Gene therapy for cancer

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Initiation of clinical trials of gene therapies for cancer has been made possible by two major technological advances: the ability to clone genes that constitute the genetic basis of carcinogenesis or that have therapeutic potential, and the development of an increasing number of gene transfer methods. As a result, 30 experimental trials of gene therapy for the treatment of human cancer have been approved in the United States of America. Here, we discuss the current status of gene therapy for cancer together with future directions for its development.

During the past two decades, there have been major advances in our understanding of how cancer develops, proving that cancer has a genetic basis[1]. A series of genetic abnormalities that accumulate in one cell may result in a pattern of abnormal clonal proliferation. Our growing understanding of the genetic basis of cancer offers new opportunities for the molecular prevention and treatment of cancer. For example, an early assessment of an individual's genetic predisposition to cancer may allow changes of life-style to avoid certain environmental exposures (e.g. cigarette smoke). Diagnosis of cancer at the molecular level should allow more accurate prognosis. Since the development and progression of cancer involves a complex genetic system, the ability to dissect the basis of factors such as invasion, metastasis and drug resistance should enable oncologists to use more selective therapies, develop genetic vaccines and account for variable responses to standard therapies.

One of the most promising approaches to emerge from the improved understanding of cancer at the molecular level is the possibility of using gene therapy to selectively target and destroy tumor cells. For example, the loss of tumor suppressor genes (e.g. the p53 gene) and the overexpression of oncogenes (e.g. K-*RAS*) have been identified in a number of malignancies. It may be possible to correct an abnormality in a tumor suppressor such as p53 by inserting a copy of the wild-type gene; indeed, insertion of the wild-type p53 gene into p53-deficient tumor cells has been shown to result in the death of tumor cells[2]. This has significant implications, since p53 alterations are the most common genetic abnormalities in human cancers. The overexpression of an oncogene such as K-*RAS* can be blocked at the genetic level by integration of an antisense gene whose transcript binds specifically to the oncogene RNA, disabling its capacity to produce protein. Experiments *in vitro* and *in vivo* have demonstrated that when an antisense K-*RAS* vector is integrated into lung cancer cells that overexpress K-*RAS*, their tumorigenicity is decreased[3]. Despite the promise of such approaches, a number of practical difficulties remain to be overcome, the most important of which is the need for more efficient systems of gene delivery. No gene transfer system is 100% efficient, unless germ-line therapy is contemplated. However, some non-integrating vectors, such as those derived from adenovirus, may be sufficiently efficient for long enough to destroy a tumor. If a highly efficient, integrating system of *in vivo* gene transfer can be developed, genes might be delivered prospectively, preventing malignant transformation in susceptible patients. Gene therapy for the treatment of cancer has a wide variety of potential uses, several of which are listed in Box 1.

Box 1. Potential strategies for gene therapy in the treatment of cancer

- Enhancing the immunogenicity of the tumor, for example by introducing genes that encode foreign antigens
- Enhancing immune cells to increase anti-tumor activity, for example by introducing genes that encode cytokines
- Inserting a 'sensitivity' or 'suicide' gene into the tumor, for example by introducing the gene that encodes HSVtk
- Blocking the expression of oncogenes, for example by introducing the gene that encodes antisense K-RAS message
- Inserting a wild-type tumor suppressor gene, for example p53 or the gene involved in Wilms' tumor
- Protecting stem cells from the toxic effects of chemotherapy, for example by introducing the gene that confers MDR-1
- Blocking the mechanisms by which tumors evade immunological destruction, for example by introducing the gene that encodes antisense IGF-1 message
- Killing tumor cells by inserting toxin genes under the control of a tumor-specific promoter, for example the gene that encodes diphtheria A chain

The first experiment in human gene therapy began in 1990, with the aim of treating adenosine deaminase deficiency, a rare immunodeficiency disorder[4]. Since that time, there has been substantial growth in gene therapy, especially in the field of oncology (Fig. 1). This is, in part, because tumor cells can be manipulated *ex vivo*, while the affected tissues (e.g. lung, CNS) from individuals with other genetic diseases often cannot. Moreover, therapy using immunostimulatory genes such as interleukin (IL-2) may potentially be useful in many different cancers, while molecular treatment of genetic diseases would typically require the isolation, cloning and functional characterization of the gene involved in each disorder. Trials for cancer gene therapy that have been approved in the USA have involved malignancies that are considered incurable. This clinical situation, which is unlike many genetic diseases for which life expectancy is measured in years rather than weeks or months, has been considered more appropriate ethically for untested technologies. For these reasons, we expect that applications of gene therapy to cancer will continue to be the fastest growing area of human gene therapy.

