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The IGF-I Receptor and Cancer

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Introduction

It has been known for a long time that the growth of tumors depends not only on the rate of cell proliferation, but also on the rate of cell death *(1)*. An increase in the former and a decrease in the latter cumulatively result in an increase in cell number, which is the most relevant characteristic of tumors. From this perspective, the insulin-like growth factor I receptor (IGF-IR) is an important component of tumor growth (and a good potential target for therapeutic interventions), because it both stimulates cell proliferation and protects cells from cell death in general, and apoptosis in particular. Indeed, a functional impairment of the IGF-IR actually achieves the dual goal of inhibiting cell proliferation and of inducing massive apoptosis. In this presentation, the authors will briefly summarize the functions of the IGF-IR related to growth, and then focus on some aspects of its targeting that are unusually interesting.

Functions of the IGF-I Receptor

Mitogenicity

The IGF-IR activated by its ligands plays a substantial role in the control of cell proliferation in mammalian cells both in vivo and in vitro *(2,3)*. The essential role of the IGF system in vivo has been formally demonstrated by the elegant experiments of Efstratiadis and coworkers *(4,5)*. These investigators have shown that a targeted disruption of the IGF-II gene results in progeny, which, at birth, have a body weight that is 60% the body weight of wild-type littermates. When both the IGF-II and the IGF-IR genes are disrupted by homologous recombination, the homozygous mutant embryos (no IGF-II and no IGF-IR genes) at birth have a body weight that is only 30% the weight of wild type littermates. Since IGF-II is the predominant ligand of the IGF-IR in mouse embryos (which express negligible amounts of IGF-I), it can be stated that the activated IGF-IR accounts for 70% of embryonal murine growth. In vitro, it is known that many cell types in culture require IGF-I for optimal growth *(6)*, and these cell types include human diploid fibroblasts, epithelial cells, smooth muscle cells, endothelial cells, T lymphocytes, myeloid cells, chondrocytes, and osteoblasts, as well as the stem cells of the bone marrow *(7)*.

The requirement for a functional IGF-IR for growth in serum-free medium supplemented by purified growth factors, has been formally demonstrated by the use of R^- cells *(8,9)*, generated by a 3T3-like protocol from mouse embryos with a targeted disruption of the IGF-IR genes *(4,5)*. R^- cells do not grow in serum-free medium supplemented by the growth factors that sustain the growth of mouse embryo cells derived from wild type littermates or of other 3T3 cells. R^-cells, however, grow in 10% serum, and they do so at a reduced rate in comparison to wild type cells, in a remarkable agreement with the growth rate of mouse embryos null for the IGF-IR *(4,5)*. These in vivo and in vitro experiments indicate that the IGF-IR is not essential for growth, although it may be required for optimal growth.

Transformation

Overexpression and/or constitutive activation of IGF-IR in a variety of cell types leads to ligand-dependent growth in serum-free medium and to the establishment of a transformed phenotype; i.e., ability to form colonies in soft agar and/or to produce tumors in mice *(7)*. Although a great number of overexpressed gene products can transform cells, including proto-oncogenes, activated cellular oncogenes, signal transducing molecules, even glycolytic enzymes, the IGF-IR differs from other transforming agents in two crucial characteristics: R^- cells (i.e., mouse embryo cells with a targeted disruption of the IGF-IR genes) are refractory to transformation by certain viral and cellular oncogenes. The list of oncogenes that fail to transform R^- cells *(7,10)* include the SV40 large T antigen, an activated ras, or a combination of T antigen and ras, the bovine papilloma virus E5 protein, human papilloma virus, and overexpressed growth factor receptors, such as the EGF receptor, the PDGF β receptor and the IR, all conditions that readily transform cells with a physiologi-

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cal number of IGF-IRs, like 3T3 cells of various derivations. The second crucial finding is that the transformed phenotype can be reversed to a nontransformed phenotype in a variety of tumor cells by decreasing the number of IGF-IRs, or by interfering with its function *(7,10)*.

Antiapoptotic Signaling

The protective effect of the activated IGF-IR on cell survival has been known for some years, especially in the central nervous system *(11)*. IGF-I also inhibits apoptosis caused by IL-3 withdrawal in hemopoietic cells *(12,13)*, by an overexpressed c-myc *(14)*, or by TGF-β *(15)*. A logical corollary is that an overexpressed IGF-IR can also protect cells in vitro from apoptosis induced, for instance, by etoposide *(16)*, tumor necrosis factor *(17)*, IL-3 withdrawal *(18,19)*, p53 *(19)*, high concentrations of serum *(20)*, serum withdrawal *(21)* and several injurious agents in neuroblastoma cells *(22)*. Even more dramatic are the in vivo data. A decrease in IGF-IR levels below normal levels *(23,24)*, or the use of a dominant negative of the IGF-IR *(25)* cause massive apoptosis of tumor cells in vivo. The role of the IGF-IR in apoptosis, and the possibility that it may discriminate between normal and tumor cells, have been discussed in two recent reviews by Baserga *(10,26)*.

