Gene Therapy in the Treatment of Cancer
Progress and Prospects

Edited by
Brian E. Huber & Ian Magrath
The clinical applications of human gene therapy have been particularly fruitful in oncology, and in the last two decades there has been explosive growth in understanding of the genetic lesions leading to neoplasia. This volume in the series *Cancer: Clinical Science in Practice* reviews progress in the basic and clinical science of gene therapy in oncology, and looks forward to future developments. It considers what has worked and what has not in the fast-evolving field of gene therapy, drawing on laboratory studies and clinical trials, including the ground-breaking work of the contributors themselves. Elucidation of fundamental genetic differences between normal and tumor cells, and identification of tumor-specific DNA sequences are being exploited in novel therapies by a number of targeting strategies outlined here.

Up-to-date and authoritative, volumes in this series are intended for a wide audience of clinicians and researchers with an interest in the application of biomedical science to the understanding and management of cancer.
GENE THERAPY IN THE TREATMENT OF CANCER:
PROGRESS AND PROSPECTS
CANCER: CLINICAL SCIENCE IN PRACTICE

General Editor
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Department of Clinical Oncology
Royal Postgraduate Medical School
Hammersmith Hospital, London

A series of authoritative review volumes intended for a wide audience of clinicians and researchers with an interest in the application of biomedical science to the understanding and management of cancer.

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GENE THERAPY IN THE
TREATMENT OF CANCER
Progress and Prospects

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Preface

Cancer is now the biggest target disease for gene therapy protocols worldwide. Despite significant advances in the treatment of cancer in the last two decades, most tumors remain resistant to all current treatment modalities — surgery, radiotherapy, chemotherapy, and biotherapy. Local methods such as surgery and radiotherapy are often only effective in the absence of metastatic disease. Despite tremendous effort over the last fifty years, only a very small proportion of human cancers are cured by chemotherapy.

Gene-based therapy is a novel therapeutic approach to treating cancer which has been made possible only by recent and remarkable progress in our understanding of the molecular biology of cancer. Gene therapy can be described as the transfer to human cells and the expression of genetic material for a therapeutic purpose. Currently there are over two hundred gene therapy protocols active worldwide specifically aimed at single gene defects such as cystic fibrosis and a growing number of cancers. This book outlines the diverse approaches being attempted to develop effective future cancer therapies.

The last decade has seen dramatic advances in our understanding of the mechanisms involved in the control of cell growth and their deregulation in cancer. Certain classes of genes encode proteins that play distinct roles in the processing of signals from the outside of the cell to the nucleus. Any changes to the delicate system of control by these oncogenes or tumor suppressor genes may result in the formation of cancer. It is becoming increasingly clear that pre-existing genetic factors and environmental events combine to cause the series of molecular changes that are necessary for tumor formation. Elucidation of fundamental genetic differences between normal and tumor cells and identification of tumor-specific DNA sequences are being exploited in novel therapies by a number of targeting strategies outlined here.

*Karol Sikora*
Introduction: gene therapy approaches to cancer

BRIAN E. HUBER

It is interesting to reflect back on the literature written between 1980 and 1987 regarding the prospects and anticipated first applications of human gene therapy. What were predicted with a fair degree of accuracy were the specific gene transfer technologies that would first be utilized in the initial human gene therapy protocols: liposomal-mediated gene transfer and gene transfer via replication-defective retroviral and adenoviral vectors. The vast majority of human gene transfer protocols to date have used these transfer technologies. However, it was not originally anticipated that naked DNA would be efficacious in certain specific tissues, such as muscle, for gene transfer and expression.

What were neither anticipated nor predicted with any degree of accuracy were the target diseases for the initial human gene therapy protocols. These were originally predicted to be primarily monogenetic diseases, some of which are listed in Table 1.1. However, although the very first pioneering human gene therapy protocol, performed on September 14, 1990, was for correction of adenosine deaminase deficiency disease—a milestone event in the continual evolution of modern medicine—this prediction has proved to be incorrect. Instead, reflecting back over the last few years, it is clear that most of the initial applications have been in the oncology area, and it is interesting to examine the evolution of human gene therapy over this period.