Approaches to *ex vivo* gene transfer

Genetically engineered tumor cells

Various groups are investigating the production of autologous cellular vaccines for the treatment and

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prevention of cancer. This is most commonly attempted by surgically removing tumor cells from the patient, growing them in tissue culture and inserting immunostimulatory genes *in vitro*. These cells are then reinjected into the patient in an effort to induce a significant systemic immune response that will both destroy tumor cells and vaccinate the patient against recurrence of the tumor. Vaccination with cells that produce cytokines has been shown to result in systemic immunity in mice. Alteration of syngeneic tumors with the genes that encode IL-1β (R.M. Blaese *et al.*, unpublished), IL-2, IL-4, IL-6, TNFα, GM-CSF or γ-interferon (Refs 5–10) results in immunological destruction of the tumor cells *in vivo*. On the basis of these studies, several gene therapy trials in humans have been approved, in which patients are injected with either autologous or allogeneic genetically modified tumor cells (Table 1). These trials involve the insertion of retroviral vectors carrying the gene that encodes either IL-2 (Refs 11–14), TNFα (Ref. 15) or GM-CSF into melanoma, colorectal, renal cell carcinoma, neuroblastoma or breast cancer cells *in vitro*. Melanoma and renal cell carcinoma have been the primary focus of tumor vaccine studies since they are believed to be more immunogenic than other tumors and might therefore be most likely to respond to this therapeutic approach. One modification of this technique is the insertion of the gene for either IL-2 or IL-4 into autologous fibroblasts, which are then mixed with irradiated tumor cells from the patient and reinjected. This approach has the advantage that growing fibroblasts *in vitro* is much easier than culturing tumor cells from a large number of individuals. The results of several of these clinical trials should be published within the next year, and should provide a foundation for developing new, improved vaccination protocols.

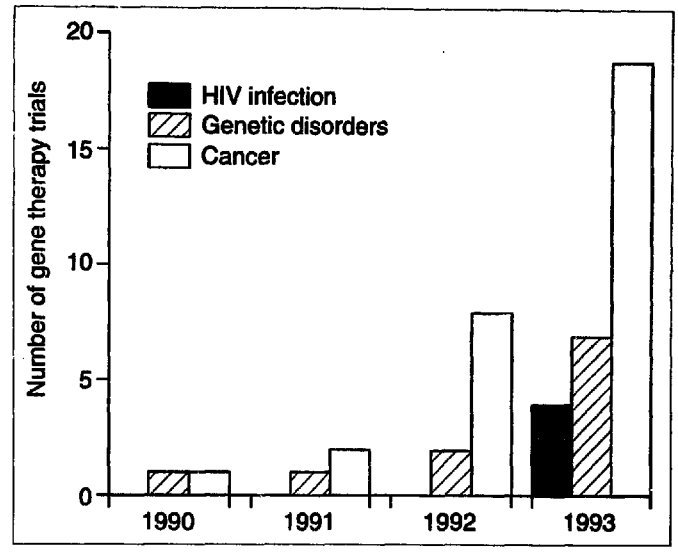

FIGURE 1. The number of gene therapy trials approved by the US Recombinant DNA Advisory Committee has been increasing steadily since the first trial was approved in 1990. As of December, 1993, there are 45 approved trials in the USA, 30 of which are for the treatment of cancer. Other trials test approaches to the treatment of HIV infection or inherited genetic disorders.