Mutational Analysis of the IGF-I Receptor

A number of laboratories have carried out a mutational analysis of the IGF-IR, in order to determine the domains responsible for the different functions. The results of different laboratories on the effect of various mutations on 3 properties of the IGF-IR, mitogenicity, transforming activity, and protection from apoptosis, can be summarized *(7)* as follows: the C-terminus of the receptor is dispensable for mitogenesis, but is necessary for transforming activity *(27)*. The transforming domain can be tentatively located between residues 1245 and 1310. The domain(s) for protection from apoptosis can be separated from the mitogenic and transforming domains *(18)*. One has therefore to conclude that the 3 functions of the IGF-IR that relate to cell proliferation are spatially separated on the receptor, presumably signaling through distinct pathways. The fact that the three pathways originate from different domains of a single growth factor receptor suggests the possibility of interfering with one pathway, and at the same time sparing the others.

The Major Substrates of the IGF-I Receptor Do Not Protect Cells from Apoptosis, as Efficiently as the Receptor Itself

As mentioned above, an overexpressed IGF-IR protects murine hemopoietic cells from apoptosis caused by IL-3 withdrawal *(18,19)*. This protective effect does not extend to its two major substrates, IRS-1 and Shc proteins. An overexpressed IRS-1 actually does partially protect 32D cells from apoptosis caused by IL-3 withdrawal *(19)*, although not as efficiently as a wild type IGF-IR. But overexpressed Shc proteins have no protective effect at all on survival of 32D cells after IL-3 withdrawal (Fig. 1). If anything, cells overexpressing the Shc proteins died faster than the parental 32D cells. Interestingly, a combination of IRS-1 and SV40 T antigen is much more efficient than either, given singly, in protection from apoptosis (unpublished data). This co-operation between T antigen and IRS-1 had been previously shown to induce transformation of R^- cells, that are refractory to transformation by either, again when singly transfected *(28)*. It suggests that, both for transformation and protection from apoptosis, the IGF-IR has a pathway, which is additional to the IRS-1 pathway, and that the SV40 T antigen could provide this missing pathway.

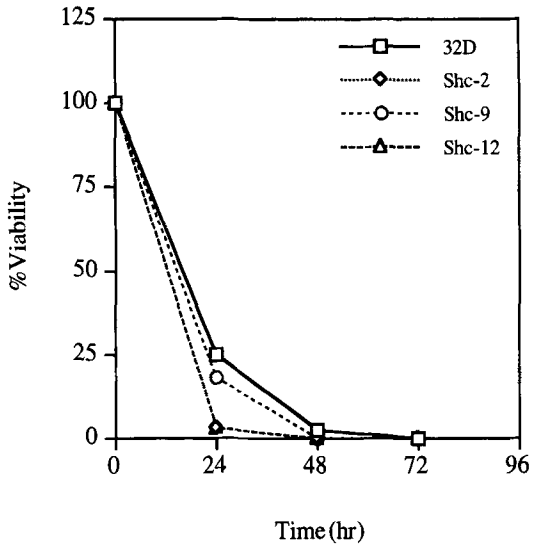

Fig. 1. Shc proteins do not protect 32D cells from apoptosis. Parental 32D cells and three clones of 32D cells overexpressing the Shc proteins (clones 2, 9, and 12) were grown in IL-3-containing medium, then carefully washed and cultured in 10% serum, without IL-3, for the times indicated on the abscissa. Percentage of viable cells was determined as previously described *(19)*.

The IGF-I Receptor as a Target for Anti-Cancer Therapy

The fact that interference with the function of the IGF-IR results in tumor cell death, inhibition of tumorigenesis (*see* above) and prevention of metastases *(10)*, in itself, is not especially remarkable, since many agents and many modalities can do the same. But there is something unique about the IGF-IR *(26)*. Interference with the function of the IGF-IR:

1. Causes massive apoptosis of tumor cells in vivo;
2. Inhibits tumorigenesis;
3. Elicits a host response leading to the eradication of surviving cells; and
4. Has only a moderate effect on normal cells

In this last section, we will focus on the host response, that has all the characteristics of an immune response *(7)*, and is a peculiar one. Rats previously injected with, or carrying in a diffusion chamber for 24 h, tumor cells with a targeted IGF-IR (antisense strategies or dominant negatives) become totally resistant to a subsequent challenge with wild type C6 tumor cells *(23,25)*. Untreated wild type cells do not confer this resistance. This would not be particularly surprising, except that the animals become resistant to challenge with wild type C6 rat glioblastoma cells, even when they are pre-treated with unrelated tumor cells, indeed even tumor cells of different species *(7)*. In these experiments, the immunizing tumor cells were either injected subcutaneously or loaded in a diffusion chamber, which is then inserted into the subcutaneous tissue of a rat for 24 h. The chamber is then removed, and from 1–6 wk later, the rats are challenged with wild-type C6 cells. Provided the immunizing cells had a decrease in the number or a functional impairment of the IGF-IR, they invariably elicited a response that made the rats resistant to subsequent challenge with C6 cells.

