We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Cancer Gene Therapy

Hakan Akbulut, Muge Ocal and Gizem Sonugur

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/61775>

Abstract

Cancer treatment has been the major goal of the gene therapy studies over the decades. Although there is no cancer gene therapy drug in the market yet, substantial progress has been made in defining potential targets and in developing viral and nonviral gene delivery systems recently. Numerous genes have been studied as the targets for cancer gene therapy so far. Various gene therapy strategies, including suicide gene therapy, oncolytic viral therapies, antiangiogenesis, and gene therapy vaccines have been developed. The combination of gene therapy with conventional methods, such as chemotherapy, radiotherapy, and immunotherapy, has further improved the therapeutic efficacy. Although the preclinical and experimental studies have yielded highly encouraging results, there are still few gene therapy agents at phase III trials. In the current chapter, we will review gene transfer systems, targets, gene targeting strategies, and cancer gene therapy in the clinic.

Keywords: Cancer gene therapy, viral vectors, nonviral vectors, gene targeting

1. Introduction

The improvements in the past 20 years in the molecular biology have evoked optimism in the treatment of cancer and yielded a number of targeted drugs in the market. However, the curative treatment of the cancer has still been possible with only the early diagnosis and early intervention in the vast majority of the solid tumors. Almost half of the cancer patients diagnosed each year have been dying of the disease throughout the world. In particular, the patients with distant metastasis have no hope of cure with the current treatment modalities.

2. Cancer is a complex genetic disease

It has long been suggested that the cancer has evolved from a single cell transformed by the influence of the environmental factors such as physical, chemical factors, and viruses. Changes in hundreds of genes, so-called mutations, are required to transform a normal cell into a cancer cell. The major functional changes that transform a cell are mainly the activation of oncogenes or inactivation of tumor suppressor genes.

The overexpression of oncogenes and loss of function of tumor suppressor genes usually induce malignant transformation. Those changes are also required for further growth of tumor cells.

A transformed cell usually gains some important biological properties to establish a malignant disease. Those properties, including uncontrolled proliferation, evasion of growth suppressors, inhibition of apoptosis, replicative immortality, angiogenesis, proliferative signals, invasion, and metastasis, are discussed in detail in a recent review of Hanahan and Weinberg [1]. Although the conventional chemotherapy has mainly focused on direct tumor cell killing, a vast majority of current targeted therapies have aimed to eliminate one or more of the above-mentioned properties of cancer cells.

The targeting of angiogenesis, proliferation pathways, and immune system has yielded a number of drugs that are already in the market. Nodules of cancer cells cannot grow beyond 1–2 mm without expanding their blood supply to access every increasing need for oxygen and nutrients. In order to generate the additional blood supply, the tumor tissue stimulates the elaboration of its own vessel network, through a process called angiogenesis [2]. If one could cut the blood supply of the tumor, it cannot grow beyond 1–2 mm, which means that they cannot grow enough to be diagnosed by the current diagnostic technology and cannot cause a clinical disease. The tumor vascular targeting therapy or antiangiogenetic therapies like bevacizumab and aflibercept targeting ligands of angiogenesis or small tyrosine kinase inhibitors of angiogenesis pathway receptors or signaling molecules have already emerged as standard therapeutic drugs in various tumors [3].

The overexpression of oncogenes and the loss of function of tumor suppressor genes are usually involved in both malignant conversion of the cells and further growth of tumor cells. A new generation of small molecules targeting proliferation pathways, like gefitinib, erlotinib, and imatinib, has been developed to block the cancer-causing signals within cancer cells and become standard treatments in those patients with mutations of EGFR or c-KIT [4]. Antibody molecules, targeting the EGFR family of receptors like trastuzumab, cetuximab, and panitumumab also block the growth-promoting signals that push cancer cells into an unregulated pattern of growth [5]. In contrast to standard chemotherapy, which is quite damaging to the normal tissues of the body as well as the cancer tissue, the targeted drugs are quite specific for the cancer cells and therefore relatively free of side effects.

Although majority of the cancer patients has a fairly intact immune system, the cells of the immune system do not usually respond to tumor cells because the immune system cannot differentiate the normal and cancer cells and therefore cannot fight against them. Immuno-

therapy or cancer vaccine therapy aims to activate immune system against tumors. Recently, ipilimumab/tremelimumab and pembrolizumab/nivolumab targeting checkpoints of immune response such as CTLA-4 or PD1 have also been approved [6]. Likewise, a dendritic cell-based vaccine, sipuleucel T, for the treatment of metastatic prostate cancer has been approved 2 years ago [7].

Hundreds of genes have been involved in the action and regulation of those pathways. The generation of cancer through a series of changes in the normal cellular genes makes the disease a genetic disease at the cellular base. The involvement of genes in the development of the disease also makes the disease a good candidate for gene therapy. Therefore, gene therapy has emerged as the hope of curative treatment modality in cancer.

3. What is gene therapy?

Gene therapy can be defined as the delivery of genetic elements to the cancer cell or to the cells of the immune response in order to correct the abnormalities in the cancer tissue or to induce an immune response against the cancer cells. The corrective strategies can involve replacing missing or defective genes, i.e., tumor suppressor genes [8], suppressing the action of cancer promoting oncogenes [9], or programming normal or cancer cells to release into the systemic circulation molecules which suppress the growth of cancer cells or their vasculature [10].

There are some prerequisites for a successful gene therapy program in cancer, such as a suitable target to be replaced or modified, a carrier to reach the interest of gene to the cell, a successful targeting of the vector, and a sufficient expression of the therapeutic genes in the target cells. Besides a strong therapeutic efficacy, safety is also mandatory for the success of the treatment.

Unraveling the mystery of the genetic changes in the development of cancer has been proposed many genes as targets for gene therapy studies. The second step in gene therapy following the identification of a suitable gene is to introduce it into the target cell. Different vehicles (vectors) have been used to introduce the genes into the cells, such as viral vectors, nonviral vectors, and cell-based carriers. The mainly used viral vectors in cancer gene therapy are retroviruses, adenoviruses, and adeno-associated viruses. The gene therapist uses the capability of the virus to enter and reprogram the action of cells for purposes of therapy. The therapeutic genetic element is first placed into a viral backbone to produce a complete therapeutic viral vector. Alternatively, the therapeutic genetic elements can be delivered into the cancer cells through droplets of fat called liposomes or nanoparticles. The genes themselves, in the form of naked DNA or DNA packed into particles can be administered locally or systemically.

A third way of delivering genes to the target tissues is accomplished by using living cells such as irradiated tumor cells, blood cells, and mesenchymal or neuronal stem cells. All of these cells have the capability to home to particular types of target tissue through the blood stream. In this way, the therapeutic genes can be placed into the brain or other target tissues because of the homing properties of those cells.

For the safety of the procedure and the increased therapeutic efficacy, the genes of interest should be expressed in only target cells or tissues. Sparing of the normal cells and tissues is one of the keystones in their clinical use. The target specificity of the vectors could be achieved by the targeting of those specific to the tumor cells or tissues.

4. Gene transfer systems of cancer gene therapy

There are three main ways of transferring genes into the tumor cells: nonviral vectors, viral vectors, and cell-based vehicles. For most of the tumors, a relatively short-term expression of therapeutic genes may be sufficient to kill the tumor cells. Rapid clearance of viral vectors from the blood stream has enabled the development of synthetic gene delivery vectors. However, an important drawback for these approaches is to carry the DNA of interest to the distant metastatic deposits. The nonviral gene delivery vectors have usually been injected locally to the tumors. Although local injection is reasonable for tumors as melanoma, head and neck cancers, or peritoneal carcinomatosis; it is not suitable in patients with hematogenous metastases. The limitations of the viral vectors are also valid for the nonviral vectors for gene therapy. They have to survive through the blood stream to be arrested in the target tumor tissue, to extravasate, and to bind to specific cells and to enter the cells and then to reach the nucleus.

4.1. Nonviral vectors

Plasmid DNA, which is mostly used as nonviral gene therapy modality, is easily degraded by nucleases [11]. Therefore, some strategies to reduce the size and prevent the degradation have been developed. The most commonly used agents for gene delivery are *cationic lipids* [12]. The cationic head group of the lipids binds to DNA and the lipid tail enables the collapse of the DNA lipid complex [13]. Cationic lipid DNA complexes (lipoplexes) (LPD/DNA) enter the target cell through an endosomal pathway. However, the transgene expression efficiency is very low with lipoplexes. It has been shown that only a very small portion of the systemically injected DNA could be reached to tumor tissue [14].