Besides modifying tumor cells to produce 'immune activating' cytokines, another strategy is to block the production of insulin-like growth factor-1 (IGF-1). Many tumors, for example glioblastoma multiforme and breast cancer, produce high levels of IGF-1. Insertion of an antisense gene that stops production of IGF-1 in the tumor allows immunological rejection of the genetically altered tumor after reimplantation[16]. Destruction of the tumor is mediated by cytotoxic T lymphocytes. The precise mechanism by which IGF-1 mediates tumor protection *in vivo* remains unclear. This approach has been approved for trials in treating glioblastoma multiforme, which are expected to begin this year.

Genetically engineered T lymphocytes

T lymphocytes have the capacity to home in on tumor tissue. Several strategies for human gene therapy hope to exploit this property to deliver cytokines directly to tumor masses. The hypothesis is that secretion of cytokines locally at the tumor site by the effector T lymphocytes will enhance their anti-tumor activity and avoid the side-effects that result from the systemic administration of cytokines. A clinical trial of TNF-modified tumor infiltrating T lymphocytes (TILs) has been in progress since 1991. Unfortunately, T lymphocytes are difficult to transduce with retroviral vectors and tend to downregulate expression of the cytokine gene carried by the vector[17]. These two problems of poor gene transfer efficiency and poor cytokine expression have so far limited the application of this approach, and have shifted the emphasis from modification of T lymphocytes toward the genetic alteration of tumor cells, which are much easier to grow in culture and more readily engineered.

Insertion of a sensitivity gene

Gene therapy using genes that activate a relatively nontoxic pro-drug to form a highly toxic agent may be very valuable[18]. The most widely studied system uses the thymidine kinase gene of the *Herpes simplex* virus (HSVtk). The HSVtk gene confers sensitivity to the anti-herpes drug, ganciclovir (GCV), by phosphorylating GCV to a monophosphate form (GCV-MP). Phosphorylation to the triphosphate form (GCV-TP) by cellular kinases results in inhibition of DNA polymerase, and leads to cell death. One interesting feature of the way that GCV kills tumor cells expressing HSVtk is that adjacent cells that lack the gene are also destroyed, a phenomenon termed the bystander effect[19]. The mechanism of this effect is thought to be related to the diffusion of GCV-TP to adjacent tumor cells, perhaps via gap junctions. In one approved clinical trial, investigators will attempt to exploit the bystander effect to kill human ovarian cancer *in vivo*[20]. Irradiated ovarian tumor cells that contain the HSVtk gene will be injected into the peritoneal cavity of women with stage III ovarian cancer, who will be given GCV. It is hoped that these HSVtk-expressing cells will destroy bystander tumor cells *in vivo*. The HSVtk–GCV system has

also been used in the treatment of brain tumors, as discussed below.

Protection of hematopoietic stem cells

Another possible strategy for cancer therapy attempts to protect hematopoietic stem cells (HSCs) from the toxic effects of chemotherapy by using the gene that confers multiple drug resistance type 1 (MDR-1). The MDR-1 gene[21] has been isolated from tumor cells, where it functions to pump chemotherapy drugs (including daunorubicin, doxorubicin, vincristine, vinblastine, VP-16, VM-26, taxol and actinomycin-D) from within the cell. In mice, *ex vivo* transfer of a retroviral vector carrying the MDR-1 gene into bone marrow stem cells and their subsequent reintroduction into animals has been shown to protect stem cells *in vivo* from the effects of large doses of taxol. Clinical trials are approved to evaluate this form of stem cell protection in women who are receiving high-dosage chemotherapy for disseminated breast cancer or ovarian cancer, and for patients with brain tumors. It is unclear whether in other organ systems, the dose at which the toxic effect of chemotherapy occurs is significantly different from that which induces damage in the bone marrow. The relationship between these doses will determine whether this approach will be successful.

