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Chapter 6

Gene therapy for cancer treatment

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Advances in knowledge and techniques for manipulating genes have lead scientists to alter genetic information to treat or prevent disease. Gene therapy for cancer is a novel and experimental treatment that involves transferring nucleic acids into cells to treat cancer. Gene therapy has been studied in clinical trials for different types of cancers but has not yet been integrated into standard treatments. Some hurdles must be overcome before the clinical application of this novel treatment becomes realistic. This chapter discusses the current advances and trends in cancer gene therapy and its clinical outlook through recent Phase III trials.

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The broad definition of cancer includes malignancies such as leukemias and lymphomas, which are derived from blood-forming tissues, and carcinomas and sarcomas, which are derived from solid organs. Here, we use the term cancer in a narrow sense as a malignancy derived from solid organs. Despite developments in the prevention, early diagnosis and treatment of cancer, there has been a steady rise in the occurrence of cancer along with the extension of life expectancy. Thus, cancer remains a serious worldwide health problem. Owing to the asymptomatic nature of cancer, it is often diagnosed at an advanced stage, which limits the possibility of a complete resection and increases the possibility of a recurrence after treatment. Although surgery, chemotherapy and radiotherapy constitute the conventional cancer treatment modality, complete surgical resection remains the most effective treatment and the only way to cure cancer. However, in cases of advanced-stage cancer, daughter cells from the primary tumor have metastasized to distant locations in the body, and the metastatic disease may not necessarily be visible by imaging studies. The majority of advanced cancers remain resistant to conventional chemotherapy and radiotherapy, thus novel treatments are needed.

Gene therapy emerged as a promising alternative for the treatment of cancer more than two decades ago, and numerous clinical trials of gene therapies have been conducted. Gene therapy approaches to cancer treatment include the replacement of mutant or defective genes, enhancement of the immune response, targeted killing of cancer cells and inhibition of angiogenesis. This chapter focuses only on representative cancer gene therapies that have reached Phase III clinical studies.

Gene replacement or addition strategy with nonreplicative virus vectors

Ad-*p53*

The direct application of the initial concept of gene therapy for genetic disease is to replace mutant or defective genes with wild-type genes. Because missing or altered genes, such as *p53*, may cause cancer, the substitution of these genes may be used to treat cancer. This intuitive approach was initially promising, and several clinical trials have been conducted. The most widely studied application of this approach is *p53* gene therapy. *p53* gene is a tumor suppressor gene that is crucial to the regulation of the cell cycle and the control of apoptotic cell death [1,2]. *p53* functions to maintain the genetic integrity of the cell and to induce apoptosis when DNA damage is too severe to produce normal progeny cells. It controls cell-cycle regulation, apoptosis and DNA repair. Thus,

abnormalities in *p53* cause deregulations of the cell cycle and apoptotic pathways, which are among the most common and fundamental molecular mechanisms of cancer pathogenesis and treatment resistance [3]. These observations formed the rationale for developing *p53* cancer therapy [4–6].

Head and neck squamous cell cancer (HNSCC) frequently exhibits inactivation of the tumor suppressor gene *p53* by mutation [7], overexpression of the primary negative regulator of *p53* [8], inactivation of the inhibitor of the negative regulator [9,10] and interference with *p53* posttranslational modifications that may be necessary for the gene to function [11]. Ad-*p53* is a replication-defective adenoviral vector that consists of the cytomegalovirus promoter, wild-type human *p53* cDNA, and a SV40 polyadenylation signal inserted into the E1-deleted region of modified adenovirus-5 [12,13]. Ad-*p53* treatment, in combination with radiation and/or chemotherapy, results in dramatic apoptosis in *p53*-deficient cancer cell lines [14,15]. Extensive animal studies revealed significant efficacy after intratumoral injection with Ad-*p53* in human cancer xenograft models. Repeated intratumoral injections of Ad-*p53* were well tolerated in Phase I trials, resulted in *p53* transgene expression, and were associated with antitumor activity [16]. The main side effects were transient fever and local inflammatory responses [17]. Phase II trials of Ad-*p53* have been conducted in various types of cancers including unresectable recurrent HNSCC [18].

