Cancer Gene Therapy
Contemporary Cancer Research

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The field of cancer therapy is beginning to reap the benefits of our increasing understanding of the molecular basis of cancer. In contrast to conventional surgical interventions or cytotoxic chemotherapy and radiation therapy, a new generation of targeted cancer therapeutics is being specifically directed toward molecular pathways that underlie the malignant phenotype. In this regard, the management of patients with Philadelphia chromosome-positive chronic myeloid leukemia has been profoundly changed by Gleevec® (Novartis), a small molecule that specifically inhibits the Bcr-abl tyrosine kinase that is central to the pathogenesis of this disease. Particularly noteworthy is the rapid translation of this molecular targeted agent from the laboratory to clinical trials and thence to regulatory approval. Other novel targeted therapeutics that are currently approved by the FDA for treatment of patients with cancer include Rituxan® (Genentech), a humanized monoclonal antibody that binds to the CD20 antigen present on B-cell lymphomas and is currently approved for the treatment of patients with relapsed or refractory low-grade or follicular CD20-positive B-cell non-Hodgkin’s lymphoma. The humanized anti-HER-2/neu monoclonal antibody Herceptin® (Genentech) is approved for use in patients with metastatic breast cancer that demonstrates overexpression of HER-2/neu. These therapies target specific tumor cell receptors or signaling events that are critical to tumor progression while reducing toxicity to normal cells.

Within this context of targeted molecular interventions with the potential to achieve a much higher level of specificity of action than afforded by conventional drug therapeutics, we can view cancer gene therapy as the transfer of genetic material to the cells of an individual with the goal of eradicating cancer cells. This can be accomplished directly by transferring genetic material into the cancer cells themselves to bring about their destruction, or indirectly, either by stimulating the immune system to recognize and eliminate the cancer cells or by targeting the nonmalignant stromal cells that support the growth and metastasis of cancer cells. Each of these approaches exploits our expanding knowledge of the genetic basis of cancer, thereby allowing rationally targeted interventions at the molecular level. These cancer gene therapy strategies are discussed in the first contributions to Cancer Gene Therapy.

Any gene therapy strategy is dependent on the safe and efficient transfer of the therapeutic gene selectively to the target cell. Indeed, one of the main lessons learned from the success of clinical gene therapy trials for monogenic inherited disorders, such as severe combined immunodeficiency and hemophilia, is that therapeutic advances are predicated on improvements in the design of gene delivery vehicles, or vectors. Hence, two chapters focus on the development of vectors for cancer gene therapy, both viral and nonviral, emphasizing how the properties of a given vector favor its application in a particular therapeutic approach.

The recognition of the limitations of replication-defective vectors, which are incapable of delivering therapeutic genes to more than a small proportion of cancer...
cells in a 3D tumor mass, has led to the development of a new class of anticancer therapeutic agents, oncolytic replication-competent viruses, which are also described. The safety of oncolytic viruses derives from the restriction of their replication to tumor cells, while sparing normal cells. A number of naturally occurring viruses possess intrinsic selectivity for replication in tumor cells, while advances in the molecular characterization of viruses and cancer cells have enabled other lytic viruses to be genetically engineered to selectively replicate in, and thus destroy, tumor cells.

It is apparent that therapeutic gene delivery designed to eradicate cancer cells in the clinical setting would benefit from noninvasive techniques to monitor the extent of gene transfer and disease regression during the course of treatment. Hence, a chapter describes imaging of cancer gene therapy. After a chapter discussing the lessons learned to date from clinical trials for cancer gene therapy, the final chapter reviews the regulatory guidelines with which future trials should comply.

Thus, the contributions to this book demonstrate that cancer gene therapy strategies are founded on an understanding of the molecular basis of disease that, together with improvements to the safety and efficacy of vectors, will provide a rational basis for their application in the clinic setting. It is anticipated that gene therapy will ultimately take its place in the clinic alongside other targeted molecular inventions for cancer.

David T. Curiel, MD, PhD
Joanne T. Douglas, PhD
Contents

Preface ................................................................................................................... v
Contributors ....................................................................................................... ix

1 Cancer Gene Therapy: Historical Perspective
   Malcolm K. Brenner ............................................................................................ 1

2 The Genetic Basis of Cancer
   Akseli Hemminki and Kari Hemminki ........................................................... 9

3 Tumor Suppressor Gene Replacement for Cancer
   Jack A. Roth and Susan F. Grammer .............................................................. 19

4 Antisense Technology
   Ruiwen Zhang and Hui Wang ........................................................................ 35

5 Cancer Therapeutic Applications of Ribozymes and RNAi
   Lisa Scherer and John J. Rossi ....................................................................... 51

6 Fusogeneic Membrane Glycoproteins for Cancer Gene Therapy:
   A Better Class of Killer
   Andrew Bateman, Vy Phan, Alan Melcher, Emmanouela Linardakis,
   Kevin Harrington, and Richard Vile ............................................................ 65

7 Suicide Gene Therapy
   Caroline J. Springer and Ion Niculescu-Duvaz ........................................... 81

8 Molecular Chemotherapy Approaches
   Daniel H. Palmer and David J. Kerr ............................................................. 109

9 Genetic Immunotherapy Approaches
   Denise R. Shaw and Albert F. LoBuglio ....................................................... 129

10 Immunotherapy of Cancer by Dendritic Cell-Targeted
    Gene Transfer
    Tanja D. de Gruijl, Herbert M. Pinedo, and Rik J. Schepers .................... 143

11 Dendritic Cells
   Weiping Zou, Shuang Wei, and Tyler J. Curiel ......................................... 173

12 Polynucleotide Immunization for Cancer Therapy
   Theresa V. Strong ............................................................................................ 185