Table 1. Trials of gene therapy for cancer approved by the US Recombinant DNA Advisory Committee

Type of cancer	Tissue[a]	Gene encoding	No. of participating centers
Brain tumors	Tumor cells	HSVtk[b]	6
	Tumor cells	Antisense IGF-1	1
	HSCs	MDR-1	1
Breast cancer	Fibroblasts	IL-4	1
	HSCs	MDR-1	2
Colorectal cancer	Tumor cells	IL-2 or TNF	2
	Fibroblasts	IL-2 or IL-4	2
	Tumor cells	HLA-B7 and β2-microglobulin[b]	1
Malignant melanoma	T cells	TNF	1
	Tumor cells	TNF or IL-2	6
	Fibroblasts	IL-4	1
	Tumor cells	γ-Interferon[b]	1
	Tumor cells	B7 co-stimulatory molecule	1
	Tumor cells	HLA-B7 or HLA-B7 and β2-microglobulin[b]	2
Neuroblastoma	Tumor cells	IL-2	1
Non-small-cell lung cancer	Tumor cells	Antisense K-RAS[b]	1
		Wild-type p53[b]	1
Ovarian cancer	HSCs	MDR-1	2
	Tumor cells	HSVtk	1
Renal cell carcinoma	Tumor cells	IL-2, TNF or GM-CSF	4
	Fibroblasts	IL-4	1
Small-cell lung cancer	Tumor cells	IL-2	1
Solid tumors	Tumor cells	HLA-B7 and β2-microglobulin[b]	1

[a]HSCs, hematopoietic stem cells.
[b]By *in vivo* gene transfer.

Genetic alteration of cancer cells *in situ*

Liposome-mediated gene transfer

The first attempt to genetically modify tumors *in situ* involved the direct injection of liposomes containing an allogene that encoded HLA-B7 (Ref. 22), a foreign antigen that is transiently expressed on the cell surface and induces an immune reaction against the altered tumor cells. Studies in animals have shown that the anti-tumor immune response is significantly increased when some of the tumor cells express foreign antigens on their cell surface. Three patients have been treated using this approach, with increasing dosages of liposomes. One patient appears to have developed a substantial systemic immune response to both liposome-treated and non-treated melanoma lesions[23]. These encouraging results suggest that the transient expression of immunostimulatory genes in tumors might have potential as a treatment and as a vaccination against certain malignancies. A further trial has been approved, and will combine transfer of the genes for HLA-B7 and β2-microglobulin in an attempt to further enhance the anti-tumor immune response.

Retrovirus-mediated gene transfer

In vivo gene transfer using murine retroviral vectors has been applied to the treatment of brain tumors[24]. In this protocol, murine fibroblasts that are actively producing retroviral vectors, so-called retroviral vector producer cells or VPCs, are implanted directly into growing brain tumors (Fig. 2). The gene transferred by the retroviral vectors into the surrounding brain tumor cells is the HSVtk gene. Since retrovirus-mediated gene transfer is limited to mitotically active cells, the HSVtk gene should integrate only into the proliferating tumor cells, sparing normal brain tissue.

In a series of experiments using a rat model, this technique resulted in transfer of the gene for HSVtk into 30–60% of brain tumor cells and was capable of mediating complete tumor destruction in 80% of animals[19]; these effects were seen despite the fact that not all tumor cells contained the gene. Additional

animal studies have suggested that if at least 10% of cells in a tumor contain HSVtk, more than 50% of the cancers can be eliminated completely: adjacent tumor cells that do not contain HSVtk are destroyed through the bystander effect (see above). No associated systemic toxicity or evidence of systemic spread of the retroviral vectors was seen with this form of *in vivo* gene transfer[25].

While the results from these studies are encouraging, the rat model is not an ideal approximation of recurrent brain tumors in humans. While human tumors infiltrate the surrounding brain, the rat tumor model does so to a much lesser extent. Therefore, it is not clear whether this gene delivery system will suffice to eradicate the larger, infiltrative human tumors. Unfortunately, a more suitable model for preclinical study has yet to be found. Because of the very poor prognosis for patients with recurrent brain tumors, a clinical trial of this approach was initiated at the National Institutes of Health in December, 1992.