Lipid-based formulations of gene delivery have been predominantly limited to the intratumoral or local applications. The systemic administration carries the potential risk of adverse inflammatory and immune reactions. The development of systemic lipid delivery systems with the modifications to reduce the systemic toxicity could have the potential for clinical use in cancer gene therapy. In an animal model of breast cancer, folate-targeted lipid-protamine DNA complexes (LPD-PEG-folate) have been shown to reduce the tumor volume and increase the survival when administered systemically [14].

Neutral liposomes composed of DOPC (1,2-dioleoyl-sn-phosphatidyl choline) and DOPE (1,2-dioleoyl-sn-phosphatidyl ethanol amine) and polycationic carrier proteins as protamine, polylysine, polyarginine, polyhistidine, or polyethylenimine (PEI) are also suitable to carry the DNA [15–18]. The *hydrophobic polymers*, such as polyethylene glycol (PEG), polyhydroxy propylmethacrylamide (pHPMA), and polyvinyl pyrrolidone (pVPyrr), have also been

used to mask the positive charge of DNA to extend its half-life in the blood [19,20]. Both the neutral liposomes and hydrophobic polymers yield less toxicity when administered systemically. The leaky nature of the blood vessels of the tumors allows the influx of macromolecules as polymer shielded DNA into the tumor. The PEGylation of plasmid DNA has been reported to circulate in the blood several hours and passively accumulate in the subcutaneous tumors in animals [21].

4.2. Viral vectors

Viruses have the natural ability to deliver the nucleic acids within its own genome to specific cell types, including cancer cells. This ability makes those attractive and popular gene-delivery vehicles. Retroviruses, adenoviruses, adeno-associated viruses, herpes simplex virus, poxviruses, and baculoviruses are commonly modified and used as gene therapy vectors in cancer. Additionally, chimeric viral-vector systems combining the properties of two or more virus type are also developed.

Retroviral vectors derived from retroviruses contain a linear single-stranded RNA of around 7–10 kb and have a lipid envelope. The viral particles enter the mammalian cells expressing appropriate receptors for retroviruses [22]. After entering the cell, the viral reverse transcriptase transcribes the virus RNA into double-stranded DNA (dsDNA). The dsDNA transcribed in the cytoplasm forms a nucleoprotein preintegration complex (PIC) by binding cellular proteins [23]. The PIC migrates to the nucleus and thereby integrates the host genome. The ability of transgene expression in only dividing cells is an advantage of retroviral vectors for cancer gene therapy to avoid undesired expression in nondividing cells of surrounding tissues. The incorporation of retroviral genes into the host genome provides long-term expression of transgenes. Although this is advantageous, a nonspecific incorporation of viral DNA could impair the function of host gene or induce aberrant expression of a cellular oncogene [24]. Although retroviral vectors have been the most widely used gene transfer vehicles in the clinic, the risk of insertional oncogenesis seen in the trial of X-SCID infants in 2003 has limited the use of retroviral gene transfer systems in humans [25]. The possibility of generating replication-competent retroviruses is another safety issue regarding the clinical use of those vectors [26].

Lentiviral vectors derived from retroviruses can cause stable integration of the transgene into the host genome with long-term gene expression. The ability of transducing both dividing and nondividing cells make those vectors more suitable and efficient gene transfer vehicle over retroviruses. Targeting strategies of vectors at the level of cell entry and transgene transcription improved the use of lentiviral vectors in gene therapy trials [27]. However, the biosafety concerns of random integration to the host genome as in retroviruses are the limitations of those vectors.

Adenoviral vectors are widely used to introduce the therapeutic genes into the tumor cells. They can infect a broad range of cell types, transfer the genes being not dependent on cell division, and have high titers and high level of gene expression [28]. The most widely used serotypes of adenoviruses to develop vectors in human cancer gene therapy studies are type 5 (Ad5) and type 2 (Ad2). They have the capacity of approximately 8–10 kb of therapeutic genes with first-

generation vectors and up to 36 kbp with gutless third generation adenoviral vectors [29]. However, along with the immunogenic potential, the broad range of host cells by adenovirus limits its systemic use in human cancer gene therapy trials [30]. Targeting strategies have enabled the use of adenoviral vectors in human gene therapy trials. Adenoviral vectors cannot integrate to cellular genome and express the transgene episomally. They cannot induce random mutations. However, the transgene expression is limited to 7–10 days postinfection [31]. Therefore, repeated administrations of the vector are needed to achieve sustainable responses in cancer treatment. Adenoviruses could be engineered either as replication deficient by deleting the immediate early genes of E1 or replication-competent keeping the E1 region. Replication-competent adenoviral vectors will be further discussed in the section of oncolytic viruses.

Adeno-associated viruses (AAV) are simple viruses with approximately single-stranded DNA of 4.7 kb in size [32]. They belong to parvovirus family and require a helper virus such as adenovirus or herpes virus for lytic replication and release from the cell [33]. They can infect a wide variety of cells independent of cell cycle. This property makes AAV as suitable vectors for cancer gene therapy. Furthermore, unlike adenoviruses, they elicit little immune response when infect the normal host cells. Another advantage of AAV over adenoviruses is their ability to integrate the transgene into a particular spot on the 19th chromosome of human cells [34]. Unlike retroviruses, AAV cannot induce mutations. However, the major drawback of AAV is its limited cargo capacity of approximately 4 kbp of therapeutic genes. AAV could transduce certain cell types. Therefore, targeting strategies such as modification of viral capsid proteins, binding monoclonal antibodies, or bispecific proteins have been developed to improve the efficiency of AAV systems in cancer gene therapy [35,36].

Baculoviruses are enveloped viral particles with a large dsDNA of approximately 80–180 kb. They naturally infect insect cells. There have been no diseases related to baculoviruses in humans. Along with their highly safety profile in humans, they seem very useful gene therapy vehicles with their highly large cargo capacity of approximately 40 kb with possible multiple inserts, easy manipulation, and production [37]. *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is the most widely used types of baculovirus in gene therapy studies. It has a circular dsDNA genome of 135 kb [38]. They can easily transduce mammalian cells, including many types of cancer cells, and cause high transgene expression in the host cell [39]. They are already approved for the production of human vaccine components such as Cervarix (GlaxoSmithKline) in cervical cancer and Provenge (Dendreon) in prostatic cancer [40].

Herpes simplex virus (HSV) is a large DNA virus with approximately 152 kb of dsDNA genome. It has a natural tropism to nerve tissues and cannot integrate into the host genome [41]. The HSV vectors can be designed in three different types as amplicons, replication-defective, and replication-competent vectors [42]. In general, the replication-competent HSV vectors are used as oncolytic agents in cancer gene therapy studies [43].

Poxviruses were the first viruses to be used as gene therapy vectors. They have been used in the in vitro production of proteins and as live vaccines. The attenuated forms of poxviruses have been developed and used in the development of genetic cancer vaccine trials [44]. The immunostimulatory properties of poxviruses make them preferable agents to induce immun-

ity against tumors. In particular, the attenuated MVA virus derived from chorioallantoic vaccinia Ankara (CVA), a Turkish smallpox vaccine strain, has been widely used in cancer vaccine development strategies [45].

5. Cells as the carriers of cancer gene therapy vectors

The systemic administration of the gene therapy vectors usually failed because of low titer achieved in the target tissue and insufficient transgene expression. The clearance of the vector by the immune system, sequestration, and nonspecific binding to nontarget tissues are the major drawbacks of viral and nonviral vectors [46,47]. In general, *in vivo* targeting has relied mainly upon the enhanced leakiness of the tumor vessels, allowing the extravasation and access to tumor cells. Besides, the target tropism, extravasations in tumor site, and poor penetration of the vectors into the tumor tissue are the major problems for the vectors to eradicate the metastatic tumor deposits.

Cell carriers have the potential of eliminating those problems. They are stable and most of them have tumor homing properties and can be administered locally, such as intraperitoneal or intratumoral injections or systemically. In case of the use of autologous cells, they will not be cleared by the immune system. Macrophages, bone marrow mesenchymal stem cells (MSC), T cells, NK cells, and eosinophils are the known cells infiltrating the tumor tissues. Also, the tumor cells themselves naturally have the potential of homing to the tumor deposits throughout the body.