13 Development of Oncolytic Replication-Competent
    Herpes Simplex Virus Vectors: The G207 Paradigm
    Tomoki Todo and Samuel D. Rabkin ......................................................... 199
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Development of Oncolytic Adenoviruses: From the Lab Bench to the Bedside</td>
<td>John A. Howe, Robert Ralston, and Murali Ramachandra</td>
<td>211</td>
</tr>
<tr>
<td>15</td>
<td>Conditionally Replicating Adenoviruses for Cancer Treatment</td>
<td>Ramon Alemany</td>
<td>235</td>
</tr>
<tr>
<td>16</td>
<td>Reovirus as an Oncolytic Agent</td>
<td>Megan K. Patrick, Kara L. Norman, and Patrick W. K. Lee</td>
<td>249</td>
</tr>
<tr>
<td>17</td>
<td>Antiangiogenic Gene Therapy of Cancer</td>
<td>Steve Gyory, Jack Gauldie, A. Keith Stewart, and Xiao-Yan Wen</td>
<td>261</td>
</tr>
<tr>
<td>18</td>
<td>Proapoptotic Strategy in Cancer Gene Therapy</td>
<td>David H. Holman, Marc L. Hyer, Ahmed El-Zawahry, Gina M. Keller, and James S. Norris</td>
<td>273</td>
</tr>
<tr>
<td>19</td>
<td>Antimetastasis</td>
<td>Duen-Hwa Yan, Kung-Ming Rau, and Mien-Chie Hung</td>
<td>287</td>
</tr>
<tr>
<td>20</td>
<td>Antimetastatic Gene Therapy: Prostate Cancer Theory to Therapy</td>
<td>Thomas A. Gardner, Juan Antonio Jiménez, Leland W. K. Chung, and Chinghai Kao</td>
<td>299</td>
</tr>
<tr>
<td>21</td>
<td>Drug Resistance Gene Transfer as an Antitumor Strategy</td>
<td>Colin L. Sweeney and R. Scott McIvor</td>
<td>321</td>
</tr>
<tr>
<td>22</td>
<td>Chemosensitization</td>
<td>Per Eystein Lønning</td>
<td>335</td>
</tr>
<tr>
<td>23</td>
<td>Radiosensitization by Gene Therapy</td>
<td>Steven J. Chmura, Michael Garofalo, and Ralph R. Weichselbaum</td>
<td>349</td>
</tr>
<tr>
<td>24</td>
<td>Nonviral Vector Systems for Cancer Gene Therapy</td>
<td>Greg F. Walker and Ernst Wagner</td>
<td>367</td>
</tr>
<tr>
<td>25</td>
<td>Viral Vectors for Cancer Gene Therapy</td>
<td>Joanne T. Douglas and David T. Curiel</td>
<td>379</td>
</tr>
<tr>
<td>26</td>
<td>Bacterial Systems for Tumor-Specific Gene Therapy</td>
<td>J. Martin Brown, Shie-Chau Liu, Jan Theys, and Philippe Lambin</td>
<td>393</td>
</tr>
<tr>
<td>27</td>
<td>Molecular Imaging of Cancer Gene Therapy</td>
<td>Harvey R. Herschman</td>
<td>405</td>
</tr>
<tr>
<td>28</td>
<td>Cancer-Related Gene Therapy Clinical Trials</td>
<td>Robert J. Korst and Ronald G. Crystal</td>
<td>427</td>
</tr>
<tr>
<td>29</td>
<td>Regulatory Aspects in the Development of Gene Therapies</td>
<td>Rosemarie Aurigemma, Joseph E. Tomaszewski, Sheryl Ruppel, Stephen Creekmore, and Edward A. Sausville</td>
<td>441</td>
</tr>
<tr>
<td></td>
<td>Index</td>
<td></td>
<td>473</td>
</tr>
</tbody>
</table>
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1. INTRODUCTION

Although it is always tempting to skip the history of a field, this is particularly unwise for a discipline as young as cancer gene therapy. Indeed, it is the history of the last few years that is largely dictating the research directions that will likely be both profitable and permitted in the future. This brief introductory chapter outlines the early days of cancer gene therapy—the successes and the setbacks—and suggests how the remaining challenges may be faced.

2. BACKGROUND

When the possibility of human gene therapy was first mooted (and illicitly attempted) in the 1970s, it was assumed that inherited single-gene disorders would be the target of the approach (1). The obvious elegance of repairing or replacing the root cause of a disease had and retains an enormous appeal to researchers, patients, and public alike. Unfortunately, it soon became obvious that the tools available were simply not up to the job.

Effective gene therapy of genetic disorders requires a vector that can efficiently transduce the desired cell type, in a targeted manner, preferably in vivo. Moreover, the gene product usually would need to be produced in substantial quantities for a long time, often in a regulated manner. Above all, the process and consequences of gene transfer should be safe.

Sadly, the gene transfer vectors available for clinical use, then as now, possess none of these desirable properties. They are diffusely targeted, inefficient at making transgene products, and difficult to regulate. As the gene therapy community has painfully learned, they are not even all that safe for they have the potential to produce immediate (adenovectors) or delayed (retroviral vectors) severe or lethal adverse events.

Many of these limitations were obvious to early workers in the field and led them to concentrate on disorders in which low-frequency transduction of stem cells would lead to a selective growth advantage and repopulation of the host and in which unregulated expression of even small quantities of the transgenic material would be of therapeutic benefit. The group of disorders that most clearly met these criteria was the inherited severe combined immunodeficiency syndromes. But, although these remain of great interest as a possible “proof of principle” for establishing the value of this new technology, they are exceedingly rare, and there was a strong feeling that the technology should be applied to more common conditions. Although these included more widespread inherited genetic disorders such as cystic fibrosis, the prospect of treating cancer with gene therapy grew increasingly justifiable in the 1980s.
Several factors led to this change in perception:

1. The realization that cancer too was a genetic disorder, albeit one that was acquired and multigenic.
2. The existence of a profoundly unmet therapeutic need because conventional treatments were toxic, ineffective, or, most commonly, both.
3. The high incidence of the disorder, making it an appealing area for research and development support from industry.
4. The availability of an established community of researchers used to clinical trial development and monitoring.
5. A general agreement that the risk-to-benefit ratio was likely to be acceptable to patients, regulatory agencies, and the general public given the immediate life-threatening nature of most of these disorders and the paucity of safe alternatives.

As a consequence, the very first gene transfer protocol approved was in cancer patients (2), and the dominance of this area in gene therapy has persisted to this day, with more than 80% of gene transfer subjects falling into this disease category (3). However, it must also be pointed out that cancer gene therapy has a number of drawbacks, and as evident from the chapters in this book, these have led to an underappreciation of its achievements to date and an underestimation of its likely future importance.

Cancer, even of a single cell type in a single organ, is a molecularly heterogeneous disease. Although there is extensive categorization of the molecular basis of hematologic malignancies, this process is just beginning in solid tumors. Hence, no single therapeutic approach is likely to be effective in more than a minority of patients with a given broad histologic category of disease, so that a low success rate even for effective gene therapies is currently to be expected.

Even when effective, single therapies are rarely curative. Cancers evolve and escape from all therapeutic agents. Instead, combined modalities must be used in which resistance to one does not predicate resistance to the others. Although gene therapies have the great advantage of non-cross-resistance to most conventional treatments, combination clinical studies that convincingly show the benefits of adding gene therapy are slow and expensive to perform. End points are often either vague (e.g., increased tumor “response” rates) or delayed for months or years (e.g., prolongation of survival).

