosis, since the prevention of apoptosis by IGF-I is diminished in cells expressing dominant negative Akt mutants [28]. Two recent studies have led to better understanding of the role of Akt in inhibiting apoptosis [29]. Current models of the regulation of apoptosis suggest that the balance between members of the Bcl-2 family plays a critical role in determining whether a cell survives or activates the cell death machinery. Several members of the Bcl-2 family (Bcl-2, Bcl-x_L, MCL-1, A1, and BAG-1) appear to promote survival, whereas others (Bcl-x_S, BAD, BAX, and BAK) promote apoptosis. The balance between hetero- and homodimerization of the various Bcl-2 family proteins is believed to be critical in determining cell fate [30]. The stimulation of Akt activity following IGF-I receptor activation culminates in the phosphorylation of BAD on ser-136. Serine phosphorylated BAD can then form a complex with 14-3-3z, thereby sequestering BAD and preventing its pro-apoptotic actions. IGF-I can also promote cell survival by increasing the expression of Bcl-2 family members that inhibit apoptosis [31].

Three isoforms of the SHC family are known to exist (p46, p52 and p66 kDa). IGF-I receptor activation results in the phosphorylation of SHC on tyrosine residues that then acts as a docking site for the SH2 domain of GRB2. The guanine nucleotide exchange protein mSos, bound to the SH3 domains of GRB2, then catalyzes the exchange of GTP for GDP on the small GTP-binding protein Ras, resulting in the activation of Ras and subsequently the activation of the mitogenic Ras-Raf MAPK[5] pathway. Inhibition of the SHC pathway by the microinjection of antibodies inhibits the mitogenic response of Rat1 fibroblasts to IGF-I [32]. The SHC-GRB2 pathway has been shown to be the predominant activator of p21Ras for the IGF-I, insulin and EGF[6] receptors. Recent data suggest that the SHC isoforms may have distinct roles in signal transduction, with the p66 isoform having a negative effect on the activation of MAPK by the EGF receptor.

Our laboratory has focused on the roles of the CrkII and CrkL adapter proteins in IGF-I receptor signal transduction [33]. CrkII is the cellular homologue of the viral oncoprotein, v-Crk, while both CrkII and CrkL share a similar structure with a single N-terminal SH2 and two C-terminal SH3 domains. The SH3 domains of CrkII and CrkL bind to mSos and the related C3G, thus the CrkII- and CrkL-C3G/mSos might function in a manner analogous to the GRB2-mSos complex. IGF-I stimulation of cultured embryonic kidney (293) and NIH-3T3 cells induces the tyrosine phosphorylation of CrkII [33]. In the rat uterus, IGF-I stimulation has markedly different effects on CrkII and CrkL tyrosine phosphorylation and association with paxillin, a protein that localizes to the focal adhesion plaque [34]. The tyrosine phosphorylation of CrkL is increased following IGF-I stimulation, while CrkII is not affected. Whereas CrkL dissociates from paxillin, CrkII association with paxillin is stimulated. These data indicate that CrkII and CrkL might have distinct roles in IGF-I receptor signal transduction. Our laboratory is also pursuing data indicating that IRS-4 binds CrkII and CrkL in a manner regulated by IGF-I. CrkL is apparently the more "oncogenic" of these two adapter proteins. Overexpression of CrkII or CrkL in cells resulted in different phenotypic responses. Cells overexpressing CrkL responded to IGF-I stimulation with a more robust proliferative index, whereas CrkII-overexpressing cells actually blocked the mitogenic response to IGF-I. The CrkL-overexpressing cells formed colonies in soft agar, an indirect measure of a transforming phenotype, and CrkII-overexpressing cells failed to form colonies. We interpret these findings to suggest that CrkL is involved in the mitogenic response of cells to IGF-I, and CrkII is involved in the differentiative response of cells to IGF-I [35,36].

Does the GH/IGF Axis Affect Tumorigenesis?

At the clinical level there is increasing evidence that the GH/IGF axis is related to enhanced tumor growth. Acromegalic patients with elevated circulating GH levels are prone to precancerous colonic polyps. When treated with ablation to the pituitary gland, patients who continue to show elevated circulating GH levels are more prone to develop repeated polyp formation than patients whose GH levels return to the normal range. GH administration has also been shown to cause hyperplastic changes in prostatic and mammary glands, suggestive of a pre-cancerous lesion [37]. These effects of GH are most likely mediated by local production of IGF-I. Slight elevations in serum levels of IGF-I are associated with increased risk for prostatic, breast, colon and lung cancer. Interestingly, in the case of prostatic cancer the circulating IGF-I levels correlated better with risk of cancer than the correlation of cancer risk and prostate-specific antigen levels [38].

In addition to its effects on cellular proliferation in culture, the administration of recombinant human IGF-1 enhances tumor growth in nude mice. In these studies, mice were inoculated with fibroblasts that express different levels of the IGF-I receptor, and followed over the subsequent 3 months to await the expected appearance of fibrosarcomas at 4–6 weeks. The administration of rhIGF-I,[7] in varying doses, resulted in a shortened latency period — i.e., an earlier appearance of tumors — and a more rapid growth of the tumors. These effects were particularly noticeable in tumors resulting from those cells that expressed higher levels of the IGF-I receptor [39]. Thus, systemic administration of rhIGF-I enhanced tumor

[5] MAPK = mitogen-activated protein kinase

[6] EGF = epidermal growth fator

[7] rhIGF-1 = recombinant human IGF-1

growth in the face of enhanced IGF-I receptor expression — levels commonly seen in cancers.

