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In situ Delivery of Suicide Genes for Cancer Treatment

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INTRODUCTION

THE TRADITIONAL treatments for cancer—surgery, radiation and chemotherapy–have shown only a modest increment in improved therapeutic outcomes during the past decade, and may be nearing their maximum effectiveness as therapy for many cancers. Clearly, new approaches are needed if better results are to be obtained for several tumour types. The past decade has seen early development of several biologically-based cancer treatment strategies, particularly immunotherapy, with great anticipation that these techniques will provide a fourth significant treatment option for cancer. Unfortunately, with a few notable exceptions, the results of biologically-based treatments for most cancers have been disappointing.

There has been a recent upsurge in excitement in this area because the initial clinical applications of gene therapy have rekindled the hope that biological treatments for cancer may finally become the effective weapon against cancer that has been so long anticipated. Two broadly-defined strategies for gene therapy of cancer are currently being explored. In one, which can be defined as an indirect or adjuvant approach, gene insertion is directed at enhancing the anti-tumour response of a patient. As outlined by other contributors to this volume, this strategy may include attempts to use gene insertion to improve the anti-tumour response of a patient's T-cells by modifying them directly, by introducing genes to render normal tissues (e.g. bone marrow) resistant to chemotherapy, or by genetically modifying the tumour in order to enhance its immunogenicity and induce an effective systemic anti-tumour immune response.

This report will discuss another strategy for gene therapy of cancer—the attempt to directly treat cancer by gene insertion. The power of gene transfer offers a unique opportunity to develop entirely new treatment strategies for cancer. However, many formidable obstacles will have to be overcome in order to make these approaches clinically useful.

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REVIEW

The approach to direct gene therapy for cancer has the basic premise that if exogenous genes could be successfully delivered to every cancer cell in a patient, then a whole range of new treatment options might become possible. For example, wild type tumour suppressor genes might be introduced into malig-

nant cells to cause them to revert to a non-malignant phenotype. Anti-sense contructs might be targeted to activated oncogenes or growth factors that are driving the growth of the cancer. Gene systems to induce apoptosis might be preferentially activated in cancer cells. Genes encoding any of several potent toxins, perhaps under the transcriptional control of a tumour-specific promoter, could be used to kill the cancer. It might be possible to fundamentally alter the metabolic pathways of a tumour cell so that it becomes susceptible to agents which are relatively benign when given to unmodified, normal cells.

There are many challenges to the successful development of any of these strategies. In addition to the problem of the selection of a therapeutic gene, it would seem obvious that direct gene therapy would be absolutely dependent upon the successful delivery of the gene or its product to all the tumour cells or else the treatment is very unlikely to succeed. At the present time, no technique is available which can accomplish this goal for systemic delivery to disseminated cancer. Even for treatment of localised cancer, some mechanism to amplify the anti-tumour effect is needed because, even here, universal gene delivery is very unlikely.

As a first tentative step along the road to development of this general strategy, we have been exploring the use of "suicide genes" for treatment of localised deposits of cancer. These were called "suicide genes" because they originally developed as a safety feature for inclusion into retroviral vectors. Because retroviral vectors integrate randomly into the chromosomes of the cells into which they insert, the promoter of the vector might activate a gene downstream from the site of integration. If such a cellular gene were an oncogene or growth factor, uncontrolled cell growth through insertional mutagenesis might be the result. As a possible counter to this problem, one strategy would involve engineering the retroviral vector to also contain a latent suicide gene which could be activated on demand if needed to eliminate a cell transformed by insertional mutagenesis. Preferably, this latent gene would encode for a benign gene product, such as an enzyme, which would have no direct effect on cellular function but which would be capable of activating a non-toxic prodrug into a cellular toxin, active only in the gene-modified cells. Then a cell transformed by insertional mutagenesis could be killed by simply administering the prodrug when a malignancy involving the gene-modified cells was detected.

Our group has studied two such suicide systems. Herpes simplex virus is killed by the anti-viral drug ganciclovir. Herpes thymidine kinase (TK) phosphorylates ganciclovir to produce the nucleotide toxin ganciclovir phosphate. This compound functions as a chain terminator and DNA polymerase inhibitor, and, via these actions, is cytotoxic. Thus, as Moolton first demonstrated [1], a tumour cell engineered to express herpes TK should be killed upon subsequent exposure to the prodrug ganciclovir. Another suicide system involves the use of the fungal or bacterial gene encoding the enzyme cytosine deaminase (CD). CD, which is not found in mammalian cells, deaminates the anti-fungal drug 5′-fluorocytosine (5-FC) and thereby generates the anti-cancer drug 5′-fluorouracil (5-FU). Mullen and colleagues [2] first showed that tumour cells, engineered to express CD, will generate their own chemotherapy and be killed when exposed to the prodrug 5-FC. Thus, our work and that of other groups has demonstrated that each of these suicide systems can have profound anti-tumour activity *in vivo* as evidenced by the elimination of sizeable deposits of cancer that were generated from tumour cells previously transduced *in vitro* to express either of these suicide genes [3–18].

