Cancer gene therapy is based on the principle of altering a tumor cell genetically to improve cancer treatment. This strategy works because the tumor cells can be made to express a new gene, for example, from bacteria that other cells in the body do not express, that would render them susceptible to a drug or other treatment. In the years to come this technology is expected to make significant impact on how cancer patients are being treated.
18.1 Introduction

The last decade has seen tremendous growth of studies attempting to take gene therapy of cancer into the clinical arena. The major objective is to introduce genetic material into cancer cells with the intent of sensitizing them to chemo- and radiation therapy. A number of strategies have now reached phases I and II clinical trials, with some showing promise. In addition, using molecular tools and engineering, a major thrust in the field is to image affected cancer cells and their fate during therapy, for example by positron emission tomography (PET) or magnetic resonance imaging (MRI). Occasionally, this can be achieved by the conversion of an image probe by the therapeutic protein itself resulting in an imageable feature and sometimes a co-expressed protein can accomplish this. This chapter reviews the status of the cancer gene therapy field, with focus on new technology and directions, existing problems, and highlights and discusses areas with gene therapy applications in radiation therapy and imaging.

18.2 Vectors

The vector is the vehicle that carries the DNA into the cells (Valerie 1999). One overriding technical difficulty shared by all gene therapy vectors is the relative inefficient delivery of DNA into the tumor. All tumor cells need to be affected or the cancer would recur. Virus vectors including adenovirus (ADV), adeno-associated virus (AAV), and retrovirus (RV) have traditionally been the vectors of choice for introducing genetic material (e.g., suppressor genes, dominant-negative genes, and “suicide genes”) into cancer cells to make them more sensitive to chemo- and radiation therapy. Viruses have evolved highly effective mechanisms for infecting cells, on which has been capitalized. However, each vector has its pros and cons (Table 18.1). By molecular engineering, these viruses have been altered to accommodate the therapeutic gene and at the same time allow for efficient growth and safe handling.

A significant problem using viral vectors for cancer gene therapy are production and quality control issues, safety, and cost associated with obtaining clinical grade preparations suitable for human use. In terms of the efficiency of delivery, ADV remains the most effective viral vector because of the ability to obtain high titers and higher multiplicities of infection ([MOI] i.e., virus per cell ratio) than other viral vectors, and the relative ease by which large quantities of virus can be isolated and purified (Table 18.1). Retrovirus, and more recently lentivirus (LV), remain promising vectors. However, whereas ADV is not typically integrated into the genome of infected cells, both RV and LV integrate and potentially could be a safety concern due to the possibility that a critical cellular gene is inactivated by insertion of the virus. Because of these advantageous properties,

| Vector(s)     | Pros                                      | Cons                                      |
|---------------|-------------------------------------------|-------------------------------------------|
| ADV           | Efficient gene transfer                    | Lack of cell type specificity             |
|               | Infects nonreplicating cells               |                                           |
|               | High titers                               |                                           |
|               | Transient expression                      |                                           |
|               | Large gene capacity                       |                                           |
| AAV           | Single, site-specific integration         | Difficult to purify                       |
|               | Relatively small gene capacity            |                                           |
| RV, LV        | Efficient gene transfer                    | Risk of insertional mutagenesis          |
|               | Relatively small gene capacity            | Relatively low titer and expression       |
|               | Infects nonreplicating cells (LV)         | Cumbersome to purify for clinical use     |
| Lipid–DNA     | Unlimited gene capacity                   | Relatively poor in vivo gene transfer     |
|               | Excellent scale-up capability             |                                           |
| Nanoparticles | Unlimited gene capacity                   | Relatively poor in vivo gene transfer     |
|               | Excellent scale-up capability             |                                           |
|               | Imageable                                |                                           |
|               | Cell targeting                           |                                           |
ADV is considered a more suitable vector for cancer therapy than RV and LV. However, the efficient delivery of viruses into the tumor is still a major problem despite viruses’ ability of infecting cells in culture at high levels. The effective delivery of virus or by any other means (physical or chemical) within the tumor bed remains a major technical hurdle (Freytag et al. 2004). Penetration of ADV within tissue rarely exceeds a volume larger than a cubic centimeter (Barton et al. 2007), thus limiting potential success only to small tumors.

