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Perspectives in Cancer Research

The Insulin-like Growth Factor I Receptor: A Key to Tumor Growth?

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Abstract

The insulin-like growth factor I receptor (IGF-IR) belongs to the family of transmembrane tyrosine kinase receptors, like the receptors for platelet-derived growth factor, the epidermal growth factor, insulin, and others.

Genetic evidence has shown that the IGF-IR is required for optimal growth *in vitro* and *in vivo*. Even more important, however, have been recent findings from several laboratories clearly showing that the IGF-IR is an absolute requirement for the establishment and maintenance of the transformed phenotype, both *in vivo* and *in vitro* and in several cell types. These findings indicate that the IGF-IR plays a central role in the mechanism of transformation and, as such, could be a preferred target for therapeutic interventions.

Introduction

IGF-IR[1] is a tyrosine kinase receptor with a 70% homology to the insulin receptor (1). When activated by its ligands (IGF-I, IGF-II, or insulin at supraphysiological concentrations), the IGF-IR transmits a signal to its two major substrates, insulin receptor substrate 1 and Shc (2, 3), a signal which is subsequently transduced via the common signal-transducing pathway, through ras and raf, all the way to the nucleus (4). The subject of this perspective is to examine the role of the IGF-IR in transformation and tumorigenesis. The exclusion from this discussion of other growth factors and their receptors is not intended as a dismissal of their importance. However, while there have been many reviews on the mitogenic and transforming potential of several receptors for growth factors, especially those for EGF, PDGF, hemopoietic growth factors, and even insulin, the IGF-IR has always been treated as "the poor relative" in such distinguished company. It is my avowed purpose, in this discussion, hopefully to rectify this situation and place the role of the IGF-IR in a better perspective.

The IGF-IR in Mitogenesis

For many years, the activated IGF-IR has been known to be mitogenic in cells in culture; however, in growth-regulated cells (*e.g.*, 3T3 cells or human diploid fibroblasts), IGF-I, alone, cannot sustain growth of cells in serum-free medium but needs the co-operation of other growth factors, for instance PDGF, which, by itself, also fails to induce cellular proliferation (5, 6).

Recently, the importance of the IGF-IR in cell growth has been confirmed *in vivo* by the finding that mouse embryos with a targeted disruption (by homologous recombination) of the IGF-IR genes and the IGF-II gene have a size at birth that is only 30% the size of wild type littermates (7, 8). This finding is the formal demonstration that the IGF-IR and its ligands are also required *in vivo*, where they control 70% of murine embryonal growth. 3T3-like cells were subsequently derived from the knockout mouse embryos as well as from their wild type littermates and designated, respectively, R^- cells and W cells (9). Using these cells, Sell *et al.* (10) were able to define the role of the IGF-IR in cell growth: (*a*) cells lacking the IGF-IR, R^- cells fail to grow in serum-free medium supplemented with the growth factors that sustain the growth of W cells (or other 3T3 cells); (*b*) in 10% serum, R^- cells grow, albeit at a slower rate than W cells, indicating that the IGF-IR is not an absolute requirement for growth, although it is required for optimal growth; (*c*) in R^- cells, all phases of the cell cycle are elongated, suggesting a requirement for IGF-I in all phases of the cell cycle (10, 11); and (*d*) the growth deficits of R^- cells are abrogated if the cells are stably transfected with a wild type (but not a mutant) IGF-IR cDNA (9, 10, 12), unequivocally showing that the growth phenotype of R^- cells is specifically due to the absence of the IGF-IR.

Most intriguing, however, have been the findings on the effect of the IGF-IR on transformation, and the subject of this perspective is to discuss the rapidly accumulating evidence that physiological levels of IGF-IR are an obligatory requirement for the establishment and maintenance of the transformed phenotype, at least for several cell types, both *in vitro* and in the intact animal.

The Basic Observation

The basic observation (9, 10) is that cells derived from mouse embryos with a targeted disruption of the IGF-IR genes (R^- cells) cannot be transformed by the SV40 T antigen, by an activated and overexpressed Ha-*ras*, or by a combination of both, all of which transform very efficiently the corresponding W cells (or other 3T3-like cells). It is well known that rodent cells have an unfortunate tendency to transform spontaneously, to the point where investigators are extremely careful in identifying an oncogene as such, purely on the basis of the appearance of a few transformed foci in rodent cell lines. It is, therefore, quite remarkable that R^- cells cannot be transformed by the SV40 T antigen and the *ras* oncogene (we also have preliminary evidence that they cannot be transformed by bovine papilloma virus, v-*src*, and *Raf*, all oncogenes that transform wild type cells).