Most cancer patients have received multiple other toxic therapies. Their responsiveness to gene therapies designed, for example, to boost immunity may be deficient. Similarly, when toxic events occur, it may be difficult to discern whether they are attributable to the disease, to prior or concomitant therapy, or to the investigational gene drug.

It is these difficulties that lead to the failure or abandonment of many of the historically early studies in gene therapy. However, as is apparent from succeeding chapters, the ability to compensate for some of these problems now underlies many of the successes seen, together with improvements in the transgenes used and the ways in which they are delivered.

The earliest studies using gene transfer to treat cancer were all designed to compensate for the remarkable inefficiency of the available adenoviral, retroviral, and plasmid vectors. Gene-marking studies were the first out of the gate (2,4–6). These were implemented not with any direct therapeutic intent, but rather to use the transferred marker gene as a means of tracking normal or malignant cells and help validate and improve interventions already in use. The principle of gene marking is the transfer and integration of a unique deoxyribonucleic acid (DNA) sequence (e.g., a nonhuman gene) into the DNA of a host cell (e.g., T cell, hematopoietic stem cell), allowing the gene or the gene product to be detected easily, thereby serving as a marker for these labeled cells (5).

In 1988, Rosenberg proposed a protocol to genetically mark lymphocytes derived from tumor patients (tumor-infiltrating lymphocytes, TILs). These lymphocytes appeared to have antitumor activity and could be expanded ex vivo and returned to patients with tumors. However, it was unclear whether the infused cells were able to traffic to tumor sites and produce antitumor activity. Rosenberg’s group planned to expand TIL cells ex vivo, transduce them with a Moloney retroviral vector encoding the neomycin phosphotransferase gene (NEO or, as it was then written, NeoR) and return them to the patient. Any transduced cells infused and all their progeny could then be detected by subsequent
Historical Perspective

analysis of blood, tissues, and tumor sites. This would in turn reveal whether these cells persisted in vivo, expanded, and trafficked to the tumor sites. If they did relocalize, it would also be possible to see whether their presence was associated with tumor regression.

Although simple in concept, this protocol, like all early gene therapy protocols, was exposed to intense, repetitious, and prolonged public and private scrutiny. At that time, all gene transfer protocols were examined locally by institutional review boards and biosafety committees before passing on to the Human Gene Therapy Subcommittee of the Recombinant DNA Advisory Committee (RAC) of the National Institutes of Health for public review.

Usually, after several reviews, each resulting in requested changes and additional data, protocols were forwarded to the RAC for public re-review. The RAC itself usually had requests and requirements before passing the protocol on to the director of the National Institutes of Health for approval. Next would come the submission to the Food and Drug Administration, which would generally also require public review by their own advisory panel before applying their standard internal review for investigational new drug applications, using staffers specializing in pharmacology/toxicology, product manufacturing, and clinical affairs. Although the review process has since simplified, it remains more complex than for other anticancer agents, even though many of these (such as alkylating agents) may have the potential to cause equivalent oncogenic, germline (transgenerational), and environmental harm.

Finally, patient accrual began on the first protocol, and the results were published in 1990 (2). The study successfully showed the feasibility of human gene transfer in a clinical setting, and that marker studies could be safe. However, the low level of marking achieved and the limited expansion observed in vivo made it difficult to demonstrate clearly selective tumor homing or to correlate the presence of TILs with tumor response.

Later marking studies were more informative (4,5,7–9). Autologous hematopoietic stem cell (HSC) transplantation had shown promise as an effective treatment for leukemias and lymphomas and some solid tumors (10–13), but disease recurrence was the major cause of treatment failure. When the tumor originated from or involved the marrow, relapse could originate from malignant cells persisting in the patient, in the rescuing HSCs, or in both. Concern that the HSCs may contain residual malignant cells led to extensive evaluation of techniques for purging these cells (14,15). However, it had been hard to show that this reduced the risk of relapse, and the purging techniques usually slowed engraftment because of damage to normal progenitor cells.

In three early 1990 studies, of acute myeloblastic leukemia, neuroblastoma, and chronic myeloid leukemia, autologous stem cells were marked, after they were harvested and prior to reinfusion, using murine retroviral vectors encoding the neomycin resistance gene. At relapse, the investigators looked to see whether the marker gene was present in the malignant cells (7,16–18). In all three diseases, it proved possible to detect both the transferred marker and a tumor-specific marker in the same cells at the time of relapse, which provided unequivocal evidence that the residual malignant cells in the marrow were a source of malignant recurrence (5). These studies also provided information on the transfer of marker genes to normal hematopoietic cells and showed that marrow autografts contribute to long-term hematopoietic reconstitution after transplant (19). With more than 10 years follow-up, they have also so far proved safe.

Unfortunately, the poor transducibility of many other tumors by the available retroviral vectors prevented this technique from wide use in other tumor settings (8,20). However, the approach was subsequently used in a series of protocols that demonstrated the safety, persistence, and effectiveness of viral and tumor-specific T lymphocytes (9,21,22), which had been the intention of the first TIL protocol.

3. EARLY THERAPEUTIC STUDIES: THE NEED FOR AMPLIFICATION

Although informative, marker studies could never by themselves be considered gene therapy. But, the poor efficiency and lack of targeting of available vectors seemed to rule out using the transfer of directly therapeutic genes because too small a proportion of tumor cells would be affected for benefit
to be seen. Instead, investigators tried to combine gene therapy with two modalities that could amplify the effects of gene transfer.

3.1. Prodrug Metabolizing Enzymes

In the first of these modalities, a gene was transferred that acted as an enzyme capable of splitting an inactive small molecule into a lethal cytotoxin. By intercellular (gap junction) and local extracellular spread, these newly formed toxic small molecules would be expected to diffuse locally and damage even tumor cells that had not been transduced, in a so-called bystander effect. Even before development of gene therapy, more than a dozen prodrug-metabolizing enzyme (PDME) systems had been described.

Of these, the herpes simplex virus thymidine kinase system was considered most appropriate for translation into a gene therapeutic. This gene phosphorylates small-molecule drugs such as acyclovir or ganciclovir to nucleoside analogs toxic to dividing cells. For the PDME approach to be tumor selective, either the vector or the prodrug product must be targeted to the malignant cell. The first clinical studies to test this novel strategy aimed for both types of selectivity by introducing a thymidine kinase gene into a tumor cell using a retroviral vector (23,24). On exposure to ganciclovir, the transduced cells phosphorylate the drug. If the cell then divides, the product is incorporated into DNA with lethal consequences; nondividing cells are unaffected.