Figure 1.1 illustrates the increase in the number of human gene therapy protocols approved by the Recombinant DNA Advisory Committee (RAC) that has taken place over the last few years in the United States—a total of 132 protocols by November 1996. It is apparent that there was an almost yearly exponential increase in the number of protocols submitted and approved up to 1995, but that the rate of submissions and approvals significantly slowed after that. This is due to a decline in the number of protocols submitted for approval rather than to a decrease in the success rate in the approval process.
Table 1.1. *Monogenetic diseases anticipated to be the initial target diseases for the application of human gene therapy*

<table>
<thead>
<tr>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine deaminase deficiency</td>
</tr>
<tr>
<td>Purine nucleoside phosphorylase deficiency</td>
</tr>
<tr>
<td>Hypoxanthine–guanine phosphoribosyltransferase deficiency</td>
</tr>
<tr>
<td>Factor VIII deficiency</td>
</tr>
<tr>
<td>Factor IX deficiency</td>
</tr>
<tr>
<td>Sickle cell anemia</td>
</tr>
<tr>
<td>β-Thalassemia</td>
</tr>
<tr>
<td>Ornithine transcarbamylase deficiency</td>
</tr>
<tr>
<td>Argininosuccinate synthetase deficiency</td>
</tr>
<tr>
<td>α1-Antitrypsine deficiency</td>
</tr>
<tr>
<td>Glucocerebrosidase deficiency</td>
</tr>
</tbody>
</table>

Fig. 1.1. Approved gene therapy protocols in the USA. The number of approvals was determined based on summation of Review level 1 through Review level 4 approvals. Approvals are classified as follows. 

*Review level 1:* full RAC review + NIH Director approval + FDA Investigational New Drug (IND) approval. 

*Review level 2:* accelerated RAC review + NIH Office of Recombinant DNA activities (ORDA) approval + FDA IND approval. 

*Review level 3:* sole FDA review (simultaneous submission to NIH (ORDA) for the purpose of data monitoring and adverse event reporting). 

*Review level 4:* sole FDA review (submission to NIH (ORDA) not required). (For reference see: dwknorr/protocol/protocol.new/October9,1996 on the HHS/NIH/RAC home page.)
Gene therapy approaches to cancer

Table 1.2. Gene-transfer techniques and methodology for approved protocols in the United States

<table>
<thead>
<tr>
<th>Gene-transfer technique</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex-vivo gene transfer</td>
<td>80</td>
</tr>
<tr>
<td>In-vivo gene transfer</td>
<td>52</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene-transfer method</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Retro viral vectors</td>
<td>87</td>
</tr>
<tr>
<td>Adenoviral vectors</td>
<td>16</td>
</tr>
<tr>
<td>Adeno-associated viral vectors</td>
<td>2</td>
</tr>
<tr>
<td>Pox vector</td>
<td>1</td>
</tr>
<tr>
<td>Cationic liposomes</td>
<td>20</td>
</tr>
<tr>
<td>Plasmid DNA</td>
<td>4</td>
</tr>
<tr>
<td>Particle mediated</td>
<td>1</td>
</tr>
<tr>
<td>Vaccinia virus vector</td>
<td>1</td>
</tr>
</tbody>
</table>

Itself. It is anticipated that the number of submissions and approvals during the remainder of this decade will be somewhat lower than that in the first half of the decade. Approximately 40 human gene therapy protocols have been approved in Europe during the same period.

Due both to safety concerns and to the limitations of gene-transfer technology, the first human gene therapy protocols predominantly involved ex-vivo gene-transfer techniques (Table 1.2). However, as confidence in safety has increased, so has the number of approvals for protocols involving in-vivo gene transfer. As predicted, the gene-transfer methodology that has predominated in the initial protocols has involved the use of replication-deficient viral vectors and cationic liposomes, despite the many significant limitations associated with retroviral vectors (Table 1.1).