Encouraging results from Phase I and II trials lead to the development of two Phase III trials, which compared Ad-*p53* with methotrexate and analyzed the results for their correlation with *p53* biomarkers in advanced recurrent HNSCC [18]. Vast majority of responded tumors to Ad-*p53* therapy had wild-type *p53* that was inactivated by upregulation of the *p53* inhibitors Mdm-2 or Mdm-4, or had low expression of mutated *p53*. Patients with this favorable *p53* profile showed a significant increase in survival compared with those with an unfavorable *p53* profile (7.2 vs 2.7 months). The Phase III trial demonstrated a lower toxicity profile of Ad-*p53*, supporting the data of Phase I and II trials. However, application of Ad-*p53* as a local regional therapeutic did not have the expected effectiveness in patients with HNSCC. Therefore, further clinical development of Ad-*p53* for the treatment of HNSCC was stopped.

In ovarian cancer, promising preclinical and clinical data also led to the initiation of an international randomized Phase II and III trial of Ad-*p53* that was intraperitoneally administered in combination with standard chemotherapy to patients with ovarian cancers containing *p53* mutations. However, the study was closed after the first interim analysis due to the lack of adequate therapeutic benefit.

Ad-*p53* gene therapy failed, although the high frequency of *p53* mutations and the central role of *p53* in regulating growth and apoptosis suggested that the *p53* gene would be an ideal target for gene replacement therapy. There are several possible reasons for its failure. The repair of a single gene might not be a suitable strategy for the treatment of cancers, which have multiple genetic changes and epigenetic dysregulations. There is also a substantial problem in targeting tumors with adenovirus. The heterogeneity or lack of expression of receptors and cofactors in tumors and the presence of adenovirus-neutralizing antibodies in patients are inevitable [19].

TNFerade™

TNF-α is a soluble cytokine and mediator of the cellular immune response with potent anticancer activities [20]. Its anticancer activities are exerted through apoptosis, necrosis, antiangiogenesis, immunomodulation and direct antitumor toxicity. The most critical anticancer mechanism of TNF-α would be the production of hydroxyl radicals, which lead to DNA damage. Radiation therapy also produces cell damage by free-radical formation, thus a synergistic interaction between TNF-α and radiation is anticipated.

TNFerade™ biologic (GenVec Inc., MD, USA) is an adenoviral vector that contains the gene for TNF-α under the control of a radiation-inducible promoter [21]. Administration of TNFerade biologic and subsequent activation of the *TNF-α* gene by radiation provides spatial and temporal control of the expression of *TNF-α* in tumors [22]. In Phase I and II studies, TNFerade biologic was injected into locally advanced pancreatic carcinomas by using endoscopic ultrasound or percutaneous administration once a week for 5 weeks together with radiation and 5-fluorouracil. Dose-limiting toxicities were pancreatitis and cholangitis. The antitumor activities among 50 patients consisted of one complete response, three partial responses and 12 patients with stable disease [23].

These results led to a Phase III study. The Pancreatic Cancer Clinical Trial was a multicenter, randomized, active and controlled study of 330 patients designed to evaluate the safety and efficacy of TNFerade plus standard of care versus standard of care alone in patients with locally advanced pancreatic cancer. This gene therapy was injected directly into pancreatic cancer and was studied in combination with standard chemoradiation [23]. However, the study was stopped after an interim analysis because the trial could not demonstrate clinically relevant evidence of effectiveness. The data did not yield the statistical significance required for the approval of a biological license application and thereby warranted discontinuation of the trial.

The goal of this gene therapy strategy was to improve overall survival in patients with advanced cancer. Like Ad-*p53*, TNFerade biologic is a nonreplicating adenoviral vector, and the main effect of this strategy would be local disease control by direct tumor cell killing. Therefore, this kind of therapy needs to target locally advanced but limited diseases. Otherwise, advanced disease in which the symptoms are relieved by local control should be targeted, and the end point would be improved quality of life, rather than extended survival.

ProstAtak™

Suicide gene therapy is based on the transduction of a viral or bacterial gene that encodes an enzyme able to convert a nontoxic prodrug into a lethal drug. The herpes simplex virus thymidine kinase gene (*HSV-tk*) with ganciclovir (GCV) [24] and the cytosine deaminase gene of *Escherichia coli* with 5-fluorocytosine [25] are the most extensively studied suicide gene therapies. In the *HSV-tk*/GCV system, the expression of viral thymidine kinase metabolizes GCV to ganciclovir monophosphate, which is then converted to ganciclovir triphosphate by cellular kinases. The phosphorylated compounds are nucleotide analogs that are incorporated into DNA during cell division, leading to the termination of DNA replication and cell death [26,27]. The number of cells killed significantly exceeds the number of cells transduced with the *HSV-tk* gene, a phenomenon known as the bystander effect [27]. In addition to the local bystander effect, a systemic bystander effect that generates protection against tumor rechallenge was observed [28].