To maximize the therapeutic index of the approach, initial study of Tk gene transfer was made in patients with brain tumors; in this context, there is a particularly clear distinction between tumor cells (which divide and are destined to be killed by the ganciclovir) and normal neurons (which do not divide and should escape unharmed). Retroviral vectors offered additional tumor specificity in this system because they function only in dividing cells and therefore do not transduce normal neurons. In initial phase I and II studies introducing vector or vector producer cells, there appeared to be tumor regression and patient benefit. Unfortunately, this benefit was not seen in a larger scale, definitive, and extremely expensive phase III study, leading to abandonment of the approach by large pharma.

Other investigators have continued to use this system, delivering PDME genes with adenoviral vectors to sites such as the prostate, peritoneum, and lung. This loses the specificity for dividing cells at the level of infection (although retaining it at the level of drug activity), but the higher transducing efficiency of adenovectors is felt to be essential for efficacy. This approach remains in investigation for many tumors and is looking particularly promising when combined with the second amplification method, which uses the immune system.

3.2. Generation of Tumor Vaccines

Although current vectors lack the capacity to be targeted precisely and are poorly distributed, specificity and excellent biodistribution are almost the defining characteristics of the immune system. Tumor immunotherapy has had a long and somewhat tarnished history, but by the 1980s it was evident that many human tumor cells really did express tumor-associated or tumor-specific antigens, and that even when internal, these antigens could be processed to peptides and appear on the tumor cell surface. Most important perhaps, data from allogeneic stem cell transplantation and from newly developed monoclonal antibodies unequivocally demonstrated that an effectively manipulated immune system was indeed capable of eradicating even extensive malignancies.

Hence, there was great interest in using gene transfer to augment an otherwise ineffective antitumor immune response, which in turn would be sufficiently targeted and well distributed to destroy tumor sites regardless of their location in the patient. Murine studies showed that transduction of tumor cells with cytokine genes (25), allogeneic major histocompatibility complex molecules (26), or costimulatory molecules such as B7.1 or CD40 ligand (27,28) augmented immunogenicity. Injection of neoplastic cells in doses that would normally establish a tumor instead recruited immune system effector cells. In some models, established, nontransduced, parental malignant cells were also eradicated.

The first studies of this approach began in 1992 using autologous melanoma cells or allogeneic melanoma cell lines (26,29) expressing transgenic granulocyte-macrophage colony-stimulating fac-
tor or HLA-B7 (in HLA-B7-negative individuals). There were certainly clinical responses to these vaccines, but it has proved difficult to scale-up the projects and make them commercially viable. Nonetheless, the results were sufficiently encouraging that there have been well over 200 different tumor vaccine studies using a range of immunostimulatory molecules.

Results in diseases such as melanoma, renal cell carcinoma, and neuroblastoma showed that tumor cells transduced with the interleukin-2, granulocyte-macrophage colony-stimulating factor, or HLA-B7 gene can be given safely and produce immunomodulatory effects, including peripheral blood eosinophilia, a rise in natural killer and activated killer (AK) cell number and activity, and an increase in tumor-specific cytotoxic T-lymphocyte precursor frequency (29–34). There have been reports of clinical responses in distal tumor sites, although other metastases have continued to grow (perhaps because their phenotypic heterogeneity allowed them to evade the immune system). It is likely that the best application of this approach will be in the adjuvant setting to prevent relapse in patients with presumed minimal residual disease and/or in combination with cytoreductive, but not immunosuppressive, levels of chemotherapy. Unfortunately, the design, interpretation, and expense of such complex and lengthy studies represent major obstacles to drug development.

4. SELECTION OF TRANSDUCED CELLS

The application of gene therapy outside malignancy was predicated on the transduced cells having a survival advantage; hence, the earliest application to patients with inherited immunodeficiency states such as adenosine deaminase (ADA) deficiency (35). However, it was evident early on that one way of broadening these applications would be to build in a selectable marker along with the therapeutic gene of interest and then subsequently administer the selecting agent. Many cytotoxic drug resistance genes were discovered in the 1980s, making this approach a theoretical possibility.

Cancer patients were chosen to investigate the feasibility and toxicity of the selectable approach because one of the tenets of cancer therapy is that “more is better.” There is certainly good evidence for some tumors that patients given more intense therapy for longer do better than those receiving less-intense or shorter treatments. Drug dosing is limited by toxicity to normal tissues, particularly marrow progenitor cells. Hence, if it were possible to transfer genes that rendered normal cells resistant to one or more cytotoxic drugs, it might enable them to resist the toxic consequences of the relevant agent. The multidrug resistance gene 1 (MDR-1) was the most widely considered gene for human therapy. Its product, P-glycoprotein, functions as an ATP binding cassette (ABC) transporter and is a potent drug efflux pump that confers resistance to many chemotherapeutic agents (36).

After extensive preclinical testing, a clinical protocol to test the approach began in 1994 (37). Patients with breast cancer were given marrow stem cells transduced with the MDR-1 gene via retroviral-mediated gene transfer and their response to chemotherapy with taxol as the selecting agent was measured. The investigators hoped to see a progressive rise in the numbers of MDR-1 transgenic cells after taxol therapy and ultimately the ability to sustain normal hemopoiesis even after extensive treatment. Unfortunately, the low efficiency of stem cell transduction and poor gene expression observed in the earliest clinical protocols resulted in no selection of gene-modified cells and hence no in vivo protection (37,38).

The approach has been pursued with different drug resistance genes in several different malignancies. Although transfer of such genes may find a place in treatment of malignant disease, it seems increasingly unlikely that it will ever be feasible to apply the technique in nonmalignant diseases as originally intended. The combination of retroviral gene transfer and cytotoxic drugs in patients without malignancy is one that now holds little appeal given the oncogenic events observed in the severe combined immunodeficiency (SCID) trials in France (39).

5. TUMOR CORRECTION

Although there is an attractive elegance to the strategy of introducing genetic material into a malignancy to correct the specific genetic defects contributing to the neoplastic phenotype, the lack of
targeting and poor efficiency of gene transfer vectors was considered to render this approach clinically worthless: Transduction in vivo of 1–2% of tumor cells (an optimistic estimate) with a corrective gene would be of no patient benefit. There was therefore great resistance to an early protocol in which investigators proposed to replace a defective p53 gene in localized unresectable or recurrent carcinoma of the bronchus by injecting a retroviral vector encoding p53 (40).