AAV has also been considered as a vector for cancer therapy (Li et al. 2005b). However, even though AAV supposedly is integrating at a specific chromosomal site, potentially avoiding mutagenesis, production issues and relatively low expression levels of therapeutic genes from AAV vectors are shared with RV and LV (Table 18.1).

Some studies have attempted direct injection of DNA into tumors, either alone or in complex with lipids. Introducing DNA or RNA directly into the tumor limits immune responses sometimes elicited when viral preparations are administered. However, the efficiency of lipid–DNA to enter cells in a tumor remains a problem, as does the relatively nonspecific cellular uptake of lipid–DNA complexes. Attempts to incorporate molecules in the lipid bilayer that bind to cell surface receptor to improve cell specificity have been investigated, but the differential effects seen in vitro are not generally duplicated in vivo. Along the same line and more recently, nanoparticles has also been considered as vector for delivering therapeutic genes. An added benefit with nanoparticles is that they can also be imaged (Nire et al. 2007).

18.2.1
ADV and AAV

Adenovirus is a relatively large DNA virus that infects a variety of epithelial and endothelial cells expressing the Coxsackie-adeno virus receptor and the integrin receptor (Valerie 1999). Relatively large therapeutic genes can be transferred by ADV. First-generation viruses can harbor DNA inserts of more than 3-kb whereas “gutted” ADV is able to harbor up to 34-kb of DNA (Ng and Graham 2002; Ng et al. 2002). This ability of ADV in addition to the relative ease by which the virus is propagated and purified in large quantities has made ADV an attractive vector choice for cancer gene therapy. In terms of targeting therapeutic ADVs to specific cells, a number of clever approaches have been devised including altering the viral penton protein necessary for infection to alter the propensity of infection to cancer cells over normal cells (Glasgow et al. 2006). Engineered ADV vectors remain the most biologically efficient and cost-effective viral cancer gene therapy vector.

AAV is a relatively small DNA virus that integrates at a specific chromosomal site on chromosome 19, infects both dividing and nondividing cells, transduces a broad range of tissues in vivo, and initiates long-term gene expression in these tissues (Li et al. 2005b). Furthermore, wild-type AAV does not cause any known disease and does not stimulate a cell-mediated immune response. In order for AAV to propagate, it requires a helper virus such as ADV. The relatively small genome size of 4.7-kb only allows smaller therapeutic genes to be transferred. Similar to ADV, attempts to change the cell tropism for AAV infection have been successful in vitro, but these approaches have not yet been fully tested in vivo. Thus, AAV is similar to RV and LV in its properties as gene therapy vector but may not be as significant mutagenesis threat as are these other two viruses (Table 18.1).

18.2.2
RV and LV

An engineered mouse leukemia retrovirus was the first viral vector developed for cancer gene therapy (Culver et al. 1992). The retroviruses stably integrate randomly into the genome of infected cells, and thus would be a potential safety issue. Another major shortcoming of the RV as vector for cancer gene therapy is the relatively low expression levels compared with adenovirus. The typical LV used for gene therapy is a human retrovirus derived from human immunodeficiency virus (HIV-1) that is highly efficient in infecting cells. In contrast to the mouse retrovirus, human LV infects nondividing cells in addition to dividing cells, which makes this vector very attractive for somatic cell gene therapy and in vitro work. However, for cancer gene therapy, LV may not be advantageous since it infects indiscriminately, whereas RV only infects dividing cancer cells. RV and LV vectors have some very attractive features but are not ideal for human cancer gene therapy.