This resistance of R^- cells to transformation is also abolished if a plasmid expressing a wild type (but not a mutant) human IGF-I receptor cDNA is stably transfected into R^- cells (with or without the T antigen), indicating that the defect in transformability is specifically due to the lack of IGF-IRs (9, 12). R^- cells expressing the SV40 T antigen have been passaged for 2 years in our laboratory, they are still contact inhibited, and they do not form colonies in soft agar.

The implication of these observations on the growth and transformability of R^- cells is that the activated IGF-I receptor is important for growth, although not an absolute requirement (30–40% of growth continues in its absence), but is obligatory for transformation. It means that, at least in mouse embryo fibroblasts, there is an alternate pathway for the growth of cells, which is IGF-I receptor independent, but there is no alternate pathway which, by itself, is sufficient for transformation by certain oncogenes.

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[1] The abbreviations used are: IGF-IR, insulin-like growth factor I receptor; IGF-I, insulin-like growth factor I; PDGF, platelet-derived growth factor; EGF, epidermal growth factor.

The IGF-I Receptor and Other Growth Factor Receptors

Pietrzkowski *et al.* (13) and Coppola *et al.* (12) have shown that the EGF receptor, even when overexpressed, needs a functional IGF-I receptor to exert its mitogenic and transforming potential. The contrary is not true; an overexpressed IGF-I receptor does not need an EGF receptor for its actions. Similar results have been obtained with the PDGF receptor.[2] This would indicate that other growth factor receptors are dependent on a functional IGF-I autocrine or paracrine loop for their action. Of course, targeting the EGF receptor or other growth factor receptors can also inhibit growth (14), but some cells could circumvent the inhibition and make themselves independent of other growth factors by simply increasing the number of IGF-I receptors, which makes cells responsive to IGF-I only (15–17). True, it is also possible to circumvent the IGF-I receptor, but it seems that the IGF-IR is, so to speak, downstream of other growth factor receptors (including some for hemopoietic cells), arguably making it a more general and better target for growth inhibition than other receptors.

The Importance of the IGF-I Receptor in Transformation Has a Broad Spectrum

If the absence of an IGF-I receptor precludes the establishment of transformation, one can legitimately ask whether interference with its expression could bring about the reversal of the transformed phenotype. Since the IGF-IR is expressed in many cell types and IGF-I is a growth factor for a great variety of cells (18, 19), one could also ask whether its importance in transformation could be extended to other cell types besides fibroblasts. A series of experiments based on antisense strategies against the IGF-IR RNA (9–11, 20–22) have unequivocally shown that a decrease in the number of IGF-IRs causes a reversal of the transformed phenotype, as measured by colony formation in soft agar. The reduction in the number of IGF-IRs was achieved by two basic strategies: (*a*) stable transfection of a plasmid expressing an antisense RNA to the IGF-I receptor RNA; or (*b*) incubation of cells with antisense oligodeoxynucleotides against the IGF-IR RNA (wild type cells and sense controls were used in every case). These antisense strategies inhibited or completely abrogated soft agar growth in the following cell lines: human glioblastoma T98G; rat glioblastoma C6; human breast carcinoma MC-F7, cells human small cell lung carcinoma CALA 6; human melanoma FO-1; and mouse melanoma B16-F10 cells. Although, undoubtedly, tumor cell lines will be found that are resistant to the ablation of the IGF-IR, for the moment at least, we can say that antisense strategies reverse the transformed phenotype and can do so in at least 6 different cells types, from 3 species, including human.

Other Models

The above results were obtained with antisense strategies against the IGF-IR RNA, but there are other reports in the literature, based on different approaches, that abundantly confirm the notion that the IGF-IR activated by its ligands plays a major role in transformation. Thus, an antisense oligodeoxynucleotide to IGF-I (the ligand, not the receptor, this time) inhibits growth of C6 glioblastoma cells in syngeneic rats (23, 24); a dominant negative mutant of the IGF-IR inhibits tumorigenesis in nude mice (25); an antisense to IGF-II inhibits tumorigenesis *in vivo* (26), and antibodies to the IGF-IR inhibit the growth of breast cancer cells, both *in vivo* and *in vitro* (27, 28). Conversely, overexpression of the IGF-IR (12, 29, 30) or of IGF-II (26, 31) results in ligand-dependent transformation and/or tumorigenesis. Unpublished data from my laboratory and from that of Dr. Giovanni Rovera (Wistar Institute) confirm that the antisense strategy against the IGF-IR inhibits tumorigenesis in two other syngeneic systems, mouse melanoma and mouse leukemia.