Each of the 12 patients enrolled in this trial received stereotactic injections of VPCs into multiple locations throughout the growing tumor. One week afterward, they began to receive twice daily intravenous infusions of GCV. These infusions were administered for 14 days. Preliminary observations of eight patients who had recurrent glioblastoma multiforme or metastatic tumors found that five showed some degree of anti-tumor response. As in the animal experiments, there was no evidence of toxicity related to the gene transfer protocol. These early findings suggest that the treatment is selectively toxic to the tumor cells and that enhancing delivery of the VPCs is the critical factor in optimizing this approach to brain tumor therapy. Three additional clinical trials using this technique have been approved. One will treat adults who have recurrent glioblastoma multiforme using a combination of surgical resection and simultaneous direct injection of HSVtk VPCs into the walls of the tumor bed. An Ommaya reservoir will be left in place, allowing the opportunity for repeated injections of VPCs into the tumor bed without the need for more surgery. These studies will be conducted at the Iowa Methodist Medical Center in Des Moines, the University of Iowa in Iowa City and the University of California, San Francisco. The other two trials, at the Children's Hospital of Los Angeles and St Jude's Children's Research Hospital in Memphis, Tennessee, will be conducted in children with recurrent astrocytoma, and will look at the efficacy of this therapy in tumors that have not been irradiated. Unlike adults with brain tumors, affected children less than six years old are not routinely irradiated, in an effort to minimize adverse effects on brain growth. Irradiation is known to induce significant necrosis within the tumor that may significantly inhibit gene transfer and the bystander effect. These studies will provide important information about the effect of the HSVtk gene in non-irradiated human tumors, allowing this approach to be optimized for both children and adults.

Two protocols for *in vivo* gene transfer for cancer therapy have been approved for clinical trials. Both entail the direct injection into tumor deposits of a supernatant containing a retroviral vector. One group will inject two different retroviral vectors into endobronchial non-small-cell lung cancers[3,26]. The vectors will carry genes that target the genetic mechanisms responsible for the malignancy: for example, if the lung tumors are deficient in expression of the p53 tumor suppressor gene, this gene will be used. In lung cancers that overexpress the K-*RAS* oncogene, a vector containing an