18.2.3
Lipids and Nanoparticles

The discovery that positively charged lipids could be used to introduce DNA into cells in vitro opened up the possibility of using lipid–DNA complexes for direct intratumoral injection. Lipid–DNA complexes efficiently transfect cells in vitro. However, the low yield of cellular uptake in vivo remains a problem. With the advent of
using synthesized anti-sense oligonucleotides and RNA interference (see below) for manipulating gene expression, lipid vectors are probably the most promising vector in the long run, since viral vectors will most likely continue to have production and safety issues that have to be adequately addressed resulting in very high production costs. Entirely manmade DNA/RNA and lipids needed for human use would provide excellent scale-up capabilities, quality control, and increased safety, similar to the manufacturing of small molecule cancer drugs. However, the efficiency and specificity of lipids to deliver DNA into the tumor need improvement. Nanoparticles made of chemically synthesized, highly structured macromolecules able to entrap a drug, therapeutic DNA or RNA, and/or agents that can be imaged show enormous future potential (Niz et al. 2007). Completely synthetic nanoparticle vectors and DNA or RNA for gene therapy is likely the way of the future.

18.3 Strategies and Targets for Cancer Sensitization

Most cancer gene therapy studies have up until now used viral vectors, in particular ADV, to deliver the therapeutic DNA into the tumor cells. Many clever ways have been devised, and numerous cellular processes have been explored as targets for enhancing killing of cancer cells in vitro, for example by increasing apoptosis, and many times these strategies have also shown efficacy in animal tumor models. Herein, only the most significant studies and concepts will be discussed.

To improve the transmission and spread of ADV within the tumor and design a “magic bullet” for cancer cells, the oncolytic ONYX-015 ADV was developed (Bischoff et al. 1996). The basic idea is for this ADV to replicate only in cancer cells but not in normal cells, due to a mutation in a specific viral gene, E1B, that renders ADV replication dependent on mutant or abnormal p53, a condition found in about half of all cancers. When ONYX-015 infects a p53 mutant cancer cell, it subsequently lyses or breaks open the cell and releases new ADV available for infection of additional cancer cells, thus the name oncolytic. The tumor suppressor p53 was initially believed to make the decision whether replication occurred or not—mutant p53 allowed the oncolytic virus to replicate whereas wild type did not. Since normal somatic cells express wild-type p53 they would not allow the ADV to replicate and thus would be spared. However, it turns out that other p53-related processes and modulators of cell cycle checkpoints in-cluding p14AR/p16INK4A may also play important roles in whether the oncolytic ADV replicates or not. Nevertheless, oncolytic ADV showed some positive initial clinical results, but it is not a magic bullet for cancer. However, the oncolytic virus concept remains a highly attractive strategy for cancer therapy in general since it would seek out cancer cells and destroy them, whereas normal cells would be left unharmed (Alemany 2007).

Additional permutations on the original oncolytic virus idea have been proposed and tested using a variety of different viruses. Some also include a therapeutic gene such as a tumor suppressor or suicide gene (see below) to produce a potential multi-prong therapeutic effect.

18.3.1 RNA Interference

RNA interference (RNAi) is a highly conserved mechanism by which small, nonprotein-coding RNA molecules interfere with, or modulate, gene expression. Although RNAi represents just one function of a variety of small noncoding RNA molecules, it has received the most attention largely because of its utility as a basic research tool to silence the expression of specific genes (Zamore and Haley 2005). Moreover, because of its potency and high specificity, RNAi has now emerged as a promising new therapeutic strategy to reduce or eliminate gene expression in animals. Indeed, RNAi is already undergoing human clinical trials, including pioneering work for treating macular degeneration and respiratory syncytial viral infection (Bumcrot et al. 2006).