Macrophages have been used to deliver therapeutic genes because of their naturally trafficking ability to sites of neoplastic diseases [48]. Further refinement of the targeting of these cells by using transcriptional promoters could avoid the transgene expression in other parts of the body where the macrophages naturally traveled [49].

T cells can be used to transfer the therapeutic genes to target tissues because of their ability to circulate through the body and arrest in tumor tissues [50]. T cells have the advantage of the release of vectors that they carry in an antigen-binding-specific manner. The T cells could also provide further antitumoral activity by their cytotoxic effects. Tumor infiltrating lymphocytes (TIL) are the first example of cell-based carriers in cancer therapy in which they were transfected with cytokine genes [51].

Mesenchymal progenitor cells from either bone marrow (MSC) or adipose tissue (PLA) have the potential to expand in culture and the differentiation along the adipogenic, osteogenic, chondrogenic, and myogenic lineages [52,53]. It has been shown that lentivirally transfected mesenchymal progenitors from the adipose tissue have sustained transgene expression, even after the differentiation into adipogenic and osteogenic lineages [54]. Further modifications of PLA cells transfected *ex vivo* in order to target tumor tissues of their natural potential differentiation would provide an efficient gene delivery vehicle.

Some other cells such as fibroblasts and allogeneic cells have also been used as cell carriers for gene therapy vectors [55,56]. Because of their homing properties to the tumor cell deposits,

tumor cells could be good candidates to target the established metastases. An animal model of MDA-MB-231 cells, transduced *ex vivo* by a CD carrying Ad vector, has been shown to reduce the tumor volumes in the established metastases of the tumor [57].

6. Gene targeting in cancer gene therapy

In order to maximize the therapeutic index of cancer gene therapy, the expression of therapeutic genes could be restricted to the target tissues. Therefore, the targeting of gene therapy vectors is the major key for the success of those treatments. There are two main targeting strategies: physical targeting and biological targeting.

6.1. Physical targeting

The first one is physical targeting by means of some physical methods such as local injections, catheters, gene guns, and electroporation. This strategy is usually used for local delivery of gene therapy vectors and is therefore not suitable for most of the cancer patients who may have cancer spread throughout the body. Supercoiled DNA molecules and oligonucleotides are also successfully delivered to the cells of the skin following intradermal injection to the tumor deposits accessible by local injections. However, intratumoral injection might have only the transducing capacity of the cells neighboring the needle. The tumor deposits in the body cavities such as peritoneum, pleura, and meninges and in subcutaneous tissues are the potential targets for the physical targeting of the gene therapy vectors in the clinic [58,59].

6.2. Biological targeting

In a second strategy, the viral or nonviral carriers of the genes are modified in such a way that they can only bind to tumor cells but not the normal cells. Because of the low transduction efficiency of the currently used gene therapy vectors in distant tissues when administered systemically, the specific transgene expression or viral replication in target tissues could provide an opportunity to achieve sufficient antitumor activity. To achieve this goal, *transcriptionally* and *transductionally* targeted vectors have been developed. For safety reasons, mostly the replication defective vectors have been used to transfer the therapeutic genes into tumor cells. However, because of limitations of vector delivery and relatively low levels of gene transfer capacity, replication-deficient vector systems are usually inefficient for the treatment of large solid tumors. Therefore, replicating vectors could efficiently transfer genes and also increase the therapeutic efficiency by means of its oncolytic effect. Such vectors could be targeted in such a way that they can replicate within the tumor cells but not in normal cells and cause no local or systemic toxicity.

6.3. Transcriptional targeting

The clinical utility of a cancer gene therapy program will be dependent on its therapeutic index. In order to maximize the therapeutic index, the expression of therapeutic genes could be

restricted to the target tissues. The selective targeting of gene therapy vectors to specific cells enables the delivery of therapeutic genes to the target cancer cells while sparing the normal tissues. This has the potential of the reducing the dose of vectors and toxicity.

Transcriptional targeting, which utilizes DNA regulatory (promoter/enhancer) elements that enable the expression of transgenes within specific cells, would probably decrease the toxicity of the treatment while increasing the specificity. The promoters used to drive the transgenes in viral or nonviral vectors targeted in cancer therapy could be tumor-selective, inducible, or cell cycle regulated. Certain genes have been expressed specifically in tumors such as L-plastin, survivin, telomerase, and midkine [60]. The vector constructs carrying tumor-specific promoters such as L-plastin, survivin, and midkine have been shown to efficiently eradicate tumor cells while sparing normal cells [61–63].

Likewise, the tumor-type-specific group of selective promoters shows a pattern of tumor tissue specificity. The promoters of oncofetal antigens such as carcinoembryonic antigen (CEA) and alpha-feto protein (AFP), mucin 1 (muc1), and oncogenes such as c-erbB2 and MYC have been used widely in the transcriptional targeting of gene therapy vectors to achieve specific transgene expression in tumor tissue [64–68].

The phenotypically heterogeneous expression of certain genes in certain tissues constitutes the basis of tissue-specific promoters in cancer gene therapy. The tissue specificity of those genes is largely regulated at the transcriptional level. Therefore, the promoters of those genes have been used to target cancer gene therapy vectors to specific tumor types in a specific manner of their origin of tissues. The tissue-specific promoters such as PSA in prostatic cancer [69], tyrosinase in melanoma [70], albumin in hepatocellular carcinoma [71], thyroglobulin (TG) in thyroid cancers [72], glial fibrillary acidic protein (GFAP) in glioblastoma [73], and osteocalcin (OC) in osteosarcoma [74] have been used to specifically target those tumors.

The inherent problems of tissue or tumor-specific promoters such as relative weakness and lack of true restriction of gene expression to the tumor tissues have led to the use of new promoters whose activity can be controlled exogenously. These systems also provide temporal control of gene expression. Various stress genes of the body are usually silent under normal conditions, but they are activated during the stress to protect the tissues. The stress genes up-regulated during stress such as heat, hypoxia, glucose deprivation, irradiation, and chemotherapy have opened a new avenue to the development of the tumor-specific targeting of gene therapy. The use of human heat shock protein (HSP)-driven HSV-TK or CD suicide gene therapy vectors has been a significant activity when combined with hyperthermia [75]. The promoter region of the hypoxia-inducible factor (HIF-1), a key regulator of the transcriptional response to oxygen, has been successfully used to target tumor cells [76]. MDR-1 gene encodes a 170-kDa P-glycoprotein and belongs to the ATP-binding cassette (ABC) family of transporters, which mediates the transport of some chemo drugs out of the cells thereby decreasing the efficacy of the treatment [77]. Therefore, the use of vector constructs carrying therapeutic genes under the control of MDR-1 promoter could efficiently target chemo-resistant tumor cells [78].

Uncontrolled cell proliferation is the prominent feature of cancer cells. The retinoblastoma family of proteins and their upstream regulators such as cyclin D, CDK4, and p16/INK4 regulate the G1 checkpoint in the cell cycle. Tumor suppression by Rb has been linked to its

ability to repress E2F-responsive promoters such as E2F-1 promoter. It has been shown that Ad vectors that contain transgenes driven by E2F-1 promoter can mediate tumor-selective gene expression *in vivo* in glioma cells [79]. The promoters of cell cycle genes such as cyclin D, cyclin A, cdc25c, cyclin-dependent kinase inhibitors, p16/INK4, p27, and p14 could be expected to exert cell cycle arrest, thereby increasing the apoptosis when used in vector targeting strategies in proliferating tumor or endothelial cells [80–83]. Additionally, drug-inducible systems such as tat-on/tat-off regulated by tetracycline or rapamycin could provide a wide-dose response range in the treatment [84].

6.4. Transductional targeting

The second strategy of biologic targeting is to engineer the either viral or nonviral vectors in such a way that they can be captured only in tumor tissues, and therapeutic genes are produced only in the environment of the tumor tissue. There have been numerous attempts to modify the vectors with tumor cell-specific ligands that would increase the specific binding to tumor cells and reduce the toxicity. Therefore, targeting DNA complexes to the tumor cell-specific receptors is an attractive strategy. One of the well-known strategies is coating the surface of the complexes with transferrin, an iron-binding plasma protein that is mainly an up-regulated expression on rapidly proliferating cells as tumors [85]. Likewise, coating with EGF has also been reported to cause a 50-fold increase in the transgene expression in hepatocellular carcinoma cells [86]. The suicide gene HSV-TK/PEI complex mixed with a single chain antibody (scFv) against EGFR with a negatively charged oligopeptide tail has exhibited EGFR-specific gene transfer *in vitro* and *in vivo* [87].

