Gene Therapy

Fusogenic Membrane Glycoproteins

Gene therapy for cancer is all about killing tumor cells. That destruction may be brought about through direct cytotoxicity of the protein encoded by the gene, by the initiation of cellular signaling pathways that induce apoptosis, or by expression of a protein that stimulates immune cells to recognize and kill the tumor cells.

Hundreds of genes can kill cells in which they are expressed. The pertinent issue at the moment is how to kill cells in which the gene is not expressed. This critical clinical question currently dominates the field of gene therapy for cancer because of the problems that have been encountered in finding delivery vehicles that can reliably deliver even a single copy of any gene to every tumor cell.

Activity of the gene itself can help to compensate for the paucity of its delivery into tumors. When expression of the gene in a single cell can lead to killing of its close neighbors, the effect is called local bystander killing. In other circumstances, cells in completely separate deposits from those that are accessible and targetable by gene delivery vectors can be killed. Such systemic bystander effects usually operate through activation of immune effector cells.

The specificity of appropriately activated T cells makes them the key players in generating systemic bystander responses. Most molecular immunotherapies are aimed at generating T cell responses against tumor-associated antigens. Alternatively, systemic bystander killing effects can also be raised using genes that interfere with crucial processes central to tumor development, such as angiogenesis.

Stimulation of immune cells to recognize tumor cells as foreign and therefore to attack them can be achieved by transferring genes that encode cytokines, costimulatory molecules, or potent immunogens. Our group has demonstrated that the process of killing tumor cells in situ can educate the immune system to recognize tumor cells as the source of potentially harmful antigens and therefore stimulate specific anti-tumor immune responses.

A key element in this immune activation is that the tumor cells must be killed in a manner that resembles what is seen in infections. Programmed cell death or apoptosis, typical of normal developmental processes, is much less likely to alert the immune system to react against tumor cells and their associated antigens.

Early clinical trials of genes designed to kill tumor cells showed that the potent bystander effects seen in rodent models were much reduced in the clinical setting of advanced tumors and, frequently, suppressed immune systems. We have looked for genes with much greater levels of potency in cell killing, either directly or through bystander effects.

Some viruses infect cells by expressing envelope proteins that can bind to receptors on target cells and that then mediate fusion of the viral particle with the target cell. Expression of the envelope proteins in newly infected cells then allows the generation of new viral particles and their subsequent release for further rounds of infection.

A byproduct of cellular expression of viral envelope proteins is that neighboring uninfected cells expressing the viral receptor can also bind to the envelope protein, leading to fusion of the infected and uninfected cells. Such fusion can involve a single envelope-expressing cell and many surrounding cells, as long as they express the receptor. The result is the formation of large, multinucleated syncytia, which eventually die.

Recruitment of many bystander cells into structures destined to die is exactly what we hope to achieve with gene transfer into tumor cells at the local bystander killing level. Moreover, our notion that the immune system will be best activated by killing cells in circumstances normally associated with infection should also be satisfied by the formation of viral envelope-induced syncytia. Indeed, the immune system may not be able to distinguish between genuine virus-induced syncytia, against which it is finely tuned to respond, and gene transfer-induced syncytia, against which we would like it to respond.

We tested whether cloned viral envelope genes expressed in tumor cells could lead to aggressive cell killing with consequent activation of the immune system. Initially, we used three examples of envelope genes, called fusogenic membrane glycoproteins or FMG's, and we have shown that their ability to kill cells is much better than that of other genes that have been used for this purpose, such as the herpes simplex virus thymidine kinase gene (HSV-tk).

In culture experiments, expression of either a gibbon-ape leukemia retroviral FMG or measles virus F or H protein by a single cell was able to recruit more than 200 bystander cells into syncytia and thereby kill them. In contrast, a cell expressing HSV-tk was able to kill only about 10 other cells by the metabolic conversion of ganciclovir