The protocol only went ahead after much debate, but to almost everyone’s surprise, there were apparent tumor responses. Substitution of an adenoviral p53 vector apparently gave superior responses, and the approach appeared also to produce responses in localized head and neck cancer (41). The effects occur regardless of whether the tumors are p53 mutant or wild type and certainly cannot be attributed to the low level of tumor transduction obtained with the vectors.

Although the mechanism of action remains uncertain, the results have been sufficiently encouraging to allow the implementation of a phase III clinical study. Regardless of the eventual outcome of these trials, the data make the important point that little is still understood of the biologic interactions between ourselves and our tumors, and that in the face of such ignorance, we should be careful about the predictions we make.

6. WHAT DID THE EARLY STUDIES TEACH US?

Above all else, of course, these early studies confirmed the obvious: Available vectors were simply inadequate for our needs. The past decade has seen an explosion of work identifying new vector systems, viral and nonviral. Of particular interest for cancer therapy has been the development of conditionally replication competent vectors capable of reproducing in malignant cells while sparing normal tissues. To date, these have been used clinically for their directly lytic properties, but the potential to incorporate therapeutic genes is obvious and may substantially boost the efficiency of almost all the approaches described in this chapter. Many other groups are working on vector targeting and on transgene regulation. They have made substantial advances that are described elsewhere in this volume and that will soon be showing up in clinical trials.

The issue of vector and transgene immunogenicity is also a concern. Although an adjuvantlike effect may enhance some cancer gene therapies, readministration is severely curtailed in the presence of a neutralizing immune response. The ability to deal with this problem may ultimately be the most important factor in determining the value of cancer gene therapy.

Perhaps the most important conceptual message from these early studies is that, although a single gene therapy approach alone can undoubtedly induce a tumor response, it is unlikely to eradicate disease successfully. Instead, inter- and intrapatient tumor heterogeneity means that several different therapeutic methods will need to be combined. As discussed in this book, efforts to combine gene therapies with conventional modalities are proceeding, as are efforts to combine different types of gene therapies themselves. For example, if Tk gene transfer (followed by ganciclovir) is combined with cytokine gene transfer, the patient may benefit from both a direct cytotoxic and an immune-mediated antitumor effect.

Undoubtedly, gene therapy has had a difficult first 12 years. Understandable excitement about the approach lead to unrealistic expectations about the time it would take to apply the technology successfully, and intense scrutiny of adverse events (predominantly those in nonmalignant conditions) has been discouraging to many in academia and in industry. A brief study of the history of every other novel cancer therapy, including cytotoxic drugs, radiation, or monoclonal antibodies, reveals a 20-year gap between discovery and truly successful implementation. The complexity of gene therapies and of the current regulatory environment means that much the same can be expected of this technology. Although many industrial partners have dropped from the race, it seems likely that eventual success will encourage a resurrection of interest.

One final lesson of this early history, however, is that ultimately the successful model for cancer gene therapy may not always be the pharmaceutical one. Instead, investigators may need to assemble
components from a number of suppliers and provide an institution-based manufacturing process—the model of organ transplantation and indeed of surgery and radiotherapy in general.

7. CONCLUSION

Although the history of cancer gene therapy has emphasized individual investigators and industrial sponsors, the future is more likely to accentuate the importance of interdisciplinary teams and the institutions in which they work.

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1. INTRODUCTION

Cancer is a genetic disease in which malignant cells have undergone mutations and epigenetic changes but maintain the transformed phenotype even when cultured or when injected into immunologically tolerant experimental animals (1,2). However, most of the genetic events in tumors are somatic (i.e., not hereditary), brought about environmentally or randomly, and the identified inherited (often referred to as “genetic”) causes account for a small proportion of all cancers.

Specifically, the genes with mutations that are relevant to the carcinogenic process, fall into two classes: tumor suppressor genes and oncogenes. The distinction between heritable and environmental causes may be easily made if a hereditary cancer syndrome or an environmental exposure, such as tobacco smoking or human papilloma virus, poses a high risk. For most common cancers, this is not the case, and they are therefore considered complex diseases caused by many underlying and interacting genetic and environmental factors. Heritable effects would lead to a clustering of cancer in families (3,4). However, familial clustering can also be caused by shared environment or lifestyle, and an increased familial risk does not tell whether the reason is heritable or environmental (5).

In this chapter, we discuss causes of cancer and the underlying molecular mechanisms from the point of view of potential gene therapy approaches. Improved understanding of the causes of cancer will be helpful for scientific, clinical, and cancer preventive measures. A certain notion of cancer causation, often implicit, is embedded in many science and health policy decisions.

2. THE GENETIC BACKGROUND OF CARCINOGENESIS

In a nutshell, cancer can be considered a disease caused by mutations and epigenetic changes (e.g., methylation defects) in tumor suppressor genes and oncogenes (6). Mutations may be the more common of the two types of changes and can be missense (altered amino acid), frameshift (altered reading frame), or nonsense (truncation of protein product). Sometimes, mutations do not affect the amino acid sequence, but rather influence the promoter or splice sites. Deoxyribonucleic acid (DNA) sequence variations that do not have a direct unequivocal link to the phenotype of interest but may play a role are called polymorphisms.

There are various mechanisms that can cause mutations. These include deletions of small or large DNA segments, inversions, translocations, looping leading to truncated sequence, and so on. The initial causes for these mechanisms range from ultraviolet radiation to chemical and viral carcinogens, but for most cases of cancer, causation remains poorly defined. Probably, diet and other environmental causes play a major role, but cause–effect relationships are difficult to demonstrate conclusively because of the long time between initiation of a tumor and clinical presentation. Hereditary mutations have also been identified as a cause of cancer and are discussed here.
By definition, genetic changes important for carcinogenesis (i.e., can be detected as clonal changes in malignant tumors) inactivate tumor suppressor genes or activate oncogenes. Both groups include dozens of well-defined members, and hundreds probably remain poorly characterized. A classic example of an oncogene is RAS, which was initially identified as a gene activated in the process of virally induced tumorigenesis. Later, mutations of RAS have been commonly found in a wide variety of cancers. Most protein products of oncogenes are involved in signal transduction and growth regulation. An activating mutation of one allele is usually sufficient.