The nonviral systems usually fail in promoting the delivery of DNA to the nucleus. Almost 99% of the internalized DNA from a nonviral vector is degraded in the cytoplasm [88]. Trafficking of exogenous DNA from cytosol to the nucleus may be improved by using the nuclear localization signal (NLS) found in some nuclear proteins [89]. Dermaseptins, a family of antimicrobial peptides that destabilize the membrane, have been successfully linked to NLS of SV40-T antigen and HIV-1 Rev protein [90]. Likewise, mellitin, which is a membrane-active protein, and viral protein r (vpr) of HIV-1, which binds directly to nucleoporins of the nuclear pore complex, have been successfully bound to PEI/DNA complexes to improve nuclear transport [91].

The selective targeting of viral vectors to specific cells permits the cell-specific expression of transgenes and enables the systemic administration of the vectors. Avoiding the targeting of the native receptor found on immune and inflammatory cell surfaces also reduces the immunity and inflammation to those vectors. Replication-competent retroviral vectors (RCR) based on murine leukemia virus (MLV) represent an attractive system for gene delivery through their ability to replicate and provide long term transgene expression in rapidly proliferating cells [92]. However, the uncontrolled spread of the RCR might cause the infection of nontarget cells. In order to develop tumor-selective RCR vectors, several modifications have been made such as a modification of the envelope protein by inserting single chain antibodies (scFv) [93] and peptide ligands [94]. Also, the specifically targeted entry of replication-deficient retroviral vectors has been accomplished by combining cell-specific monoclonal antibodies [95,96].

The capability of an Ad vector to infect a cell is mainly based on CAR and integrin expression. Following the attachment of an adenoviral vector to the target cell via C-

terminal part of the fiber protein (knob) and CAR (Coxsackie's B adenovirus receptor), the $\alpha V \beta 3$ and $\alpha V \beta 5$ integrins mediate the internalization of the vector [97]. The CAR deficiency of the primary tumor cells limits the success of the gene therapy protocols using Ad vectors [98]. Redirecting the Ad vectors to bind other cellular receptors would allow CAR independent virus entry into the tumor cells. There are mainly two strategies to redirect the viral vectors to the cells: conjugate-based and genetically modified viral membranes. Adenoviral vectors have been targeted to different cells by genetic modification of the capsid or by using adapter molecules. In the conjugate-based strategy, it is aimed to complex the vector with the targeting molecule that redirects the vector to the cell-specific receptors. Bispecific molecules containing a first specificity for the fiber knob to block binding to CAR and the second specificity for a cell-specific receptor, such as bispecific fusion proteins (antibodies), bispecific peptides, polymer mediated ligand coupling, and chemical modifications (biotin-avidin bridges), have been utilized to target adenoviral vectors [99–101].

Adeno-associated vectors (AAV) possess a highly favorable safety profile and have the unique potential in certain cancer models. However, they have a restricted range of cells to transduce transgenes to the target tissues. In order to augment the transduction efficiency of AAV in various tissues retargeting strategies such as engineering of viral capsid, monoclonal antibodies and specific peptides have been used to successfully retarget the AAV vectors [102,103].

7. Targets for gene therapy of cancer

Current gene therapy studies have mainly focused on introducing the genes into the tumor cells to block the action of oncogene expression and the development of tumor vasculature, or to induce the development of an immune response against the cancer tissue. The major targets of gene therapy are shown on Table 1.

Tumor suppressor genes (p53, RB, APC, BRCA1)
Oncogenes (RAS, BCL-2, MET, MYC, ERBB2, HPV E6E7, etc.)
Drug-metabolizing enzymes (cytosine deaminase, HSV-thymidine kinase, cytochrom p450, purine nucleoside phosphorylase, carboxypeptidase A)
Direct cell killing (oncolytic vectors)
Angiogenesis (endostatin, angiostatin, VEGF, tissue factor, Tie2, etc.)
Cytokines (IL-2, IL-12, GM-CSF vb)
Immune system (T-cell receptor)/cancer vaccines (tumor-specific antigens, polynucleotide vaccines, genetically modified dendritic cell-based vaccines, and adoptive immunotherapies)

Table 1. The major targets of gene therapy of cancer

7.1. Tumor suppressor genes

Loss of functions of tumor suppressor genes have crucial role in the development and spread of cancer. Therefore, those genes were among the first targets of gene therapy studies. *P53* is

mutated in almost 60 percent of solid tumors. Reintroducing wild-type p53 has been one of the common gene therapy approaches within the last two decades. The introduction of wild-type p53 by retroviruses or replication-deficient adenoviral vectors into the cancer cells inhibits tumor growth both in vitro and in vivo [104]. The use of adenoviral vectors carrying p53 has yielded some clinical activities, particularly in patients with head and neck cancers and lung cancers used either as a single agent or in combination with chemotherapy or radiotherapy [105,106]. Likewise, strategies aiming at the activation of p53 pathway in patients with p53-mutated tumors have also been tried. The introduction of small synthetic peptides like CDB3 derived from p53-binding protein 2 or p53 C terminal peptide have been shown to reactivate the mutant p53 functions in vitro [107]. Furthermore, transductions of other family members of p53 like p63 and p73, which are known to transactivate the downstream genes of p53 pathway, have been shown to induce apoptosis of tumor cells [108,109].

RB1 is a tumor suppressor gene involved in cell cycle regulation. Constitutively active RB1 potently inhibits cellular proliferation and induce persistent cell cycle arrest [110]. Since the first cloning of the RB gene at the beginning of the nineties, researchers have tried to activate the tumor suppressor function of the RB pathway. Gene transfer of truncated RB protein, such as RB94, has been shown to restore the RB pathway and to induce potent tumor growth inhibition both in vitro and in vivo [111]. However, these strategies have not been tested in the clinical setting yet.

The restoration of functions of other tumor suppressor genes such as adenomatosis polyposis coli (APC) in colorectal cancer cells [112] and BRCA1 in breast and ovarian cancers [113] has been shown to slow the growth of tumor cells.

7.2. Oncogenes

The targeting of oncogenes has long been at the focus of drug development studies in cancer. Small molecules of inhibitors of oncogene functions such as tyrosine kinase inhibitors have already been used in the routine treatment of various cancers. Gene therapeutic strategies to suppress oncogene functions are usually focused on the inhibition of those genes at mRNA level. Usually small oligonucleotides or RNA inhibitors such as short-interfering RNA (siRNA), short-hairpin RNA (shRNA), or micro-RNA (miRNA) have been used to interfere the actions of oncogenes [114].

Chemically modified or unmodified small single-stranded DNA molecules, antisense oligonucleotides inhibit protein translation through the disruption of ribosome assembly or utilization of RNase H enzymes to destroy mRNA. Numerous oligonucleotides and RNA inhibitors have been designed to inhibit oncogenes, including RAS, MYC, BCL-2, or cell signaling molecules survivin, IGF, VEGF, and PKC α , have been tested. Although the efficacy of these oligonucleotides has shown a great diversity, some of them have been tested in phase II/III clinical trials in various cancer types [115]. Oblimersen, an antisense oligonucleotide targeting Bcl-2, is one of the oldest agents that have already tested in phase III studies of Chronic Lymphocytic Leukemia CLL and multiple myeloma [116,117]. The members of the RAS family of oncogenes have been found mutated in various solid tumors. Therefore, the targeting of RAS would have been a hot topic in the development of recent therapeutics.

Targeting RAS with an anti-RAS mRNA plasmid yielded significant tumor inhibition when used alone or in combination with chemotherapy in hepatoma cells [118]. Antisense oligonucleotides targeting survivin, which are highly expressed in various cancer types, including liver, lung, breast, and prostate, have been employed successfully to inhibit the expression of the gene [119]. The phase I/II clinical trials have also shown some responses in cancers [120].