Thus, the evidence from different sources and by different methods points to the crucial importance of the IGF-IR in transformation, both *in vitro* and *in vivo*, at least in several cell types.

Tumorigenesis in Syngeneic Animals

In *in vivo* tumorigenesis, the most complete and most dramatic results have been obtained with the C6 rat glioblastoma (21). Using C6 cells and a plasmid expressing an antisense RNA against the IGF-IR RNA (wild type and sense cells were used for control), it was shown that: (*a*) cells expressing an antisense to the IGF-IR RNA did not grow at all when injected s.c. into syngeneic rats, whereas wild type and sense cells gave palpable tumors in 5 days. Some of these rats have now gone for 8 months, without any evidence of tumor growth; (*b*) injection of antisense cells inhibited the growth of subsequently and contralaterally injected wild type cells; and (*c*) injection of antisense cells caused complete regression of well established wild type tumors in the opposite flank.

Thus, we have here 3 different effects of an antisense strategy against the IGF-IR RNA: (*a*) inhibition of tumorigenesis. Cells expressing the antisense RNA undergo massive apoptosis *in vivo* (22) and fail to produce tumors; (*b*) prevention of tumorigenesis by wild type cells. Rat given injections of antisense C6 cells did not develop tumors when subsequently challenged with a contralateral injection of wild type cells. This effect is almost certainly an immune response, because in some instances the rats were challenged with wild type cells 4–6 weeks after the injection of the antisense cells and still failed to develop tumors. We do not know how specific is this protective effect, but it is not aspecific, since irradiated wild type cells, injected s.c., did not protect rats from a subsequent challenge with wild type cells; and (*c*) induction of tumor regression. This was quite spectacular and highly reproducible. The wild type tumors regressed completely and never recurred (some of the rats have been kept for several months). Neither wild type nor sense cells showed any of these effects, and the same results were obtained when wild type C6 glioblastoma cells were incubated, prior to injection, with an antisense oligodeoxynucleotide to the IGF-IR RNA.

Tumorigenesis in Nude Mice

A plasmid expressing an antisense RNA to the IGF-IR RNA or an antisense oligodeoxynucleotide also inhibit the growth of human melanoma cells in nude mice (22). In 3 of 9 nude mice, the melanomas eventually became palpable, although after a latent period of 28 days, instead of the latent period of 4 days, customary for wild type cells. Interestingly, when the tumors from these 3 mice were analyzed, they were found to have lost the plasmid; and the number of IGF-IRs had returned to normal levels. These experiments have shown dramatically that it is the decrease in the number of IGF-IR that is crucial in determining the reversal of the transformed phenotype. Although it has been repeatedly demonstrated that these antisense strategies cause a marked decrease in the number of IGF-IRs (15, 21, 22), it is not necessary to reduce the number of receptors to zero; in most cases, a 60–70% reduction is sufficient to achieve reversal of the transformed phenotype.

A very important point in the case of human melanoma cells is that, *in vitro*, the antisense plasmid had practically no effect on their growth in monolayer, yet it was quite effective in preventing tumorigenesis. This again emphasizes the fact, mentioned above, that the IGF-IR has

[2] Manuscript in preparation.

Table 1 *Importance of the IGF-I receptor in the establishment and maintenance of the transformed phenotype*[a]

Strategy	Cell type inhibited	Ref.
Antisense to the IGF-IR	Rat glioblastoma (SA, T)	21
	Human glioblastoma (SA)	9
	Human melanoma (T)	22
	Human breast carcinoma (SA)	20
	Human lung carcinoma (SA)	20
	Mouse melanoma (SA, T)	Unpublished
	Mouse leukemia (T)	Unpublished
Antisense to IGF-I	Rat glioblastoma (T)	23, 24
Antisense to IGF-II	Pancreatic cells (T)	26
Antibody to IGF-IR	Human breast carcinoma (T)	27, 28
Dominant negative of IGF-IR	3T3 cells (T)	25
Genetic deletion	Mouse embryo cells (SA)	9

[a] Detailed explanations are in the text. SA, inhibition of growth in soft agar; T, inhibition of tumorigenesis.

a more important role in the maintenance of the transformed phenotype than in the growth of cells.