The normal functions of proteins coded by tumor suppressor genes are often related to important regulatory or housekeeping functions crucial to the integrity of cellular functions, including cell division and programmed cell death. Therefore, the loss of these functions is beneficial to malignant progression. In most cases, both alleles of a tumor suppressor gene must be lost for loss of function of the protein product. Often, one allele is lost because of a “local” mutation; the other allele is lost because of a large deletion (loss of heterozygosity). Classic tumor suppressor genes include p53 and APC (adenomatous polyposis coli). The former has a wide variety of functions associated with cell cycle control and programmed cell death (apoptosis). Mutations of p53 have been identified in more than half of all cancers.

APC was initially identified as the gene harboring germline mutations in patients with familial adenomatous polyposis, a hereditary disorder that leads to formation of hundreds of intestinal polyps that, when untreated, eventually undergo malignant transformation and cause death at a young age. APC has multiple functions involved with cellular signaling and adhesion. The APC example is interesting for two reasons. First, it is a useful example of a rare genetic disorder revealing the molecular background of common disease. Although familial adenomatous polyposis is rare, mutations of APC (or members of its signaling pathway) were consecutively identified in virtually all colorectal cancers. In fact, the same is true for most of the cancer syndromes discussed in this chapter. Although the syndromes are rare, the causative genes are commonly involved in sporadic carcinogenesis as well.

Second, studies (many of which were performed by Bert Vogelstein and colleagues at Johns Hopkins in Baltimore, MD) of APC and the genetic basis of colorectal cancer have revealed another aspect that may be common for many types of malignant tumors. Inactivation of APC may be the initial or an early step in many colorectal cancers, but it is not the only change found in advanced tumors. Instead, carcinogenesis may often be a multistep process in which additional mutations confer features useful for increased growth and decreased susceptibility to growth regulation (Fig. 1).

It is unlikely that all occurring mutations are beneficial to the malignant clone. Instead, the majority may give rise to subclones that have reduced viability or perhaps increased detection by the immune system. Nevertheless, the rare beneficial changes gain a growth advantage and can thus be detected in the end product of the multistep process of carcinogenesis, which in most cases is an aggressively growing tumor capable of invasion and metastasis.

The gene that sustains the initial mutation that allows the carcinogenic process to proceed has been called the gatekeeper gene (Gene 1 in Fig. 1). The theory is that each cell type may have a crucial growth regulatory circuit; its inactivation may be necessary for carcinogenesis. For example, APC has been suggested as the gatekeeper for the colorectal epithelium. Another suggested class of tumor suppressor genes is the caretaker genes; their inactivation may facilitate the multistep process of carcinogenesis by allowing rapid accumulation of further mutations (2). These genes are often involved with DNA repair and maintaining the integrity of the genome.

For gene therapists, an important question is how many steps of the multistep process need to be blocked for effective intervention? Presently, the complete answer is not known. Nevertheless, most available evidence suggests that correction of a single defect, such as replacement of a defective tumor suppressor gene or inactivation of an overactive oncogene, can be sufficient for controlling the malignant process. For example, when p53 is expressed in p53 mutant cancer cells (with many other mutations as well), the cells undergo apoptosis and may in fact trigger neighboring cancer cells to do the same.
Thus, perhaps the malignant phenotype can be compared to a house of cards, for which removal of any card causes the whole structure to collapse. This is not completely surprising considering the various defenses the human body has against malignant cells. Fittingly, malignant cells can be detected circulating in healthy individuals who never develop cancer. Further, cancer typically arises in advanced age, when the body’s defense mechanisms have slowed, but the cancer has had decades to develop a delicately balanced combination of features that allow sustained growth while remaining undetected by the immune system.

An increasing number of genes are identified as tumor suppressor and oncogenes, and the respective protein products seem to have a wide variety of functions (1,6). Nevertheless, many cancer-associated genetic changes seem to fall into six categories (Fig. 2), which include (1) aberrant adhesion
properties (e.g., loss of contact inhibition), (2) exaggerated or unphysiological response to growth-promoting signals and reduced responsiveness to growth-regulating signals, (3) failure to undergo programmed cell death on genetic damage (dysfunction of cell cycle checkpoints), (4) immortalization (gain of telomerase activity), (5) avoidance of immune defenses, and (6) factors promoting neovasculogenesis (rapidly growing tumors need an ample supply of oxygen and nutrients). Importantly, all of these features are distinct from characteristics found in most nonmalignant cells and thus may allow intervention.

3. APPORTIONING CANCER CAUSATION

Although most cases of cancer are somatic, that is, they do not have an identifiable familial component, studies of hereditary syndromes have produced or initiated much of today’s understanding of cancer as a genetic disease. It is not unreasonable to assume that this will be true in the future as well; therefore, we briefly discuss hereditary cancer here.

Two studies have provided unique insight into the familial component of various common cancers. The first study used the classic twin design, that is, comparison of correlation of cancer in monozygotic and dizygotic twins from three Nordic countries (7). In this model, it is assumed that both types of twins equally share the environmental effects; monozygotic twins are genetically identical, whereas dizygotic twins are like any siblings, sharing by average 50% of their genes. The second study was based on the nationwide Swedish Family-Cancer Database of 3 million families (8). It compared correlation of cancers between all family members using the same statistical model used in the twin study. It had a much higher statistical power than the twin study because the whole Swedish population and its 1 million tumors were scrutinized. On the other hand, most sex-specific cancers could not be assessed in the model.

The results from both models are presented in Table 1. For stomach cancer, heritability was estimated to account for 28% from the twin study and 1% from the family study. The remainder, 72% and 99%, respectively, could be the total environmental effect, of which the majority were because of nonshared or random environment. The twin study gave statistically significant heritability estimates (for which the 95% confidence interval did not include zero) only for cancers of the colorectum (35%), breast (27%), and prostate (42%). The family study gave an identical estimate for the breast, but a lower estimate for the colorectum. The heritability of cervical cancer was 22%, but that of lung and bladder cancer and leukemia was less than 10%.