7.3. Gene-Directed Enzyme/Prodrug Therapy (GDEPT)

Conventional chemotherapeutic drugs are mainly directed to nonspecific direct cell killing. However, dose-limiting toxicities avoid the use of higher doses of those drugs to eradicate the disseminated cancer. However, if the drug was synthesized within the tumor tissue, then the toxicity level would only increase in tumor cells but not other parts of the body. The tumor-specific targeting of drug-metabolizing genes and the systemic use of a prodrug that is converted to a cytotoxic agent by the action of transduced enzyme called gene-directed enzyme/prodrug therapy (GDEPT) enable the achievement of that aim. GDEPT is also known as suicide gene therapy. A lot of drug-metabolizing genes have been used to develop suicide gene therapy/prodrug systems. Cytosine deaminase (CD) and herpes simplex virus 1 thymidine kinase (HSV1-TK) are the most widely studied ones in cancer gene therapy [121,122]. CD, an enzyme found in fungi and bacteria, converts the nontoxic 5-fluorocytosine into a toxic chemotherapy drug of 5-fluorouracil. The lack of this enzyme in mammalian cells makes it a convenient gene therapy tool to achieve intratumoral chemotherapy. Others and we have designed suicide gene therapy vectors to avoid systemic toxicity of 5-FU. We have shown that Lp-driven CD carrying adenoviral vectors (AdLpCD) specifically target the epithelial cancers, including breast, ovary, prostate, and lung [123]. It is possible to achieve a 5-FU dose in tumor tissue as much as 200-fold of the dose when the drug is used intravenously at the standard dose [123]. The 5-FU produced in the infected tumor cells can diffuse into the neighboring tumor cells and kill them even not infected by the vector, which is called bystander effect [124]. Likewise, the combination of CD carrying vectors with conventional chemotherapy or radiotherapy yields synergistic efficacy [125–127].

TK, one of the immediate early (IE) genes of HSV, converts ganciclovir (GCV) into a triphosphated form of GCV, which is an analogue of purine and inhibits DNA polymerase [128]. HSV1-TK suicide gene therapy loaded onto either adenoviral vectors or retroviral vectors has been used to treat various tumors, including pancreatic cancer, hepatocellular carcinoma, lung cancer, glioma, and leukemia [129–133]. Although the exact mechanism of HSV-TK carrying vectors to kill tumor cells is not completely understood, they can induce apoptosis sensitizing the TNF-related ligands or the sensitization of CD95-L, TNF-related apoptosis inducing ligands may contribute to cell death [134]. The transcriptional targeting of HSV1-TK vectors using tumor-specific promoters has decreased the potential side effects [130]. HSV-TK/GCV prodrug systems have also been modified with other genes such as addition of E-cadherin to increase the bystander effect of the vector [129].

Other prodrug-activating enzymes such as purine nucleoside phosphorylase to convert 6-methylpurine-2-deoxyribose to 6-methyl purine, cytochrome p450 cyclophosphamide and ifosfamide to active metabolites of phosphoramidate mustard and acrolein cyanide, and

carboxypeptidase methotrexate- α peptides to methotrexate have also been reported to decrease tumor burden in various preclinical models [135–137].

Dying tumor cells during suicide gene therapy could induce a tumor-specific immune response. Therefore, combining prodrug/enzyme systems with an immunomodulating cytokine would further improve the efficacy. The addition of an IL-2 gene to the HSV-TK has yielded more potent antitumoral activity when compared the each strategy alone [138]. Similarly, GM-CSF, IL-12, and IL-18 have also been used to increase the antitumoral activity of suicide gene therapy [139,140]. Suicide gene therapy also successfully combines with other strategies such as targeting tumor angiogenesis or adoptive transfer [141,142].

7.4. Oncolytic viral vectors

Viruses have long been recognized tumor cell lytic agents and tried to treat cancer patients. However, the use of unmodified oncolytic viruses usually failed in the clinic. The engineering of those viruses to increase their therapeutic index have been possible in the last two decades. Herpes simplex virus (HSV), adenoviruses, parvoviruses, Newcastle disease virus, and retroviruses have been modified as oncolytic viral vectors.

HSV with its high infective capacity of a large number of cell types has been one of the popular oncolytic agents in the treatment of cancers. By deleting the genes thymidine kinase (TK), ribonucleotide reductase (RR), or ICP34.5 alone or in combination, HSV vectors could be selectively targeted many cancer types [143,144]. In order to further increase the cancer cell specificity of the replicating vector, engineering of the expression of surface glycoproteins, attachment of a novel receptor, or other macromolecules such as bispecific antibodies have been tested [145]. Likewise, tumor cell-specific promoters to drive the immediate-early gene expression, which is essential for viral replication, has been another effective strategy to obtain tumor-selective HSV [146].

Adenoviruses can infect a wide variety of dividing and nondividing normal and tumor cells. They can be engineered to have tumor-selective oncotropic properties or to be conditionally replicative (CRAds) for selective cancer gene therapy.

In type I CRAds, usually a mutant Ad vector that replicates specifically in tumor cells with aberrant cell cycle regulation has been developed. A deletion in the E1B 55-kDa region abrogates the p53 binding of the vector, and therefore, the vector cannot replicate in cells with intact p53 [147]. Therefore, this mutant Ad vector (dl1520) could replicate in only p53-deficient tumor cells. However, further studies revealed that E1B 55-kDa mutant CRAds could also replicate in p53 intact tumor cells [148,149]. The CRAds are already tested in phase II/III clinical trials with some success in patients with p53-deficient tumors [150]. Accordingly, the combination of CRAds with conventional treatment modalities provided better tumor control [151]. Although the combination of E1B-55kD mutant Ad vector with chemotherapy has yielded a promising result of 63% partial response in patients with head and neck cancer administered intratumorally [151], no objective responses were seen when the vector used alone [152,153].

Another way to achieve tumor-specific adenoviral replication is to take the advantage of altered cell cycle regulation at G1-S phase checkpoint in which the retinoblastoma 1 (RB1) gene

functions. In most of the cancer cells, there is a mutation in RB1 gene. Therefore, an Ad vector having a mutation in the RB-binding site of E1A cannot induce the quiescent cells to pass the checkpoint. A mutant CRAd carrying an E1A deletion, Ad5- Δ 24, is unable to replicate in normal cells with the wild-type RB1 gene [154]. It has been shown that this E1A mutant Ad vector has strong oncolytic activity in in vitro experiments of glioblastoma cells. Also, a similar vector with E1A mutations at RB-binding sites (dl922-947) has also been shown to have strong antitumor activity in other tumor models such as breast and colon cancer [155]. An additional promising strategy to achieve specific oncolytic activity to the CRAds is the use of tumor-specific promoters that drive the genes of the vector responsible for the replication, referred to as type II CRAds. There have been many replication-competent vectors carrying tumor- or tissue-specific promoters such as prostate-specific antigen (PSA), alphafeto protein (AFP), Tcf4, MUC1, and CEA that have been developed [156–160]. We have designed replication-competent adenoviral vectors carrying Lp-driven E1A, which are specifically replicated in various tumor cell lines but not in normal cells [161]. We have also constructed a bicistronic CRAd vector carrying both cytosine deaminase (CD) gene and E1A linked by an IRES component driven by the Lp promoter (AdLpCDIRESE1A) [162]. The new bicistronic construct also has been shown to have significant oncolytic activity in the colon (HTB-38), breast (MCF-7), ovary (Ovcar 5), and prostate (LNCaP) cancer cell lines but not in normal human mammary epithelial cells [162]. Also, the combination of the construct, AdLpCDIRESE1A/5flourocytosine system, and chemotherapy has shown synergistic activity [163].

Different replication-competent viruses are currently being studied for their potential use in cancer gene therapy. The naturally occurring tumor-selective viruses in their replication and cytolysis might have the potential in cancer treatment. Autonomous parvoviruses (APV) have been shown to replicate more efficiently in transformed cells than normal cells [164]. The members of the rodent group of APVs such as LuIII, MVM (minute virus of mice), and H1, which can infect human cells, are currently being studied as vectors for cancer gene therapy. The replication of APV depends on cellular functions expressed during the S phase of the cell cycle. The oncogenic transformation of cells favor the replication of APVs and therefore makes them as oncolytic viruses [165]. The overexpression of the RAS signaling pathway [166] and the defects in the interferon pathway of the transformed cells [167] could possibly enhance the oncolytic activity of the APVs. Further manipulation of the specific targeting of those vectors to achieve tumor-specific transgene expression such as inserting binding sites for the heterodimer beta-catenin/Tcf transcription factor to the MVM P4 promoter to make it responsive to wnt signaling would make those attractive vectors for cancer gene therapy [168].