RNAi is catalyzed by RNA molecules approximately 22 nucleotides in length that can originate from both exogenous and endogenous sources (Kim 2005) (Fig. 18.1). The incredible specificity inherent in RNAi is derived from its basic mechanism of action, whereby the small interfering RNA (siRNA) molecule hybridizes specifically with its cognate messenger RNA, resulting in degradation of the mRNA. This endogenous silencing mechanism, found in all multicellular organisms, can be exploited to achieve silencing of specific genes by introduction of synthetic siRNA molecules redesigned to hybridize to target genes (Fig. 18.1). These siRNA molecules also hold significant promise for gene therapy approaches. (For a recent review of siRNA biogenesis and the mechanism of inhibition by RNAi, see Liu et al. 2008). In addition to extracellularly introduced siRNA, there exists another class of small RNA molecules, termed micro-RNA (miRNA), which is encoded by the genome of an organism. miRNA represent the most abundant class of naturally occurring small RNAs
but differ from siRNA in their origins. Unlike siRNA, miRNA is initially transcribed as part of a much longer primary transcript and then processed to liberate a hairpin precursor of ~65 nucleotides in the nucleus (Liu et al. 2008). This precursor is then exported to the cytoplasm where it is processed by the enzyme Dicer to produce a ~22-bp RNA duplex. Thus, at this point, miRNA and siRNA is similar and both enter a common pathway in which one strand of the RNA duplex becomes incorporated into a protein complex known as RISC (for RNA-induced silencing complex). The function of RISC is to guide the interaction between the siRNA or miRNA and the mRNA and catalyze either the degradation of the mRNA or inhibit its translation (Fig. 18.1) (Hutvagner and Simard 2008).

Once the basic mechanism of RNAi was uncovered, it was clear that siRNA could be introduced into cells to silence the expression of specific genes. Indeed, synthetic siRNA is now commercially available to silence most human genes. Another strategy is the manipulation of miRNA to alter gene expression. It has been estimated that the human genome encodes hundreds of forms of miRNA, which regulate the expression of a large number of protein-coding genes (for review, see Ambros 2004). Indeed, miRNA has been found to control a wide range of biological processes, including development, metabolism, cell growth, cell death, and cell fate determination (Ambros 2004), and altered expression of miRNA has been associated with human diseases including cancer (Hammond 2006). One strategy to inhibit the function of miRNA is the use of antagonirs, which hybridize to miRNA and prevent its incorporation into RISC (Mattes et al. 2007) (Fig. 18.1). Using this strategy, it is possible to increase the expression of specific genes (Mattes et al. 2007).

RNAi potentially has several major advantages compared with traditional therapeutics. First, siRNA can be designed to target genes with unparalleled specificity. Second, all proteins can be inhibited, including proteins that are not amenable for traditional drug inhibition such as structural proteins, etc. As a result, an increasing number of proof-of-principle studies have been conducted delivering siRNA to mice. Examples include systemic administration of siRNA in mouse models of hypercholesterolemia and rheumatoid arthritis (for a recent review, see Bumcroft et al. 2006). Along the same line, short hairpin RNA (shRNA) expressed from viral vectors such as RV or LV has been shown to inhibit specific gene expression, providing a more stable inhibition of gene expression than transiently transfected siRNA. siRNA delivered systemically to animals is degraded rapidly (Kim and Rossi 2007), making it more feasible to deliver siRNA with the help of a vector, e.g., lipids or nanoparticles.

The success of animal studies has now fueled the application of RNAi for use in primates and humans for the testing of treating various diseases (Dykhxoorn and Lieberman 2006). For example, it was shown that local delivery of siRNA to the lung was able to protect primates from the severe acute respiratory syndrome (SARS) coronavirus (Li et al. 2005a). Furthermore, human studies have shown that the delivery of uncomplexed, naked siRNA had success in the treatment of various human diseases (Dykhxoorn and Lieberman 2006). Survivin, telomerase, MDR1, and other genes critically involved in cancer growth and regulation, have been targeted by siRNA in vitro and in vivo with encouraging results (Martin and Caplen 2007; PutraI et al. 2006).

Altogether, significant progress has been made in applying RNAi as a therapeutic strategy in a relatively short period since its initial discovery. Undoubtedly, the biggest challenge for successful application of RNAi-based therapy remains in its delivery like for all other approaches. However, a variety of strategies are being explored to address this problem (Kim and Rossi 2007), and it is clear that the potential of RNAi-based technology for the treatment of cancer and other human diseases has yet to be fully achieved.