Table 1
Heritable Effects of Cancer and Some Involved Genes

<table>
<thead>
<tr>
<th>Cancer site</th>
<th>From twins</th>
<th>From families</th>
<th>Known genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>0.28</td>
<td>0.01</td>
<td>E-cadherin</td>
</tr>
<tr>
<td>Colorectum</td>
<td>0.35</td>
<td>0.13</td>
<td>Mismatch repair, APC, LKB1</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.36</td>
<td>—</td>
<td>CDKN2A</td>
</tr>
<tr>
<td>Lung</td>
<td>0.26</td>
<td>0.08</td>
<td>Metabolic low-penetrance genes</td>
</tr>
<tr>
<td>Breast</td>
<td>0.27</td>
<td>0.25</td>
<td>BRCA1/2, ATM (ataxia telangiectasia mutated)</td>
</tr>
<tr>
<td>Cervix uteri</td>
<td>0</td>
<td>0.22</td>
<td>Immune response genes?</td>
</tr>
<tr>
<td>Corpus uteri</td>
<td>0</td>
<td>—</td>
<td>Mismatch repair, PTEN</td>
</tr>
<tr>
<td>Ovary</td>
<td>0.22</td>
<td>—</td>
<td>BRCA1/2, mismatch repair</td>
</tr>
<tr>
<td>Prostate</td>
<td>0.42</td>
<td>—</td>
<td>Candidate loci</td>
</tr>
<tr>
<td>Bladder</td>
<td>0.31</td>
<td>0.07</td>
<td>Metabolic low-penetrance genes</td>
</tr>
<tr>
<td>Leukemia</td>
<td>0.21</td>
<td>0.09</td>
<td>ATM, helicase</td>
</tr>
</tbody>
</table>

Source: Based on a Nordic twin (7) and a Swedish family study (8).

*95% confidence interval does not include 0.0; that is, the estimate is statistically significant.
Caution should be used in overinterpreting these estimates from statistical modeling. However, certain common cancers showed a much higher range of heritability than that observed by comparing familial risks between first-degree relatives (9). If the estimates for colorectal, breast, and prostate cancers, showing 27–42% heritability, are confirmed, there are major gaps in the understanding of the genetic basis of these neoplasms.

Some of the genes that transmit familial risks are indicated in Table 1 (2). The frequencies of mutations in the well-known high-risk susceptibility genes BRCA1 and BRCA2 in breast cancer and DNA mismatch repair genes in hereditary nonpolyposis colorectal cancer (HNPCC) are so low that they explain at most 10% of the heritability noted, and 90% remain unaccounted (10,11). For prostate cancer, candidate genes have been mapped, but not identified (12–16). These findings suggest that other genes are yet to be identified, but because their polymorphisms are likely to be relatively common and confer only a modest risk increase, their identification will be difficult.

4. CANCER MODELS

Well-characterized cancer syndromes, such as familial retinoblastoma, BRCA-linked breast cancer, and HNPCC, follow a dominant Mendelian pattern of inheritance, with high penetrance (proportion of genotype carriers with phenotype); therefore, close to 50% of the offspring of an affected parent present with the disease. Nevertheless, these syndromes are rare, and the frequency of the mutant gene is on the order of 1/1000 (carrier frequency = 1/500) or less. The most common cancer syndromes BRCA1 and BRCA2 and HNPCC are thought to account for 1–3% all breast and colorectal cancers, respectively (10,17,18). Bloom syndrome, ataxia telangiectasia, and xeroderma pigmentosum are examples of Mendelian recessive cancer syndromes. About 25% of the offspring of two heterozygote parents display symptoms, including neoplasms. It is relatively easy to estimate the proportion of all cancer because of such well-characterized monogenic syndromes conferring a high risk, and 1% appears to be a good estimate (19).

Most common cancers are caused by alterations in many genes. According to the multistage theory of cancer, the clonal tumor emerges as a result of a number of mutations in a single cell (20–27). The first mutations occur in normal cells, creating a slow-growing preneoplastic colony. Additional changes in a cell of the preneoplastic colony are believed to be necessary to create a neoplastic cell capable of growing as a malignant tumor. The number of required mutations may vary and probably depends on the genes and cell types affected. This is probably true for cases arising as a result of hereditary mutations as well. The initial gatekeeper mutation may confer a growth advantage and thus increase the target size (number of cells with the initiating defect) for subsequent promotional mutations. Mathematical adoption of known mutation rates, number of stem cells, and normal human life-span can accommodate a carcinogenic process with three or more mutations, such as two in the initiation stage and one or more in the promotional stage (24,25).

When two or more genes are involved, it is difficult to observe Mendelian inheritance in pedigrees (27) because the likelihood decreases that an offspring will inherit the parental set of disease genes. Therefore, it is difficult to distinguish multifactorial inheritance from low-penetrance single-gene or environmental effects, which is a major challenge to current segregation analyses (28–30). In the twin model, polygenic inheritance would be expressed as a much higher risk among monozygotic than dizygotic twins (3,31). Another model in which polygenic inheritance could be distinguished is in multiple primary cancers in the same individual (32,33).

5. CANCER GENES

Only a small proportion of cancer is because of single-gene, dominant traits (6,34). However, the affected families have been helpful in the efforts of gene identification, and the majority of the tumor-related high-penetrance genes have been described from such families (2). Results can be obtained even for rare cancer syndromes, such as Peutz-Jeghers or skin and uterine leiomyomas if the families
are homogeneous and the risk is high (35,36). An interesting aspect of the leiomyoma study was that the gene turned out to be fumarate hydratase coding for an enzyme in the tricarboxylic acid cycle. Another enzyme in this metabolic pathway, succinate dehydrogenase, was implicated in hereditary paragangliomas and pheochromocytomas (37). These data have widened the scope of tumor-related genes to metabolic, housekeeping genes from the earlier cell cycle regulator, DNA repair, and signal transduction paradigms.

5.1. High-Risk, Rare Genes

Many forms of cancer in which a single gene poses a high risk have been identified. Of the 4700 dominant and 2800 recessive human genetic traits known in the early 1990s (31), some 440 were single-gene traits in which cancer was a complication; many of them were extremely rare, with a few identified families worldwide (38). Most known cancer syndromes are dominant at the population level (although recessive at the molecular level; 19), the gene carriers are type Aa, where a = mutant gene. In tumors, the normal allele is lost (loss of heterozygosity), and the tumor is therefore hemizygous a or homozygous aa if another mutation occurs instead of allele loss. In dominant cancer syndromes, the penetrance is typically high, often approaching 100%, which facilitates identification of the dominant pattern because cases are found in all generations.

Some rare cancer syndromes, such as xeroderma pigmentosum, ataxia telangiectasia, and Bloom syndrome, are recessive (aa) at both population and molecular levels. The detection of recessive conditions is difficult because the cases appear apparently randomly in pedigrees, but often reveal consanguinity at a closer inspection. Population geneticists have raised questions about the relatively small number of known human recessive syndromes. In species of experimental animals, recessive traits predominate, as opposed to humans, for whom dominant traits are more common (31). It is not excluded that this is an observation bias because of difficulties in identifying a recessive pattern. A further complication is that, in many cancer syndromes, the mutations are de novo germline mutations lacking familial pattern. This is the case for most disorders for which cancer occurs early; thus, the propagation of the defect to further generations is reduced. Examples include Wilms tumor, retinoblastoma, and neurofibromatoses 1 and 2 (39).