The promising results in the preclinical studies with the *HSV-tk*/GCV system led to clinical trials in various cancers including prostate cancer [29,30]. This approach has now entered a Phase III trial in which the local administration ProstAtak™ (Advantagene, MA, USA), an adenovirus expressing the *HSV-tk* gene, is followed by valacyclovir, a valine-ester of acyclovir as an oral formulation, in combination with standard external beam radiation therapy with or without hormonal therapy for localized prostate cancer. The results of this ongoing trial are highly anticipated.

Replication-selective oncolytic virotherapy

OncoVEX$^{GM\text{-}CSF}$

Tumor-killing oncolytic viruses are lytic viruses that replicate selectively in cancer cells and lyze them before spreading to adjacent cells [31]. JS1/34.5-/47-/granulocyte–macrophage colony-stimulating factor (GM-CSF), namely OncoVEX$^{GM\text{-}CSF}$, is an immune-enhanced oncolytic herpes simplex virus type 1. It is deleted for neurovirulence factor ICP34.5, which provides tumor-selective replication, and ICP47, which promotes antigen

Aa **Oncolytic virus:** naturally occurring or genetically engineered viruses that can selectively proliferate in and kill infected cancer cells. This selective replication in a tumor theoretically increases the therapeutic index of this agent. Oncolytic virus can also be modified as a vector to carry various therapeutic genes encoding toxic proteins or cytokines including granulocyte–macrophage colony-stimulating factor (GM-CSF).

GM-CSF: a cytokine that stimulates the differentiation of hematopoietic progenitor cells into dendritic cells, which are potent antigen-presenting cells. This property may enhance the presentation of tumor antigens in dendritic cells after immunization with tumors that express GM-CSF.

presentation [32]. The gene for GM-CSF was inserted to maximize the immune response generated following the release of tumor antigens by virus replication. The virus was tested *in vitro* in human tumor cell lines and *in vivo* in mice and demonstrated significant anti-tumor effects [33]. *In vivo*, both injected and noninjected tumors showed significant shrinkage or clearance and mice were protected against rechallenge with tumor cells [33]. The virus would therefore be expected to have a potent oncolytic anti-tumor effect and also function as a patient-specific tumor vaccine.

A Phase I study has established safety and clinical activity in various tumor types, including melanoma [32]. In a Phase II clinical trial, the direct injection of OncoVEX$^{GM\text{-}CSF}$ into melanoma lesions resulted in a 26% objective response rate [34]. Responding patients demonstrated regression of both injected and uninjected lesions, indicating that OncoVEX$^{GM\text{-}CSF}$ has both a direct oncolytic effect in injected tumors and an immune-mediated anti-tumor effect on distant tumors. Based on these preliminary results, a prospective, randomized Phase III clinical trial in patients with unresectable stage IIIb or IIIc and stage IV melanoma, the OncoVEX Pivotal Trial in Melanoma has been initiated and is now recruiting patients [35].

Reolysin®

Reoviruses (respiratory enteric orphan viruses) are cytoplasmically replicating viruses comprised of two concentric protein capsids surrounding a genome consisting of ten segments of dsRNA [36]. Studies of human volunteers in the 1960s indicated that reoviruses possibly play an etiologic role in the generation of minor respiratory/enteric illnesses, but in general reovirus infections are asymptomatic [37]. Thus, they were initially classified as orphan viruses, indicating a virus that is not associated with any known severe human disease. There are three serotypes of reoviruses that are based on their hemagglutination activity. Reovirus type 3 Dearing, a naturally occurring virus, exerted significant antitumor effects in preclinical *in vitro* and *in vivo* studies and has been developed as oncolytic viral agent, Reolysin® (Oncolytics Biotech Inc., Alberta, Canada) [38]. Hashiro *et al.* reported that transformed cell lines were susceptible to reovirus infection, whereas normal human cells were spared [39]. Transformed cells with oncogenes such as *Ras*, *Sos*, *v-ervB* and *c-myc* were susceptible to reovirus infection [40–42]. Reovirus can also

activate both innate and adaptive immune responses against murine and human tumors [43,44]. Collectively, these observations suggested that reovirus has an innate anticancer potential, which led to its use as a powerful anticancer agent against *Ras* oncogenic tumors.

Intravenous administration of Reolysin had favorable toxicity profiles with preliminary evidence of antitumor activity [45]. Interestingly, despite the presence of neutralizing antibodies, viral localization and replication in tumors were confirmed by biopsies in some patients. In addition, Reolysin combined with radiotherapy or chemotherapy has showed feasibility and safety, with a number of patients showing disease responses [46–48].