**18.3.2 Suppressor Genes**

The introduction of a tumor suppressor gene into a cancer cell slows down growth and results in a more
manageable cancer or at least that is the underlying hypothesis. The suppressor gene is not likely affecting normal cells since most types of cells in the body are already growth suppressed. One of the first tumor suppressors that was considered for gene therapy was the p53 gene (ROTH 2006). The introduction of the p53 tumor suppressor gene into tumor cells results in a complete halt of proliferation and increases apoptosis. However, clinical trials have been disappointing, primarily because of insufficient administration of virus into the tumor (ROTH 2006). A number of different permutations of the p53 approach have been attempted but in general, these strategies have not been successful primarily because of the underlying problem of poor penetration of the vector into the tumor bed (TERNOV et al. 2006). Expression of other tumor suppressor genes such as retinoblastoma, and PTEN as therapeutic proteins, aimed at slowing tumor growth have not been pursued to clinical trials mainly because of similar reasons as why p53 has not moved forward. On the other hand, significant advances have been made with melanoma differentiation-associated gene-7 (MDA-7/IL-24) perhaps due to this molecule’s multitude of attractive properties such as being a tumor suppressor and showing bystander effects that are specific for cancer cells (FISHER 2005; INOUE et al. 2006). In addition, cancer cells expressing MDA-7/IL-24 are also sensitized to chemo- and radiation therapy (FISHER 2005). A phase I trial in patients with solid tumors showed both clinical and biological effects (CUNNINGHAM et al. 2005; TONG et al. 2005), suggesting that MDA-7/IL-24 might be an excellent therapeutic molecule that might benefit patients with cancer.

18.3.3 Suicide Genes

The suicide gene concept is based on the expression of a heterologous gene coding for an enzyme that converts an inactive prodrug to an active drug that by itself or in combination with radiation results in increased cell kill (VALERIE 1999). Ideally and for maximum effect, the suicide gene should not exist in the target cells. The first suicide gene was the herpes simplex thymidine kinase (HSV-TK) gene used in combination with the antiviral drug ganciclovir (CULVER et al. 1994). The improved utilization of acyclovir, a drug currently used for treating herpes-associated encephalitis in children, with mutant HSV-TK (ROSENBERG et al. 2002; VALERIE et al. 2001), and bacterial and yeast cytosine deaminase with 5-fluorocytosine (KIEVIT et al. 1999; TRINH et al. 1995), have also been shown in animal models to be effective radiosensitizers. An added benefit of the suicide gene–prodrug concept is the fact that the active, toxic drug spreads to adjacent tumor cells by gap junctions or by cellular release that increases the toxicity to surrounding cells and improves the therapeutic effect about 10-fold. A number of phases I and II clinical studies have been conducted with suicide gene approaches. Focus here will be on those that combine the suicide gene concept with radiation therapy (see below).

18.3.4 Immunomodulatory Genes

To enhance tumor toxicity by using the cells own defense system, expression of various cytokines and other immunomodulatory proteins have been investigated as potential strategies for radiosensitization when expressed from various vectors. As with other combined modalities, the combinations of cytokine gene therapy with radiation or chemotheraphy have shown some promise in clinical settings. The most advanced studies are those based on tumor necrosis factor-α (TNF-α) in combination with radiation (KUFE and WEICHSELBAUM 2003). TNF-α has potent antitumor and antiangiogenesis activities that synergize with radiation therapy. TNFerade™ was developed as an ADV that expressed TNF-α under control of a radiation-inducible promoter that limits toxicities to the irradiated area, which is not the case with the direct injection of TNF-α into the tumor.

Others studies have combined radiation therapy with IL-3 immunotherapy in preclinical models (CHIANG et al. 2000; TSAI et al. 2006). However, these studies and similar involving other immunotherapy-based approaches in combination with radiotherapy have not moved forward to clinical trials.

18.4 Clinical Applications Related to Radiotherapy and Imaging

The number of clinical trials aimed at determining the safety and/or efficacy of cancer gene therapy continue to grow. The discussion herein is limited to trials that focus on treatments combined with radiation therapy. TNFerade™ is an adenovirus expressing the cytokine TNF-α under control of a radiation-induced promoter (MUNDT et al. 2004). A phase I trial with TNFerade™ has been completed for metastatic solid tumors (SENZER et al. 2004). The treatment was well tolerated, with only
minor toxicities with doses as high as $4 \times 10^{11}$ particle units (PU) corresponding to approximately $1\cdot2 \times 10^{10}$ infectious units (IU). Controlled prospective clinical trials have been initiated with patients with locally advanced pancreatic cancer to better define the therapeutic contribution of TNFerade™ (CHANG et al. 2008).