During the course of developing these latent suicide gene systems, it became apparent to us that they might also be used directly for cancer therapy, if the problem of gene delivery to cancer *in situ* could be solved. Since there is no current technique by which such suicide genes can be delivered systemically to target disseminated cancer, we focused our efforts on treatment models for localised cancer. Even the efficient uniform delivery of genes to localised tumour deposits presents a challenge far beyond the scope of present technology. Therefore, an amplification mechanism to provide a mechanism for killing tumour cells, which have not been successfully engineered to adequately express the suicide gene system being used, is essential with this approach. Fortunately, both of the suicide systems we have described are amplified by a form of the bystander effect which can be demonstrated when tumours established with cell mixtures, containing equal numbers of both wild type and gene-modified cells, are completely eliminated when the tumour-bearing animal is treated with the relevant prodrug [3, 10].

In addition to amplification, a mechanism for targeting the cytotoxic effect to the tumour cells while sparing the surrounding normal tissues is also a desirable component of this strategy. Targeted gene delivery, particularly to solid tissues *in vivo*, is still only a theoretical possibility is most cases. With viral gene delivery systems, most of the currently available vectors will target tumour cells specifically, although it might be possible to exploit other specific properties of the virus to accomplish such targeting. For example, retroviral vectors based on the MoMLV integrate their genes only in cells which are actively synthesising DNA. Thus, such retroviral vectors might effectively target gene delivery only to the mitotically active tumour cells present in an organ where the usual resident cells are not dividing, such as the brain.

Another strategy for targeting is to place the suicide gene under the transcriptional control of a tumour-specific promoter so that even if the gene is delivered to non-tumour cells it will be expressed only within the cancer. For example, the promoter from the α-fetoprotein gene might be used to target suicide or toxin gene expression to hepatomas occurring in adults. Another strategy is to have the suicide gene encode for an agent which is uniquely toxic to the tumour cells in the particular environment in which the cancer occurs. In this example, a cycle active cytotoxic agent would be relatively more toxic to proliferating tumour cells than to the quiescent normal tissues, when it is generated locally in an organ composed primarily of non-dividing normal cells such as brain, muscle or liver.

As an initial test of the feasibility of this general approach to the direct gene therapy of cancer, we elected to test whether retroviral vectors could be used to deliver the herpes simplex TK gene locally to solid tumours. Several characteristics of the retroviral vector system place constraints on how it can be used. Since retroviruses only integrate their genes into actively dividing cells, the use of these vectors provides a mechanism for targeting gene delivery to the tumour in organs where the resident cells are not mitotically active. However, even in the most rapidly growing tumours, the fraction of cells actually in mitosis at any one time is quite low. Retroviral vectors are difficult to routinely produce in titres significantly above 10^6/ml, and thus large volumes of vector-containing supernatant may need to be injected in order to successfully deliver a sufficient number of vector particles to transduce all of the tumour cells. In addition, retroviral vectors are relatively unstable at 37°C, degenerate rapidly, and therefore must be constantly replaced.

These factors combine to make a single injection or even continuous infusion of vector impractical.

As an alternative procedure for local delivery of vector particles, vector-producing cells (VPC) could be injected into the tumour so that a continuous supply of vector would be produced locally within the tumour mass. In this case, even tumour cells which enter the cell cycle at a relatively low rate would be more likely to be transduced because the locally-produced vector particles should provide a more consistent supply. In our studies, we have used the murine 3T3-derived amphotropic retroviral vector producer cell line PA317 to deliver a TK vector in several tumour systems in mice and rats, as well as in a clinical trial in patients with glioblastoma multiforme and metastatic cancer to the brain [3–10].

In this case, since the vector producer cells also contain and express the herpes TK gene, they are killed along with any locally-transduced tumour cells which ganciclovir is administered. Brain tumours were selected as the initial tumour candidate for several reasons. The brain is an immunologically-privileged site so that the xenogeneic murine vector producer cells should survive for a longer period before immunological mechanisms cause their rejection. If an immune reaction directed to the VPC does occur, it is possible that this reaction may also have a beneficial effect on the surrounding tumour as well. The brain is a mature organ with very few resident cells being mitotically active. Therefore, the use of retroviral-mediated gene transfer should restrict the genetic modification of the dividing tumour cells, sparing the non-dividing normal brain which will not integrate the retroviral vectors, providing a valuable targeting mechanism. Fortunately, the blood–brain barrier, which prevents many potential therapeutic agents from entering the CNS, does not block ganciclovir, so that this prodrug will be available to the CNS following systemic delivery.

As indicated earlier, an amplification mechanism is needed in the strategy of direct gene therapy for cancer since no gene transfer technology currently exists which will insure that all the tumour cells, even in a local deposit of cancer, will be successfully genetically modified. Both the CD and the TK systems have such a mechanism which we have called the bystander effect. For the cytosine deaminase system, the final chemotherapeutic product generated by the gene-modified tumour is 5-FU. This compound is freely diffusible across cell membranes, and thus can spread from the gene-modified cells where it is generated to adjacent tumour cells which have not been successfully modified. Thus, the final product of the interaction between the gene-encoded enzyme and the prodrug is able to spread locally within and potentially beyond the tumour. Although this ability to spread locally is an advantage, it could also potentially be achieved by local infusion of 5-FU by catheter, and it is unclear whether 5-FU generation via gene insertion has any intrinsic advantage over this local infusion strategy.