In the United States, almost 1000 patients have participated in in-vivo or ex-vivo gene therapy protocols at the time of writing. The toxicities that have been reported to date have been related either to physiologic responses to expression of the transgenes or to acute toxicities associated with the delivery vectors themselves. There have been no reports of viral vector recombination or permanent toxicities due to transgene integration.

Table 1.3 illustrates the therapeutic areas in which gene therapy protocols have been approved in the United States and Europe. The vast majority of the 132 approved protocols in the US has been for oncology indications (approximately 73%), as have approximately 80% of those in Europe.

Why has the oncology field dominated the initial clinical development of human gene therapy? Table 1.4 lists some of the reasons, the main one
Table 1.3. Therapeutic areas for approved gene therapy protocols

<table>
<thead>
<tr>
<th>Current roster of protocols approved by RAC (1996)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total gene-marking protocols</strong></td>
<td>29</td>
</tr>
<tr>
<td><strong>Total gene therapy protocols</strong></td>
<td>132</td>
</tr>
<tr>
<td>Cancer</td>
<td>96</td>
</tr>
<tr>
<td>Genetic deficiency diseases</td>
<td>21</td>
</tr>
<tr>
<td>AIDS</td>
<td>12</td>
</tr>
<tr>
<td>Vascular diseases</td>
<td>2</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Current roster of protocols approved in Europe (1996)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total gene therapy protocols</strong></td>
<td>40</td>
</tr>
<tr>
<td>Cancer</td>
<td>32</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>5</td>
</tr>
<tr>
<td>Adenosine deaminase deficiency (ADA)</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 1.4. Gene therapy targeted to cancer

1. Unmet medical need  
2. Life-threatening disease  
3. Very large, dispersed patient population  
4. Commercially viable  
5. Molecular mechanisms in pathophysiology  
6. Multiple diseases  
   Multiple mechanisms

concerning the current state of efficacy, or lack thereof, of conventional cancer treatments. There is a tremendous unmet medical need for new, innovative and unconventional approaches to cancer treatment, any which are developed being embraced by patients, clinicians and regulatory authorities. In most situations, clinicians are dealing with a life-threatening disease that requires the making of bold decisions about experimental therapeutic approaches. The cost (safety/toxicity)/benefit (potential cure or palliation) ratio is shifted toward experimental therapies since conventional therapies usually fail in this life-threatening situation.

The cancer patient population is large and dispersed throughout the world, permitting experimental clinical trials in the developed world. This fact, coupled with the significant unmet medical need in the developed world, has attracted both small biotechnology companies and large pharmaceutical companies to focus on this area. In addition, there has been a tremendous increase in understanding of the molecular etiology of neoplastic disease over
the last 15 years. Many causative and contributing genetic lesions have been identified for a variety of neoplasms, including the expression of oncogenes, the loss of function of tumor suppressor genes, and acquisition of resistance to chemotherapy through altered gene expression. This has given unique insights into the molecular pathophysiology of the disease, which has fostered extremely rational gene therapy approaches. Cancer has also dominated the initial clinical development of gene therapy due to its nature. Neoplastic disease is not one disease resulting from the same genetic lesions producing similar pathologic phenotypes. Rather, it may be considered to be 150 to 200 different neoplastic or paraneoplastic diseases, with different genetic lesions resulting in different phenotypic characteristics. This lends itself to a variety of different approaches and techniques depending on the particular tumor type. Hence, multiple approaches to multiple tumor types can be investigated simultaneously. Chapter 2 of this book describes the types of genetic lesions that are thought to play a causative role in cancer formation and, based on that knowledge, the development of transformation-specific therapy. A critical element in successful transformation-specific therapy is the delivery system, whereby a therapeutic agent is introduced into the target tissue. A number of delivery systems (vectors) are described in Chapter 3.