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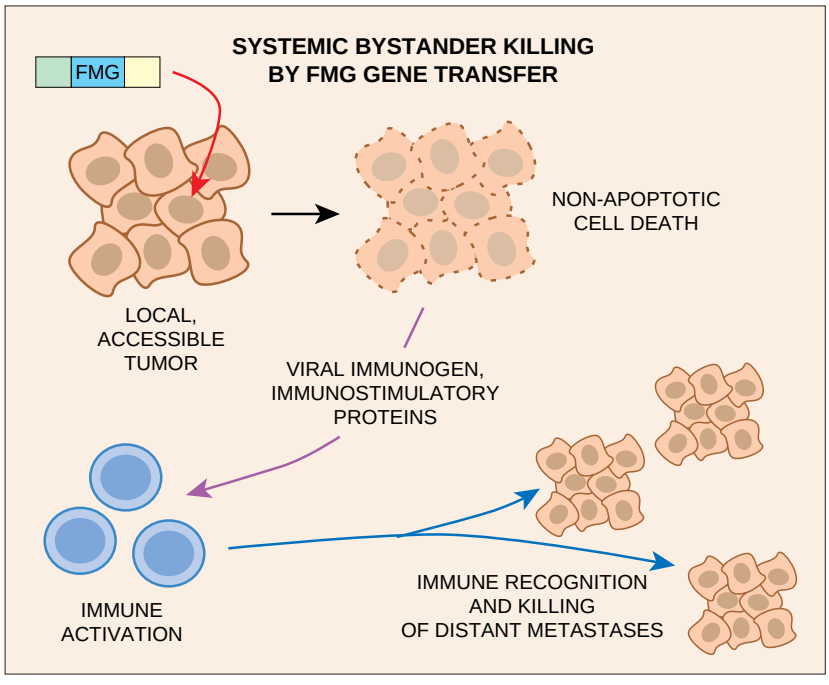

and the cell-to-cell transfer of its toxic derivative.

Perhaps of greater importance, we have shown that expression of the FMG in murine tumor cells acts as a potent immunogen, raising immune responses against unmodified tumor cells at a distant site. Almost certainly, the viral immunogen stimulates aggressive immune reactivity against the tumor cells expressing it, leading to cell killing and release of tumor antigens. These antigens are then taken up by professional antigen-presenting cells such as dendritic cells, which can present them to T cells and initiate specific anti-tumor immunity.

We have studied the mechanisms by which the developing syncytia actually die. Interestingly, it seems clear that syncytial development does not trigger apoptosis but rather a series of metabolic processes that result in effective exhaustion and self-digestion of the syncytia. Additionally, development of syncytia is accompanied by the induction of various stress-response proteins, including heat shock protein 70, which we and others have shown to be associated with increased immune recognition of tumor cells. It is as if the tumor were itself the source of a pathogen infection.

Using FMG's instead of other better-known cytotoxic genes has two principal advantages. First, increased killing potency per se means that fewer copies of the gene have to reach the tumor site. Against the background of problems with vector efficiency, this is a substantial advance for the treatment of local disease.

Second, the presence of highly immunogenic foreign viral proteins expressed in the tumor cells, along with the nonphysiological mechanisms of syncytial killing and induction of immunological alarm signals, means that even a small amount of tumor killing in the context of an intact immune system may be enough to trigger immune responses that at least contain if not clear tumor cells throughout the body. It is also possible to combine FMG's with other genes, principally cytokines, that will further augment the immune stimulatory properties of FMG gene therapy.

It is clear that FMG's alone do not represent the solution to all our problems. Potent as they are, sufficient numbers of these genes must still be delivered to tumor cells in situ. We have incorporated FMG's into retroviral and adenoviral vectors and found that direct injection of vectors expressing FMG's can clear small but established tumor burdens. However, in these experiments, our targeting has been solely at the end of a needle inserted into a subcutaneous tumor.

Targeting of delivery vectors remains a priority, and it may become more of a problem with FMG's, because the very potency of the genes means that it is more important that targeting be precise to prevent toxicity to normal cells. To that end, we have shown that it is possible to restrict expression of FMG's to certain cell types.

Gene therapy must measure its value against existing therapies that it hopes to improve upon or replace. Therapeutic tumor targeting is currently defined by the limits of the surgeon's cut, by the cell cycle selectivity of anti-proliferative drugs, or by the precision of a beam of radiation. Realistically, current gene transfer methods are unlikely to match the efficacy of, for example, a closely focused dose of radiation.

However, gene therapy offers the potential of precise targeting through the engineering of tissue-specific or tumor-specific promoter elements (for restricting expression) and of specific surface markers (for increasing specificity of vectors). But until technical advances in vector development can achieve those goals, the use of genes with high local potency and systemic immune activating properties represents one important step along the path that will some day make gene therapy for cancer a genuine clinical option.

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