Newcastle disease virus (NDV) is an animal virus showing oncolytic activity in transformed cells. In murine tumor xenograft models, the intratumoral administration of NDV has caused significant tumor reduction [169]. Also, the intraperitoneal injection of the virus has resulted in complete regressions of tumor xenografts. A replication-competent strain of NDV, PV701, has been shown to replicate in tumor tissues of patients with solid tumors when administered intravenously [170]. In that phase, trial objective responses have also been achieved at higher and repeated doses of the virus.

The murine hepatitis coronavirus (MHV), an oncolytic virus, is a positive-strand RNA virus displaying strong species specificity with a replication cycle of 10–15 h and efficiently kills cells by fusion of the infected cells with their neighboring cells [171]. Substituting its spike protein by the other species such as porcine amino peptidase could change the host cell tropism of the MHV. The resulting recombinant corona virus pMHV thus only infects porcine cells via the porcine amino peptide N (pAPN) receptor. In vitro studies have shown that the tumor cells could be more susceptible to that recombinant corona virus [172]. It is also likely to further manipulate those vectors by using specific antibodies.

7.5. Tumor vascular targeting therapy

Unraveling the mechanisms of tumor-induced angiogenesis, which is a key event in tumor growth and metastasis, has opened a new therapeutic era in cancer treatment. The antiangiogenic gene therapy approaches have been reported to inhibit the tumor-induced angiogenesis and therefore tumor growth. The main strategies in antiangiogenic gene therapy are targeting specifically the endothelial cells (direct antiangiogenic gene therapy) and interfering with a tumor-derived angiogenic factor or the receptor for it or delivery of genes that encode angiogenesis inhibitors (indirect antiangiogenic therapy).

Proangiogenic cytokines such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) mainly secreted from tumor cells are required for the new vessel formation. The indirect strategies were mainly focused on the inhibition of proangiogenic cytokines or receptors involved in VEGF pathway or basic fibroblast growth factor (bFGF). VEGF binds two high affinity receptors (VEGFR1/FLT-1 and VEGFR-2/KDR) that are expressed on endothelial cells. An adenovirus-mediated transfer of a secreted form of the extracellular domain of the FLT-1 (AdsFLT) has been shown to inhibit the growth of metastatic tumor deposits when administered intravenously to preestablished splenic and liver metastases from a murine colon carcinoma cell line in syngeneic mice [173].

Likewise, the delivery of genes encoding antiangiogenic proteins such as endostatin, angiostatin, platelet factor 4, interferon alpha, and thrombospondins have also been tested [174]. The intratumoral administration of a plasmid encoding murine endostatin under the control of a CMV promoter has provided elevated concentrations of endostatin high enough to obtain growth arrest of murine renal carcinoma cells and breast cancer model [175]. Likewise, an adenoviral vector carrying human endostatin gene markedly reduced the blood vessel density of the tumor in an orthotopic liver tumor model [176].

The viral vector constructs of other angiogenesis inhibitors such as angiostatin, thrombospondin, platelet factor 4, and hepatocyte growth factor antagonists have also been shown to successfully inhibit endothelial cell proliferation and tumor growth [177–180]. However, there are conflicting results regarding the tumor inhibiting activity of antiangiogenic gene therapy modality in experimental models. The combination of antiangiogenic gene therapy with chemotherapy or radiation could be an efficient way of the inhibition of tumor growth [181].

Many vector constructs carrying therapeutic or reporter genes driven by endothelium-specific promoters such as preproendothelin-1 (PPE-1), VEGFR kinase insert domain receptor

(KDR), VEGF, E-selectin, and endoglin/CD105 have been reported to specifically target endothelial cells [182,183]. The replication-competent adenoviral vectors driven by the regulatory elements of FLK-1 and endoglin have successfully been targeted to the dividing endothelial cells, and therefore, this strategy could be used as an antiangiogenic treatment for cancer [184]. The activation of proapoptotic caspases such as caspase 9, driven by endothelium-specific promoters such as VEGF and FGF, could be another strategy to destroy endothelial cells [185].

Antisense approaches also are being tested for the inhibition of VEGF. A recombinant adeno-associated virus (rAAV) vector encoding an antisense mRNA against VEGF has been shown to inhibit the production of endogenous tumor cell VEGF [186]. The adenovirus-mediated delivery of an uPA uPAR antagonist, which inhibits FGF, has been shown to inhibit angiogenesis-dependent tumor growth and metastasis in mice [187].

7.6. Immune system as the target of cancer gene therapy

The immune system is the most important defense mechanism of the body against cancer. Recent developments in gene therapy have suggested to many cancer therapists that cytokine–chemokine-based gene therapies, tumor antigen-specific vaccination strategies, and gene-modified cellular therapies have great potential for future use either in the treatment of an established disease or in the prevention of cancer in people having high risk of developing cancer.

Cytokine/chemokine-based gene therapy has been widely used to induce immune system against tumors. The delivery of immunomodulatory cytokines by gene therapy vectors has opened a new avenue both to decrease the toxicity of these cytokines when used systemically and to augment antitumor immunity. A wide variety of cytokines such as GM-CSF, IFN- α , IFN- γ , IL-2, IL-4, IL-12, IL-18, and IL-24 have been tested so far [188–191]. Also, the vector constructs, including the combination of these cytokines, have also been tested in cancer. The coexpression of IL-12 and GM-CSF has been reported to yield significantly more immune response than the either cytokines alone [192]. In particular, implementing the cytokine genes into oncolytic viruses has great potential for use in clinical trials [193]. Chemokines recruit the immune effector cells to the tumor microenvironment. The delivery of chemokines such as CCL-5 using viral vectors has also resulted in significant tumor reduction through increasing tumor infiltration of DCs, macrophages, and CTLs [194].

Tumor-associated antigens (TAA) loaded on to gene therapy vectors have been tested in cancer treatment (DNA vaccines) [195,196]. However, the efficacy of using TAA alone is not enough to get a sufficient immune response to decrease tumor size. Therefore, researchers have focused on the augmentation of the immune response by combining immune cytokines or costimulatory molecules and TAA. This strategy seems much better than using either gene alone. We have previously shown an increased efficacy of an adenoviral vector encoding a fusion protein of CD40L and MUC1 in preclinical models [197]. The addition of prodrug/enzyme system to DNA vaccination further increased the efficacy [198]. This strategy has also been tested in early clinical trials with some success. Vector vaccinations using cytokines or costimulatory molecules and tumor-associated antigens (TAA) have increased the immune responses and

caused antitumor responses in preclinical models and even some responses in earlier clinical trials. In a small clinical trial, an attenuated vaccinia vector carrying IL-2 and MUC1 has been found effective in a small group of patients with advanced prostatic cancer [199]. Likewise, a vector vaccine of canary poxvirus encoding B7.1 and CEA has been tested in a group of patients with epithelial tumors [200]. Hundreds of different DNA vaccines have been tested in clinical trials so far [202]. However, no DNA vaccine is available in the market.

Gene therapy vectors have also been used to transduce either autologous tumor cells or dendritic cells. In the earlier studies, irradiated autologous tumor cells transduced to express immunostimulatory molecules have been tested. In a syngeneic colon cancer model, the subcutaneous injection of CT26 colon cancer cells transduced with an adenoviral vector carrying GM-CSF gene has eliminated both the established tumors and prevented the growth of new tumor nodules when rechallenged with tumor cells [201]. Later on, this strategy has also been tested in human tumors. Autologous tumors transduced with GVAX, an adenovirus carrying GM-CSF, have induced tumor-specific immunity in a variety of tumors, including melanoma, prostate, and lung cancers [203]. Although a slight increase in overall survival has been reported in those trials, no significant tumor responses observed [203,204].