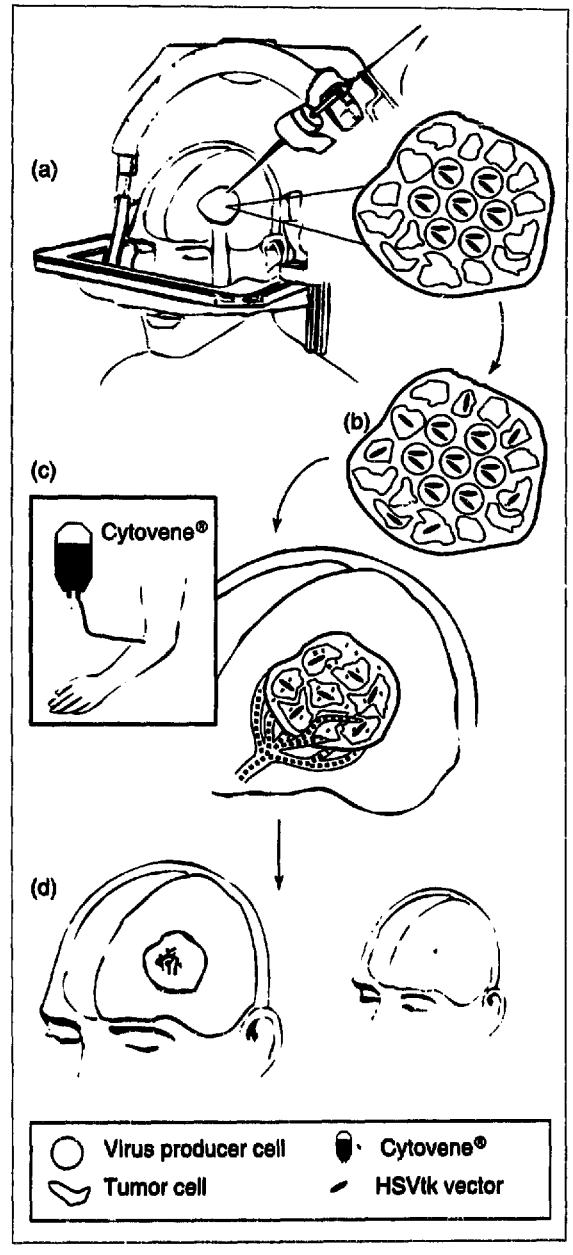

FIGURE 2. Diagram outlining the procedure for intratumoral injections of HSVtk vector producer cells (VPCs) into human brain tumors. A stereotactic apparatus is fixed to the skull. (a) Guided by nuclear magnetic resonance imaging, a needle is inserted into the tumor through a small hole in the skull. (b) Multiple deposits of VPCs are made within the body of the tumor. (c) One week later, after there has been time for the producer cells to transfer the gene to surrounding tumor cells, ganciclovir (Cytovene) is administered intravenously. (d) While the ganciclovir is administered intravenously, toxicity appears to be limited to tumor cells.

antisense K-*RAS* gene will be used. Experiments *in vitro* and in mice have demonstrated that the introduction of both such vectors can result in decreased tumorigenicity. Another group will inject a retroviral vector containing a vector that encodes γ-interferon directly into melanoma deposits.

Conclusion

A growing number of trials of gene therapy for cancer are approved or in progress. These involve the *ex vivo* genetic modification of T lymphocytes, tumor cells and stem cells; however, the trend is toward the use of *in vivo* gene transfer systems (i.e. liposomes and retroviral vectors). Increasing numbers of cytokine and sensitivity genes are being cloned, and should present new opportunities for the selective destruction of cancer cells. The primary factor hampering the widespread application of gene therapy to human disease is the lack of an efficient method for delivering genes *in situ*, and developing strategies to deliver genes to a sufficient number of tumor cells to induce complete tumor regression or restore genetic health remains a challenge. However, as these obstacles are overcome, we expect that gene therapy will become a standard part of the practice of oncology.

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BOOK REVIEWS

Sex, drugs and rolling circles

Bacterial Conjugation

edited by Don B. Clewell

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Conjugative transfer is one of the natural ways in which genetic exchange can occur between bacteria. In recent years, interest in this process has grown with the concern about the fate of recombinant DNA released into the environment in the form of genetically manipulated microorganisms. The need to define the conditions under which genetic exchange occurs in nature is providing a new dimension to the study of bacterial genetics. However, for gene transfer in the wild to be investigated fully, there must be close collaboration between bacterial geneticists and microbial ecologists based on a deeper understanding of each other's subjects. For those who (like many of the contributors to this volume) have been defining genes in *tra* operons and looking for their functions, these new environments may reveal the advantage conferred by hitherto 'cryptic' genes. By summarizing our current knowledge of the biochemical and molecular genetics of conjugative transfer, this book helps us focus on the questions that remain to be answered.

Bacterial Conjugation begins with 'A historical perspective' by Neil Willetts that is a history, a contemporary overview and an introduction to the rest of the book. In the same way as Willetts' interests started to shift towards the 'promiscuous' IncP plasmids, the book moves from consideration of the classic F system, to the P transfer system, to transfer systems in other bacteria, in particular, Gram-positive species.

The foundations are described by Karin Ippen-Ihler and Ron Skurray, who focus on the 35 kb transfer unit found in at least 120 different F-like plasmids. The single polycistronic operon contains about 40 genes involved in different aspects of transfer that are inextricably interwoven: synthesis and assembly of the pilus, the stability of aggregates, the mating signal, nicking of the origin, and unwinding and transport of DNA. This is in contrast with the 'promiscuous' IncP systems like RK2 and RP4 described by Don Guiney, in which there are two blocks of genes: one concerned mainly with mating pair formation (mpf), and the other with DNA metabolism before, during and after transfer. Relatives of both sets of these 'promiscuous' genes are turning up on many diverse plasmids; these include certain genes of the *vir* system of Ti plasmids, which transfer T-DNA from bacteria to plant cells, a topic discussed by Clarence Kado.

The first step in conjugative transfer is cell–cell contact. Although this is