Several phases I/II trials using a multiprong approach combining two suicide gene–prodrug approaches (CD+5-FC and HSV-TK+GCV) with the ONYX-015 conditionally replicating ADV and image-guided radiation therapy (IMRT) have been conducted, including one for patients with intermediate to high-risk prostate cancer that was recently completed (FREYTAG et al. 2007b). There were no dose-limiting toxicities or treatment-related serious adverse events in this trial. Relative to a previous trial using a first-generation ADV, there was no increase in hematologic, hepatic, gastrointestinal, or genitourinary toxicities. Posttreatment prostate biopsies yielded provocative preliminary results. When the results were categorized by prognostic risk, most of the treatment effect was observed in the intermediate-risk group, with 0 of 12 patients being positive for cancer at their last biopsy.

An improved ADV vector expressing yeast CD and mutant HSV-TK for better utilization of the prodrugs in an oncolytic ADV backbone were combined with co-expression of the ADV death protein (facilitates cell lysis and improves ADV spread) in a preclinical pancreatic cancer animal model. Because a substrate for HSV-TK labeled with $[{\text{18}}^F]$, 9-(4-$[{\text{18}}^F]$-fluoro-3-hydroxy-methylbutyl)guanine ($[{\text{18}}^F]$-FHGB), can be used as a probe for PET, infected tumor cells can be imaged (FREYTAG et al. 2007a). ADV was readily detected in the pancreas but not in other tissues, suggesting that this ADV can be combined with radiotherapy of the pancreas without resulting in excessive systemic toxicity. Currently, this novel ADV is undergoing a phase I trial in patients with pancreatic cancer. Other studies have tested the feasibility of using 8-$[{\text{18}}^F]$fluoroganciclovir (FGCV) as probe for imaging HSV-TK-expressing cells and tissues in preclinical animal models using microPET with similar positive results (LU et al. 2006).

Most times the therapeutic protein itself is not imageable. In this case, a second protein needs to be expressed that can be imaged, ideally with clinically suitable imaging technologies such as PET or MRI. The vector for such studies is usually limited to ADV or any other viral vector able to harbor larger DNA inserts. One example of this strategy is using the same double suicide gene prodrug with conditionally replicating ADV as mentioned above and co-expressing the human iodide symporter (hNIS) gene as a reporter for single-photon emission computed tomography (SPECT) (SIDDIQUI et al. 2007). hNIS will sequester the probe sodium pertechnetate (Na$^{99m}$TcO$_4$) to affected tissues, in this case the prostate (SIDDIQUI et al. 2007). Na$^{99m}$TcO$_4$ is an US Food and Drug Administration (FDA)-approved diagnostic imaging probe. It was found that SPECT images were readily detected up to 4 days after administering the ADV. Currently, such ADV is undergoing clinical testing. The ability to image the expression of the therapeutic gene increases the information gained from clinical trials tremendously. In the future, more, novel ways of imaging tumors by PET, MRI, or other clinically utilized imaging technologies of patients undergoing gene therapy, will likely continue to facilitate the assessment of vector penetration and treatment efficacy.

18.5

Conclusion

The idea of using cancer gene therapy to improve chemo- and radiotherapy was initially very exciting. Promising results were generated in various animal tumor models. However, clinical trials have so far shown little to no impact on patient survival, with a range of different types of cancers. Almost 20 years later, the field is still trying to deal with technical issues. Regardless of therapeutic strategy, the efficient delivery of therapeutic DNA or RNA into tumors needs to improve. RNAi-based strategies look very promising and so does the nanoparticles because of this vector’s added benefit of being able to image the targeted cells and tissues. It is very likely that many of these technical hurdles will be overcome in the future, resulting in improved clinical outcome. In addition, more insights into the unique properties of cancer cells will continue to open up new targeting opportunities and move the field forward.

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