The ex vivo transduction of dendritic cells with gene therapy vectors carrying either immunostimulatory genes or TAAs is another promising strategy. When injected subcutaneously, the dendritic cells exposed to vectors migrate to the lymph nodes where they prime cytotoxic T cells and induce a strong immune response. A number of vectors have been designed to activate dendritic cells for the past two decades. We have tested the use of ex vivo transduced dendritic cells with an adenoviral vector carrying a fusion protein of CD40L and MUC1 in a syngeneic mouse tumor model [205]. The intratumoral injection of activated dendritic cells induced a potent tumor-specific T-cell response. Furthermore, the combination of suicide gene therapy of a CD/5FU system and activated dendritic cells caused a more potent immune response and increased tumor response [205]. Likewise, retroviral vectors and lentiviral vectors are both used to transduce dendritic cells [206]. A dendritic cell vaccine based on the ex vivo activation of mononuclear antigen presenting cells by a fusion protein consisting prostatic acid phosphatase and GM-CSF has extended the progression-free survival of patients with advanced prostatic cancer and approved by FDA in 2010 (Provenge®, Dendreon, USA) [207].

Recently, an adoptive therapy of cancer using genetically modified T cells armed with chimeric antigen receptors (CAR) has gained great popularity with the announcement of success in advanced malignancies [208]. CAR is a fusion receptor of an antibody-derived targeting domain and T-cell signaling domain and expressed on T cells by a retroviral vector [209]. CARs target antigens, including proteins, carbohydrates, and glycolipids without antigen processing or HLA recognition. They can be generated in significant quantities ex vivo and used with the minimal risk of autoimmunity or graft versus host disease [210,211]. However, because of the severe side effects, the most troublesome being cytokine-release syndrome, researchers try to obtain better CAR T cells with further refinement of receptor and better targets [212].

8. Cancer gene therapy in the clinic—Future prospects

The vast majority of the clinical trials of gene therapy have been devoted to the treatment of cancer so far. The gene therapy agents have been tested in many types of cancer in the clinic. Almost 1200 clinical trials (approximately 64% of all gene therapy trials) in cancer have been started, conducted, or completed [202]. Less than 4% of those are phase II or III and only few of them are phase IV trials [202]. Although the preclinical and experimental studies have yielded highly encouraging results, the progress in the clinic is not so remarkable. There is no gene therapy agent available in the market yet.

The most important factor that has limited the success of clinical gene therapy trials in human subjects is the delivery of the vector genetic elements or their products to the target cancer cells and their vasculature. A second problem has been toxicity. Recent advances on improving the delivery and specificity of gene therapy vectors have suggested these trials may be more successful in the coming years. This is especially true of the attempts to use vectors to activate the immune response against the tumor tissue. Continued testing of these strategies in the context of clinical trials may lead to new opportunities for individuals engaged in a personal struggle with cancer to control their disease.

Indeed, the nature of the distant spread of the disease, which causes the failure of conventional treatment modalities, is also one of the main drawbacks of gene therapy of cancer.

Author details

Hakan Akbulut*, Muge Ocal and Gizem Sonugur

*Address all correspondence to: akbulut@medicine.ankara.edu.tr

Gene Therapy Research Unit, Department of Medical Oncology, Ankara University School of Medicine, Ankara, Turkey

References

- [1] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144:646–74.
- [2] Fidler IJ. Seed and soil revisited: contribution of the organ microenvironment to cancer metastasis. *Surg Oncol Clin N Am* 2001;10:257–69.
- [3] Gacche RN, Meshram RJ. Angiogenic factors as potential drug target: efficacy and limitations of anti-angiogenic therapy. *Biochim Biophys Acta* 2014;1846:161–79.

- [4] Zhang J, Hochwald SN. Targeting receptor tyrosine kinases in solid tumors. *Surg Oncol Clin N Am* 2013;22:685–703.
- [5] Cheng L, Ren W, Xie L, Li M, Liu J, Hu J, Liu BR, Qian XP. Anti-EGFR MoAb treatment in colorectal cancer: limitations, controversies, and contradictories. *Cancer Chemother Pharmacol* 2014;74:1–13.
- [6] Naidoo J, Page DB, Wolchok JD. Immune checkpoint blockade. *Hematol Oncol Clin North Am* 2014;28:585–600.
- [7] Frohlich MW. Sipuleucel-T for the treatment of advanced prostate cancer. *Semin Oncol* 2012;39:245–52.
- [8] Hong B, van den Heuvel AP, Prabhu VV, Zhang S, El-Deiry WS. Targeting tumor suppressor p53 for cancer therapy: strategies, challenges and opportunities. *Curr Drug Targets* 2014;15:80–9.
- [9] Pagliarini R, Shao W, Sellers WR. Oncogene addiction: pathways of therapeutic response, resistance, and road maps toward a cure. *EMBO Rep* 2015;16:280–296.
- [10] Katoh M. Therapeutics targeting angiogenesis: genetics and epigenetics, extracellular miRNAs and signaling networks (Review). *Int J Mol Med* 2013;32:763–7.
- [11] Low D, Parker SE, Latimer T, Abai AM, Kuwahara-Rundell A, Doh SG, Yang ZY, Laface D, Gromkowski SH, Nabel GJ, Manthorpe M, Norman J. Cancer gene therapy using plasmid DNA: pharmacokinetic study of DNA following injection in mice. *Hum Gene Ther* 1995;6:53–564.
- [12] Legendre JY, Szoka F Jr. Delivery of plasmid DNA into mammalian cell lines using pH-sensitive liposomes: comparison with cationic liposomes. *Pharm Res* 1992;9:1235–1242.
- [13] Paul FW, Dutzar B, Stayton PS, Hoffman A, Anklesaria P, Harvie P. Poly propyl-acrylic acid (PPAA) functions as an endosomal escape agent synergistically enhancing the transfection potency of folate targeted cationic-lipid-protamine-DNA (LPD) complexes. *Mol Ther* 2003;7:208.
- [14] Bruckheimer EM, Harvie P, Orthel J, Dutzar B, Furstoss K, Mebel E, Anklesaria P, Paul R. In vivo efficacy of folate-targeted lipid-protamine-DNA (LPD-PEG-Folate) complexes in an immunocompetent syngeneic model for breast adenocarcinoma. *Mol Ther* 2003;7:129.
- [15] Bailey AL, Sullivan SM. Efficient encapsulation of DNA plasmids in small neutral liposomes induced by ethanol and calcium. *Biochim Biophys* 2000;1468:239–252.
- [16] De Smedt Sc, Demeester J, Hennink WE. Cationic polymer based gene delivery systems. *Pharm Res* 2000;17:113–126.

- [17] Kircheis R, Schuller S, Brunner S, Ogris M, Heider KH, Zauner W, Wagner E. Polycation-based DNA complexes for tumor-targeted gene delivery in vivo. *J Gene Med* 1999;1:111–120.
- [18] Zou SM, Erbacher P, Remy JS, Behr JP. Systemic linear polyethynilemine (L-PEI)-mediated gene delivery in the mouse. *J Gene Med* 2000;2:128.
- [19] Oupicky D, Ogris M, Howard KA, Dash PR, Ulbrich K, Seymour LW. Importance of lateral and steric stabilization of polyelectrolyte gene delivery vectors for extended systemic circulation. *Mol Ther* 2002;5:463–472.
- [20] Ogris M, Wagner E. Targeting tumors with non-viral gene delivery systems. *Drug Discov Today* 2002;15:479–485.
- [21] Kircheis R, Wightman L, Schreiber A, Robitza B, Rouslar V, Kursu M, Wanger E. Polyethylenimine/DNA complexes shielded by transferring target gene expression to tumors after systemic application. *Gene Ther* 2001;8:28–40.
- [22] Overbaugh J, Miller AD, Eiden MV. Receptors and entry cofactors for retroviruses include single and multiple transmembrane-spanning proteins as well as newly described glycoposphatidylinositol-anchored and secreted proteins. *Microbiol Mol Biol Rev* 2001;65:371–89.
- [23] Yi Y, Jong Noh M, Hee Lee K. Current advances in retroviral gene therapy. *Curr Gene Ther* 2011;11:218–228.
- [24] Ismail SI, Kingsman SM, Kingsman AJ, Uden M. Split-intron retroviral vectors: enhanced expression with improved safety. *J Virol* 2000;74:2365–71.
- [25] Hacein-Bey-Abina S, von Kalle C, Schmidt M, Le Deist F, Wulffraat N, McIntyre E, Radford I, Villeval JL, Fraser CC, Cavazzana-Calvo M, Fischer A. A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med* 2003;348:255–6.
- [26] Baum C, Dullmann J, Li Z, Fehse B, Meyer J, Williams DA, von Kalle C. Side effects of retroviral gene transfer into hematopoietic stem cells. *Blood* 2003;101:2099–114.
- [27] Wold WS, Toth K. Adenovirus vectors for gene therapy, vaccination and cancer gene therapy. *Curr Gene Ther* 2013;1:421–33.
- [28] Lopez-Gordo E, Podgorski II, Downes N, Alemany R. Circumventing antivector immunity: potential use of nonhuman adenoviral vectors. *Hum Gene Ther* 2014;25:285–300.
- [29] Yao XL, Nakagawa S, Gao JQ. Current targeting strategies for adenovirus vectors in cancer gene therapy. *Curr Cancer Drug Targets* 2011;11:810–25.
- [30] Khare R, Chen CY, Weaver EA, Barry MA. Advances and future challenges in adenoviral vector pharmacology and targeting. *Curr Gene Ther* 2011;11:241–58.