As stated above, gene therapy can be experimentally and clinically utilized in a multitude of novel and diverse approaches to the treatment of cancer. Figure 1.2 illustrates some of these approaches, which can be separated into two broad categories based on the target cell of the genetic manipulation: either the tumor cells themselves or normal, noncancerous cells.
The tumor cell as the gene-transfer target cell

There has been proposed a multitude of gene therapy approaches that target the tumor cell as the cell that is genetically manipulated. One approach is to transfer into the tumor cells, genes which encode direct-acting toxins, such as Pseudomonas B toxin, expression of which will theoretically kill the tumor cell. The major concern with this approach is the lack of control and safety associated with overexpression or inappropriate expression of the toxin gene. For this reason, transfer and expression of genes encoding direct-acting and potent toxins have not been explored clinically. Another way to generate a cytotoxic molecule is to transfer a gene that encodes a prodrug-activation enzyme. This is a two-phase approach, which may have greater safety and control associated with it. The prodrug-activating enzyme will have no intrinsic toxicity associated with its expression. However, the presence of the enzyme results in conversion of nontoxic prodrugs into cytotoxic agents within the tumor cells but not in normal cells which lack the enzyme. Application of this approach is covered in Chapter 4.

Another approach that targets the tumor mass for gene transfer is the delivery of genes whose expression is directed specifically at interfering with the genetic aberrations that cause the transformation process. For example, the tumor mass can be targeted with genes that encode dominant negative proteins, antisense molecules, or ribozymes that inhibit specific oncogenes. Alternatively, the tumor mass can be targeted with genes that encode functional tumor suppressor genes, such as p53, to restore the function lost through mutational events. The approach of targeting tumor cells with genes that either inactivate oncogenes or restore the function of tumor suppressor genes is covered in Chapter 5.

Another approach to targeting the tumor mass is the delivery of genes expressing immunostimulators. The basis of this approach is that normally an intact, functioning immune system can effectively survey, identify, and eliminate neoplastic cells. However, in relatively rare instances, the immune system cannot recognize or is unable to eliminate these transformed cells, which results in cancer. Neoplastic cells have certain properties that may hinder the immune system from recognizing and eliminating them. For example, certain tumor cells have a decrease in expression of the major histocompatibility complex, which is essential for displaying and presenting tumor-associated antigens to cells of the immune system. In addition, tumor cells can express specific factors that may block or inhibit the cascade of events necessary for a robust cytotoxic T lymphocyte response (CTL), which is required for immunologic elimination of the tumor mass.
There have been a number of related approaches attempting to transfer into the tumor mass genes which encode factors that may increase the recognition of the tumor by the immune system or the robustness of the immune system's overall response. These approaches are described in Chapters 6 and 7.

**The normal cell as the gene-transfer target cell**

The tumor mass does not have to be the target for gene transfer. Rather, in some approaches, normal cells may be the target for genetic manipulation. One example of this involves the delivery of drug-resistance genes to normal cells that are the site of dose-limiting toxicity for specific conventional chemotherapy. Particularly relevant targets may be bone marrow, lung, cardiac tissue, and liver. Genes that encode either the multidrug resistance P-glycoprotein or very specific drug detoxification enzymes may be relevant. These approaches are discussed in Chapter 4.

Another approach targeting normal cells is the genetic manipulation of cells of the immune system, such as CTL, tumor-infiltrating lymphocytes (TIL), or hematopoietic stem cells. The very first gene-transfer experiments were performed to assess the viability, targeting, and half-life of TIL cells. Chapter 7 deals with the use of cytokine genes, and with genes encoding the

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**Fig. 1.3. Advancement of gene therapy into other therapeutic areas.** CHF, chronic heart failure; MI, myocardial infarction; RA, rheumatoid arthritis; DMD, Duchenne’s muscular dystrophy; ALS, amyotrophic lateral sclerosis.