Systemic rather than intralesional administration of Reolysin enables the virus to reach metastatic sites and makes this agent more generally applicable for clinical development. Based on the results of Phase I and Phase II studies, the agent is now in a Phase III trial with paclitaxel and carboplatin that was started in 2010 for patients with relapsed or metastatic HNSCC.

Immune vaccine gene therapy

PROSTVAC®

Prostate-specific antigen (PSA) is a serine protease secreted by prostatic epithelial cells that is widely used as a marker for prostate cancer [49]. The tissue specificity of PSA makes it a potential target for specific immunotherapy, especially in prostate cancer patients after prostatectomy and in whom the PSA-expressing tissue exists only in metastatic sites. Initial clinical studies with a recombinant vaccinia viral vector expressing PSA demonstrated an immune response and clinical efficacy [50]. PSA-targeted pox viral vaccines for prostate cancer were developed in subsequent preclinical studies. PROSTVAC® (BN ImmunoTherapeutics Inc., CA, USA) is a sequentially dosed combination of two different poxviruses which each encode PSA plus three immune-enhancing costimulatory molecules, B7.1 (CD80), ICAM-1 (CD45) and Lfa-3 (CD58), which are designated as TRICOM [51]. The first poxvirus is replication competent and is good for immune priming, which was termed Vaccinia-PSA-TRICOM. Fowlpox-PSA-TRICOM is the second poxvirus, a nonreplicating virus, which is good for repetitive immune boosting. The PROSTVAC is given as monthly injections starting with a Vaccinia-PSA-TRICOM priming dose and followed by 6-monthly Fowlpox-PSA-TRICOM boosts.

The latest trial, a randomized (2:1) Phase II placebo-controlled study of 125 patients with metastatic prostate cancer showed that patients receiving PROSTVAC had significantly longer overall survival by an average of 8.5 months as compared with the control group and that PROSTVAC had an adequate safety and tolerability profile [52]. Patients are being recruited for

a Phase III study to determine whether PROSTVAC alone or in combination with GM-CSF is effective in prolonging overall survival in men with few or no symptoms from metastatic, castration-resistant prostate cancer.

Perspectives of gene therapy for cancer

The Phase III trials described above are summarized in **Table 6.1**. Two of the trials have already failed, but the ongoing Phase III trials appear to be promising and are expected to yield good results. Most subjects in clinical trials of cancer gene therapy have not responded to standard cancer treatments, meaning that they have advanced-stage disease with an overwhelming cancer burden. In such cases, gene therapy can only provide a small improvement, if any, to the survival period, which must be difficult to demonstrate. Additionally, the patients in these trials have systemic disease that is not localized. There are two strategies to destroy cancer cells, direct cell killing by transduction of the gene of interest into cancer cells and indirect tumor suppression by eliciting an immune reaction against cancer by vaccination or by changing the microenvironment through, for instance, inhibition of angiogenesis. The former strategy represents *p53* gene therapy, which is expected to mainly kill the cancer cells in which it is expressed. *TNF-α* gene therapy is also a strategy to treat cancer cells reached by the gene therapy in combination with radiation. The lesson learned from the frontier gene therapy strategy with *p53* or *TNF-α* with replication-incompetent vectors is that local treatment for advanced cancer might rarely extend the limited period of survival, although it might bring some relief of cancer-related symptoms.

Overall, cancer gene therapy is shifting away from the local treatment model toward more systemic approaches. The recent trends are immunotherapy and oncolytic viruses that utilize the gene transfer and vector technology developed in numerous gene therapy studies. Suicide gene therapy by ProstAtak is expected to yield a systemic bystander effect, and OncoVEX$^{GM\text{-}CSF}$ and PROSTVAC are also expected to induce immune-mediated anti-tumor effects. In addition to conditionally replicative oncolytic virus, the development of intravenous, systemic administration would be a significant advance, because, so far, the existence of immunity has prevented the systemic administration of a viral agent. It will be interesting to see if Reolysin can demonstrate clinical efficacy against metastatic tumors.

In addition to the gene therapies that have reached Phase III trials, other promising gene therapies are also emerging. JX-594 is a Wyeth strain vaccine-derived oncolytic virus modified to inactivate the viral thymidine kinase gene and to express the GM-CSF and β-galactosidase genes [53]. Selective replication in cancer cells is driven by the EGFR/Ras pathway, thymidine kinase elevation and type I interferon resistance. A Phase I trial of intratumoral injection of