The relative risks (RRs) of cancer may be very high (<1000) in the rare cancer syndromes. In fact, if the penetrance is close to 100%, RR depends on the population frequency of the disease only. Most known syndromes affect young individuals, for which the population incidence is low, resulting in excessive RRs. The unusual risk of rare cancers in young individuals has facilitated identification of syndromes, including Li-Fraumeni, multiple endocrine neoplasia 2 (MEN2), and HNPCC (40,41). The RR of childhood cancers in Li-Fraumeni syndrome (hereditary p53 mutation) has been estimated at >100 (42) and that of colorectal cancer in HNPCC at 70 (17). The estimates from the Swedish Family-Cancer Database give RRs of 30 for endometrial cancer in HNPCC and 5000 for medullary thyroid cancer in MEN2 (43,44).

The proportion of gene carriers depends on the population, and the most accurate estimates are available for Europeans and European Americans. Among the known dominant cancer syndromes, the frequency of gene carriers is highest for HNPCC, about 1/500, and BRCA1 and BRCA2, each about 1/1000. For most others, such as Li-Fraumeni, MEN1 and 2, neurofibromatosis 1 and hereditary renal cell cancer (caused by mutation in VHL), retinoblastoma, Wilms, and Gorlin cancers, the frequency of carriers ranges from 1/3000 to 1/50,000 (39). In recessive conditions, such as xeroderma pigmentosum and ataxia telangiectasia, the frequency of diseased (a^2) is low (1/1 million and 1/40,000, respectively), but the carrier frequency (2Aa) of ataxia telangiectasia has been estimated at 1–5% in the US population (45). If heterozygotes are at risk for cancer, the impact may be significant. Ataxia telangiectasia heterozygotes may have an elevated risk of various cancers, such as breast cancer, and because of the large number of carriers, calculations argue that the attributable proportion of ataxia telangiectasia in breast cancer is higher than that of BRCA1 and BRCA2 (46,47).
A further aspect of familial cancer syndromes is that they often affect cancers at multiple sites, even though detected through cancers at a particular “index” site. Li-Fraumeni syndrome is an example, with more than a 100-fold RR at the index sites (childhood sarcomas), but a modest RR for more common diseases such as breast cancer. Further examples are HNPCC, BRCA1, and BRCA2. In the recessive cancer syndromes, including ataxia telangiectasia and Bloom, the affected individuals can present with almost any kind of malignancy (45,48).

Another aspect relating to the identification of a clinical entity is the presentation of other diseases in many of the known syndromes. Patients with recessive cancer syndromes are severely handicapped, as indicated by some of their descriptive names. Severe noncancer diseases beset even dominant conditions such as NF1 and NF2, MEN 1 and 2, and hereditary renal cell cancer.

5.2. Low-Risk, Common Genes

Familial effects in cancer are not only because of high-risk gene defects as discussed previously, but most likely there is contribution by more common low-risk defects, which may be frequent enough to be called polymorphisms (sometimes defined as the variant present in more than 1% of the population). Many polymorphisms have been described in the areas of drug and carcinogen metabolism, with some recent data also on hormone receptors and DNA repair genes (49–51). Although it is likely that a large number of low-risk genes modulate the carcinogenic process in humans, there has been much controversy in the current literature on the role of metabolism genes in cancer (52).

Immune surveillance plays an important role in cancer, as has been observed in immunosuppressed patients who are at a marked risk of lymphomas and many types of squamous cell carcinomas (53,54). Milder forms of immunodeficiencies probably explain some familial patterns of non-Hodgkin’s lymphoma, Hodgkin’s disease, cervical cancer, and squamous cell skin cancer (53,55,56). Suppressed immune function is also likely to modulate host response to virus, such as human papilloma virus and Epstein-Barr virus (57–60).

6. CONCLUSION

There are no data available on the etiology of cancer that would refute the predominant role of environment as a causative factor. However, since the epochal review by Doll and Peto in 1981 (61), disappointingly little progress has been seen in the search for new causes of environmental carcinogenesis. One likely reason is that environmental carcinogenesis is because of the interaction of external and host factors, which cannot be unraveled by epidemiological or molecular biological means alone. There is hope that merging of these approaches into molecular epidemiology or, even better, into molecular genetic epidemiology will tool the exogenous/endogenous interphase of human carcinogenesis. Nevertheless, there is little doubt that, regardless of the causative agents, on the molecular level the malignant process manifests as mutations and epigenetic changes in tumor suppressor and oncogenes. Further, the accumulation of mutations in these genes gradually increases the aggressiveness of the clone and therefore constitutes the multistep process of carcinogenesis.

All the main types of cancer appear to have a familial component with a frequency that varies, but often ranges from 1 to 5%. Familial risks observed among twins and among patients with multiple primary cancers provide support for the multistage carcinogenesis in human cancers at a population level (27,30). There are at least three practical implications from such findings. One is that, in the search for new susceptibility factors in cancer, low-penetrance genes may be better identified in association studies with a case–control design than in linkage studies (62–64). The second implication is that, in clinical counseling, polygenic and recessive conditions imply uncertainty (30). The disease strikes apparently randomly even though there is an inherited background.

The third problem that may have implications for gene therapy approaches involves a question: If many genes contribute to each case of cancer, is blocking or repair of one defect sufficient for reverting the malignant phenotype? Current evidence suggests that removing one “card” (mutation) from
the “house of cards” (advanced malignant tumor) can be enough. Nevertheless, considering the awesome capacity of cancers to acquire resistance, a cytostatic effect may not be desirable, and rapid killing of cells may be required instead. In addition to resistance on the cellular level, tumors can acquire resistance on the tissue level. This implies the existence of subclones that are not sensitive to the treatment. Therefore, removing multiple cards simultaneously or consecutively could have advantages.

Identification of cancer as a disease caused by mutations and epigenetic changes in genes immediately suggested gene therapy as a logical means for intervention. Thus, if the causative defects can be corrected or blocked, the disease phenotype can be reversed. Alternatively, the genetic changes present in cancer cells offer a variety of characteristics that separate them from noncancer cells. These features include dysregulated promoters and enhancers, aberrant expression of receptors and epitopes, and loss of antiviral defense mechanisms. As discussed in this book, these features can be utilized in the planning of gene therapy strategies aimed at direct killing of cancer cells.

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