As a syngeneic system, rat glioblastomas are notoriously unreliable, but the authors have obtained similar results with a syngeneic mouse melanoma model, in which C57/BL6 mice are injected with mouse melanoma cells B1792-F10. In all these experiments, the authors used antisense and sense oligodeoxynucleotides (ODN) to the IGF-IR, as described in Resnicoff et al. *(23,24)*. Whereas the sense ODN were always ineffective, the antisense ODN had little effect on the growth of B1792-F10 cells in 10% serum, but inhibited their growth in serum-free medium supplemented by IGF-I by about 70%. These cells, pretreated with either sense or antisense ODN, or untreated, were then injected subcutaneously in C57/BL6 mice (10^5 cells). The results are summarized in Table 1. Melanoma cells pretreated with antisense ODN were nontumorigenic, while all the controls had palpable tumors by d 16–18. Furthermore, injection of cells treated with antisense ODN prevented the growth of untreated cells injected subcutaneously 7 d after the first injection. If the antisense-treated cells were injected into mice already bearing a subcutaneous tumor, they inhibited the growth of the tumor, although they did not induce regression, as the authors had observed with C6 rat glioblastoma cells *(23)*. However, by the time the antisense-treated cells were injected, the tumor already had a size of 2 g, which would be the equivalent of a 5.6 kg tumor in humans. Perhaps, the authors had asked too much from the immune system.

Treatment with antisense ODN induced the immune response, even when the cells were placed in a diffusion chamber *(23)*, which was subsequently removed. When the mice treated were thus challenged 1 wk later with untreated melanoma cells, those animals that had received cells treated with 19 μM antisense ODN were fully protected, whereas the controls developed tumors very rapidly. The degree of protection seemed to correlate with the extent of apoptosis induced by the antisense ODN (Table 2).

The authors realize that this observation is somewhat unusual, but they have observed this puzzling phenom-enon now for several years, and there is no question that it is reproducible, whether the immunizing cells express an antisense RNA to the IGF-IR RNA *(23)*, or are treated with antisense OAN against the IGF-IR RNA *(24)*, or are expressing a dominant negative of the IGF-IR *(25)*.

Table 1
Effect of Antisense Strategies Against the IGF-I Receptor on the Growth of Melanoma Cells in Syngeneic Mice

Cells injected First injection (Right flank)	Second injection (Left flank)	Tumor development Number of animals (Palpable tumors in d)
Untreated		6/6 (4-5) dead by d 16
Sense ODN		6/6 (5-6) dead by d 18
Antisense ODN		0/6 (negative at d 62)
Sense ODN	Untreated	3/3 bilateral tumors
Antisense ODN	Untreated	0/3 (negative at d 55)
Untreated	Sense ODN	3/3 (dead by d 15-16)
Untreated	Antisense ODN	3/3 (same tumor weight for 1 mo)

All mice (C57/BL6) were injected subcutaneously with 10^5 B1792-F10 mouse melanoma cells. The cells were either untreated or pretreated (for 24 h before injection) with either sense or antisense oligodeoxynucleotides (ODN) to the IGF-I receptor *(23,24)*. The second injection in the left flank was given 7 d after the first. In the last two rows, the size of the tumors at the time of the second injection was 2–2.5 g.

Table 2
Immune Response Induced by Targeting of the IGF-I Receptor

Condition	Recovery (%)	Protection against challenge with untreated cells
Untreated	218	NO (tumors appeared on d 5)
Random ODN (13)	209	NO (pos. at day 5)
AS ODN (13)	114	Partial protection (pos at d 12)
Random ODN (19)	196	NO (pos at d 5)
AS ODN (19)	0.1	YES (negative for >1 mo).

In each case, the C57/BL6 mice were first treated with cells placed in a diffusion chamber that was inserted into the subcutaneous tissue and removed after 24 h *(23)*. Cells (B1792-F10 mouse melanoma cells) were either untreated or treated with random or antisense (AS) oligodeoxynycleotides (ODN) against the IGF-I receptor RNA (column 1). The numbers in parenthesis are the concentration of ODN in μM. The percentage of cells surviving in the diffusion chamber is shown in column 2. One wk after the removal of the chambers, the mice were challenged with 10^5 untreated cells, and observed for the appearance of tumors.

Conclusions

The IGF-IR is an attractive target for cancer chemotherapy for the reasons mentioned above. The targeting of the IGF-IR in transplantable tumors of rodents does not only cause inhibition of growth, but it actually induces massive apoptosis. Added to the apoptotic effect is a host response that has the characteristics of a non-MHC-restric-ted immune response, which leads to the elimination of those residual tumor cells that have escaped apoptosis. Targeting of the IGF-IR could be especially useful in those clinical situations that require the elimination of residual tumor at the primary site or of small metastatic nodules.

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