The IGF-I Receptor and Apoptosis

Leaving aside, for the moment, the immune response that is so dramatically evident in the C6 rat glioblastoma model, where it even causes tumor regression, I will now limit myself to the inhibition of tumorigenesis, which has recently been confirmed in several systems (Table 1). The question here is, "If a decrease in the number or the activity of IGF-IR has only a moderate effect on growth, why is it so effective on tumorigenesis?"

It has been known for many years (32) that the growth of tumors depends not only on the proliferation of cells (length of cell cycle and growth fraction) but also on the rate of cell death. I purposely say cell death rather than apoptosis because, although apoptosis is nowadays, for cells, the fashionable way to die, cells can die in more than one way; and in fighting cancer cells, one cannot be too particular about how cells are killed. In fact, it is possible that apoptosis and necrosis may share some of the same mechanisms (33). But apoptosis is certainly an important mechanism of cell death, and it has already been proposed as the major target for cancer therapy (34). Recently, Evan *et al.* (35) have developed a model in which an overexpressed c-*myc* induces apoptosis in cells *in vitro*, but only when growth factors are removed. These authors have now shown (36) that IGF-I (and PDGF, but not EGF and fibroblastic growth factor) prevent c-*myc* induced apoptosis. As mentioned above, cells expressing the antisense RNA to the IGF-IR RNA undergo massive apoptosis when injected into animals (22). In fact, using an *in vivo* system in rats, we have found that tumor cells expressing an antisense to the IGF-IR RNA undergo apoptosis in 27 h which is almost total (7 viable cells of 5×10^5 injected). Corresponding sense or wild type cells actually doubled in number in the same interval.[3]

By taking into consideration the results of Evan *et al. in vitro* and our own results *in vivo*, we would like to propose the following hypothesis: a decrease in the number of IGF-IRs (or activity) inhibits the growth of normal cells, which have a tendency to take refuge in the G_0 stage of the cell cycle, where they can survive in an environment poor in growth factors. However, the decrease in the number of IGF-IRs (which is the functional equivalent of growth factor removal) causes oncogene-driven cells (like those overexpressing c-*myc*) or tumor cells (most of which are presumably oncogene driven), to undergo apoptosis, much more profound *in vivo* than *in vitro*. If this hypothesis is verified, it would make the IGF-IR a preferred target for therapeutic interventions.

[3] Resnicoff, *et al.*, manuscript in preparation.

Conclusions

Antisense strategies to the IGF-IR not only inhibit tumorigenesis but also prevent the subsequent growth of wild type cells, and, indeed, can induce regression of wild type tumors. These strategies use a double-edged sword: on one side, they induce apoptosis of tumor cells; on the other, they provoke some kind of immune response that mops up any residual surviving cell. As mentioned above, the IGF-IR is not an absolute requirement for normal growth. In its absence, cells use an alternate pathway (pathways?) that allows 30–40% growth. However, the IGF-IR is an absolute requirement for transformation, at least for several cell types: in its absence, tumor cells undergo apoptosis. Therefore, it is possible that an antisense strategy to the IGF-IR may discriminate between normal and tumor cells.

The strongest reservation is that, thus far, the results have been limited to rodents (with the exception of the human melanoma cells, but in nude mice). The question now is, "How relevant are these data to human tumors?"

There are abnormalities in the expression or amplification of the genes for the IGF-IR and its ligands in several human tumors (reviewed in Ref. 37), but they are sporadic and do not seem to follow a consistent pattern. There are also intriguing reports of an increased incidence of malignancies in acromegalics, in which IGF-I levels are elevated (38). However, the important point that emerges from the experiments in rodents is that it is not the overexpression or the gain of function of the IGF-IR that is crucial but its absence. True, overexpression of the IGF-IR can lead to transformation, but the most striking observation is that transformation does not occur, or is even reversed, when the number of IGF-Rs falls below physiological levels. It is, therefore, not surprising that deletions or mutations, resulting in loss of function of the IGF-IR are not found in human tumors, since many types of tumor could not develop in the absence of a certain level of functional IGF-IRs.

In the meantime, we have to be contented with the results that have been obtained *in vitro* and in rodents, but these are dramatic enough to justify our hope that they will eventually extend to humans.

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