How can we use our Knowledge of the GH/IGF Axis–Cancer Interaction?

GH and IGFs, most likely via circulating IGF-I and local tissue IGF-I production, stimulate cellular proliferation, and IGF directly inhibits apoptosis. Together, these effects could explain the increased growth of cancer cells. It would seem reasonable to propose that inhibition of the GH/IGF axis could prevent cancer growth either by suppressing growth of cells and/or inhibiting the anti-apoptotic effect of the IGFs.

Schally and colleagues [40–42] have recently synthesized and evaluated the effects of antagonists of growth hormone-releasing hormone. Systemic administration of these analogs to mice inhibited the release of GH from the pituitary and lowered circulating IGF-I levels. In the mice that harbored prostatic and renal cancers, osteosarcomas and lung tumor xenografts, the growth hormone-releasing hormone analogs inhibited the growth of these tumors.

Another potential approach to suppressing tumor growth is to inhibit the function of the IGF-I receptor. Studies are being undertaken to decrease the expression of the IGF-I receptor, interfere with the interaction of IGFs with the IGF-I receptor, and decrease the tyrosine kinase activity of the IGF-I receptor. IGF-I analogs, IGF-I receptor-specific antibodies, and soluble IGF-I receptors have been tested *in vitro*. In addition, tyrosine kinase inhibitors such as tyrphostins are currently under investigation.

Conclusion

An increasing body of evidence is pointing to a clear relationship between cancer and the IGF family of ligands, receptors and binding proteins. This information should allow us to develop rational therapies to be used as adjuncts to chemotherapy. For example, although chemotherapy is capable of inhibiting cancer cell growth, these cells may recover from chemotherapy by growth factor-induced anti-apoptotic responses. Blocking the effects of the IGFs on apoptosis in these damaged cells may prove extremely useful in such cases.

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Capsule

A surprising partner for angiostatin

The proteins angiostatin and endostatin have generated much excitement in the past year or two following reports that they can stop or slow cancer growth in mice, apparently by preventing the birth of new blood vessels needed to nourish growing tumors. But as potential therapies they have a built-in drawback: protein drugs can be fragile and hard to produce, as the pharmaceutical company Bristol-Myers Squibb acknowledged last month when it announced that it was giving up work on angiostatin. Efforts to get around those problems by developing small molecules that would mimic the effects of these proteins have been handicapped since little is known about how they act. Now, a research team at Duke University Medical Center seems to have solved part of that puzzle, at least for angiostatin.

A team, led by Salvatore Pizzo, reports that angiostatin binds to a surprising target on the surface of the endothelial cells responsible for blood vessel growth: an enzyme called adenosine triphosphate (ATP) synthase, never before found on the outer membranes of normal cells. The team can't say if this is angiostatin's only target on the cells, but they have evidence that the binding is necessary for angiostatin's antigrowth effects.

The discovery may provide an explanation for the ability of endothelial cells to grow in very low oxygen environments such as tumors. ATP synthase manufactures the energy-rich molecule ATP and thus may be providing endothelial cells with an extra energy source. If angiostatin's binding to the enzyme blocks its activity, that could be the means by which the protein prevents blood vessel growth. What's more, the study suggests that small molecules tailored to block ATP synthase might mimic angiostatin's effects and be useful as anticancer drugs.

Tamny Moser, an associate researcher in Pizzo's lab, uncovered the enzyme in a search for endothelial cell proteins that bind angiostatin, which she undertook on the assumption that such proteins help angiostatin stop cell growth.

From a preparation of endothelial cell membranes, she fished out an angiostatin-binding protein that was actually two proteins, the alfa and beta subunits of ATP synthase.

By probing endothelial cells with antibodies that bind to the alpha subunit of the ATP synthase, the researchers soon confirmed, however, that it does indeed lie on the endothelial cell surface. They also found that the antibodies decreased angiostatin binding to the cells by more than 50%. This in turn led to an 80% decrease in angiostatin's ability to inhibit endothelial cell growth, which suggests that angiostatin works at least in part by binding to the ATP synthase. The Pizzo team recently acquired antibodies to the enzyme's beta subunit and plans to see whether those block any of the remaining effects.

Normally, oxygen-deprived cells have trouble synthesizing enough ATP to survive. But the ATP synthase in the endothelial cells' outer membranes might produce ATP in a process that does not require oxygen. During energy generation in the mitochondria, the enzyme is driven by a gradient of protons across a membrane, produced by the organelle's oxygen-burning metabolism. But in endothelial cells, the gradient could result from the lack of oxygen, which tends to acidify the inside of cells compared to the outside. Endothelial cells could also have a plentiful supply of adenosine diphosphate (ADP) for conversion to ATP, Pizzo notes, because red blood cells release large amounts of ADP in low-oxygen conditions.

If angiostatin does achieve its effects by inhibiting the enzyme, as Pizzo suspects, drug developers will likely start searching for small molecules that do the same thing. They might work as angiogenesis inhibitors that could be administered by mouth.

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