The bystander effect seen with the TK system is considerably more complex. The toxin generated in this system is a nucleotide, ganciclovir phosphate, and, as such, is unable to pass through cell membranes in substantial quantities. Nevertheless, a very significant bystander effect can be demonstrated with this system, with some tumours showing complete regression with as little as 1–2% of the cells expressing the TK gene. Recently, it has become clear that the toxic nucleotide in this system can be transmitted between cells via the gap junctions which are present in many tissues. In contrast to the situation with CD and 5-FU, this therapy is unique to gene modification, in that the nucleotide toxin must be produced inside cells since it cannot pass through the cell membrane in either direction, eliminating the possibility of systemic or even local infusion of the drug. Yet once inside the tumour cells, the toxin can be transmitted from cell to cell and thus fulfill the need for an effective amplification mechanism. A significant limitation of this strategy is that it will not be active in tumours lacking gap junctional communications. Thus, a number of types of cancer, including most lymphomas, will not be suitable candidates for this treatment strategy.

The use of xenogeneic vector producer cells with this TK system was chosen originally only for convenience as a proof of principle. Now that the preliminary results demonstrate that this strategy could be effective, it is worth considering modifications to this original plan. For example, the murine fibroblast vector producer cells are not motile and must be distributed through the tumour by injection. Perhaps a VPC which was motile might be a more efficient delivery system. Perhaps a VPC derived from human cells would survive for a longer period *in vivo* and thus result in more efficient gene transfer to the tumour.

The original experiments were set up using retroviral vector-producing cells to deliver the TK gene, with the notion that targeting would be directed to tumour cells because retroviral vectors are restricted to gene insertion in dividing cells. This approach is cumbersome and inefficient, and other gene transfer systems might have advantages if a mechanism to target gene activity could be found. For example, adenoviral vectors can be produced at titres approaching 10^{12}/ml, can transduce a wide variety of cell types and do not need integration for gene expression. These properties offer both advantages and disadvantages for cancer treatment. One mechanism for targeting might be the use of tumour-specific promoters to control expression of the introduced genes. Another strategy would use the specific characteristics of the toxic compound produced to target the effect to the tumour. Ganciclovir phosphate is a cycle-active drug that is toxic primarily to dividing cells. Thus, it may be redundant to target delivery of the TK gene to cycling cells via a retroviral vector and also have the inserted gene generate a cycle active drug. In this regard, we and others have developed data that show that an adenoviral TK gene delivery system can also be effective in this suicide gene strategy for direct cancer therapy [17, 18].

Potential applications for gene therapy of cancer are just beginning to be explored, and it is difficult to predict just what direction will ultimately prove to be of value. What does seem clear is that this technology offers unparalleled opportunities for the exploration of entirely new and unique approaches to cancer treatment and control.

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Regulation of Gene Therapy in Europe: a Current Statement Including Reference to US Regulation

O. Cohen-Haguenauer

INTRODUCTION

OVER THE past decade, methods for delivering genes into mammalian cells, with the aim of subsequent expression of the transferred sequences from the host cell, have been developed. Potential therapeutic applications have been envisaged and have raised great interest.

It is generally accepted that no attempt to introduce germ line genetic alterations should be performed. This would result in introduction of genetic changes into the reproductive cells of an individual, with the aim of modifying genes passed on to the individual's offspring. This paper will only consider somatic cell gene therapy, where genetic modifications are applied to a single patient's somatic cells.

Although procedures based on specific gene targeting will not enter clinical protocols prior to solving fundamental issues, procedures based on addition of genetic material are currently underway. The expected benefit of genetic modification of somatic cells needs to be carefully assessed with respect to its potential risks. Whatever the technological strategy, non-propagation and non-transmission of the gene transfer delivery system is mandatory.

Preclinical studies covering a large range of pathologies are currently underway. These studies are primarily aimed at demonstrating the technological feasibility of various approaches rather than the true therapeutic efficacy. Technological improvements, and the solving of basic issues dealing with both the regulation of gene expression and the biology of cell transplantation, are still required in order that gene therapy may enter a clinical efficacy phase [1]. A multidisciplinary approach is needed to allow fruitful exchange between investigators in the basic sciences and clinical therapists. The European Working Group on Human Gene Transfer and Therapy (EWGT) was set up to meet this need.

Whatever the interest raised by gene therapy and its innovatory potential, the relevance of this approach should be carefully considered, both in terms of economical constraints and ethics, and in the context of an overall therapeutic strategy. This is particularly important in the case of acquired diseases, like cancer, and this new approach should be compared to alternative treatments, such as targeted drugs.

In principle, gene transfer involves the insertion of a foreign sequence of nucleic acid into the nucleus of a deficient target

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