- [31] Kasala D, Choi JW, Kim SW, Yun CO. Utilizing adenovirus vectors for gene delivery in cancer. *Expert Opin Drug Deliv* 2014;11:379–92.
- [32] Lerch TF, Xie Q, Chapman MS. The structure of adeno-associated virus serotype 3B (AAV-3B): insights into receptor binding and immune evasion. *Virology* 2010;403[1]: 26–36.
- [33] Luo J, Luo Y, Sun J, Zhou Y, Zhang Y, Yang X. Adeno-associated virus-mediated cancer gene therapy: current status. *Cancer Lett* 2015;356:347–56.
- [34] Dunbar CE, Emmons RV. Gene transfer into hematopoietic progenitor and stem cells: progress and problems. *Stem Cells* 1994;12:563–76.
- [35] Carruthers KH, Metzger G, During MJ, Muravlev A, Wang C, Kocak E. Gene-directed enzyme prodrug therapy for localized chemotherapeutics in allograft and xenograft tumor models. *Cancer Gene Ther* 2014;21:434–40.
- [36] Kotterman MA, Schaffer DV. Engineering adeno-associated viruses for clinical gene therapy. *Nat Rev Genet* 2014;15:445–451.
- [37] Airene KJ, Hu Y-C, Kost TA, Smith RH, Kotin RM, Ono C, Matsuura Y, Wang S, Yla-Herttsala S. Baculovirus: an insect-derived vector for diverse gene transfer applications. *Mol Ther* 2013;21:739–749.
- [38] Hu Y-C. Baculovirus vectors for gene therapy. *Adv Virus Res* 2006;68:287–320.
- [39] Luo WY, Shih YS, Hung CL, Lo KW, Chiang CS, Lo WH, Huang SF, Wang SC, Yu CF, Chien CH, Hu YC. Development of the hybrid sleeping beauty: baculovirus vector for sustained gene expression and cancer therapy. *Gene Ther* 2012;19: 844–851.
- [40] van Oers MM, Pijlman GP, Vlak JM. Thirty years of baculovirus–insect cell protein expression: from dark horse to mainstream technology. *J Gen Virol* 2015;96:6–23.
- [41] Baldo A, van den Akker E, Bergmans HE, Lim F, Pauwels K. General considerations on the biosafety of virus-derived vectors used in gene therapy and vaccination. *Curr Gene Ther* 2013;13:385–394.
- [42] Manservigi R, Argnani R, Marconi P. HSV recombinant vectors for gene therapy. *Open Virol J* 2010;4:123–156.
- [43] Zhang G, Jin G, Nie X, Mi R, Zhu G, Jia W, Liu F. Enhanced antitumor efficacy of an oncolytic herpes simplex virus expressing an endostatin–angiostatin fusion gene in human glioblastoma stem cell xenografts. *PLoS One* 2014;9:e95872.
- [44] Moss B. Genetically engineered poxviruses for recombinant gene expression, vaccination, and safety. *Proc Natl Acad Sci U S A* 1996; 93:11341–11348.
- [45] Gómez CE, Nájera JL, Krupa M, Esteban M. The Poxvirus vectors MVA and NYVAC as gene delivery systems for vaccination against infectious diseases and cancer. *Curr Gene Ther* 2008;8:97–120.

- [46] Vile RG, Russel S, Lemoine N. Cancer gene therapy: hard lessons and new courses. *Gene Ther* 2000;7:2–8.
- [47] Zhang L, Dailey PJ, Gettie A, Blanchard J, Ho DD. The liver is a major organ clearing simian immunodeficiency virus in rhesus monkeys. *J Virol* 2002;76:5271–5273.
- [48] Mantovani A, Bototazzi B, Colotta F, Sozzani S, Ruco L. The origin and function of tumor-associated macrophages. *Immunol Today* 1992;10:265–270.
- [49] Pastorino S, Massaza S, Cilli M, Varesio L, Bosco MC. Generation of high titer retroviral vector-producing macrophages as vehicles for in vivo gene transfer. *Gene Ther* 2001;8:431–441.
- [50] Griffith KD, Read Ej, Carrasquilly JA, Carter CS, Yang JC, Fisher B, Aebersold P, Packard BS, Yu MY, Rosenberg SA. In vivo distribution of adoptively transferred indium-111 labeled tumor infiltrating lymphocytes and peripheral blood lymphocytes in patients with metastatic melanoma. *J Natl Cancer Inst* 1989;81:1709–1717.
- [51] Chang AE, Li Q, Bishop DK, Normolle Dp, Redman BD, Nickoloff BJ. Immunogenetic therapy of human melanoma utilizing autologous tumor cells transduced to secrete granulocyte-macrophage colony stimulating factor. *Hum Gene Ther* 2000;11:839–850.
- [52] Porada CD, Almeida-Porada G. Mesenchymal Stem Cells as Therapeutics and Vehicles for Gene and Drug Delivery. *Adv Drug Deliv Rev* 2010;62:1156–1166.
- [53] Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* 2001;7:211–228.
- [54] Morizono K, De Ugarte DA, Zhu M, Zuk P, Elbarbary A, Ashjian P, Benhaim P, Chen ISY, Hedrick MH. Multilineage cells from adipose tissue as gene delivery vehicles. *Hum Gene Ther* 2003;14:59–66.
- [55] Kang WK, Park C, Yoon HL, Kim WS, Yoon SS, Lee MH, Park K, Kim K, Jeong HS, Kim JA, Nam SJ, Yang JH, Son YI, Baek CH, Han J, Ree HJ, Lee ES, Kim SH, Kim DW, Ahn YC, Huh SJ, Choe YH, Lee JH, Park MH, Kong GS, Park EY, Kang YK, Bang YJ, Paik NS, Lee SN, Kim SH, Kim S, Robbins PD, Tahara H, Lotze MT, Park CH. Interleukin 12 gene therapy of cancer by peritumoral injection of transduced autologous fibroblasts: outcome of a phase I study. *Hum Gene Ther* 2001;12:671–684.
- [56] Löhr M, Hoffmeyer A, Kröger J, Freund M, Hain J, Holle A, Karle P, Knöfel WT, Liebe S, Müller P, Nizze H, Renner M, Saller RM, Wagner T, Hauenstein K, Günzburg WH, Salmons B. Microencapsulated cell-mediated treatment of inoperable pancreatic carcinoma. *Lancet* 2001;357:1591–1593.
- [57] Moret-Tatay I, Díaz J, Marco FM, Crespo A, Aliño SF. Complete tumor prevention by engineered tumor cell vaccines employing nonviral vectors. *Cancer Gene Ther* 2003;10:887–897.

- [58] Vasey PA, Shulman LN, Campos S, Davis J, Gore M, Johnston S, Kirn DH, O'Neill V, Siddiqui N, Seiden MV, Kaye SB. Phase I trial of intraperitoneal injection of the E1B-55-kd-gene-deleted adenovirus ONYX-015 (dl1520) given on days 1 through 5 every 3 weeks in patients with recurrent/refractory epithelial ovarian cancer. *J Clin Oncol* 2002;20:1562–9.
- [59] Li L, Liu RY, Huang JL, Liu QC, Li Y, Wu PH, Zeng YX, Huang W. Adenovirus-mediated intra tumoral delivery of the human endostatin gene inhibits tumor growth in nasopharyngeal carcinoma. *Int J Cancer* 2006;118:2064–71.
- [60] Markert JM, Medlock MD, Rabkin SD, Gillespie GY, Todo T, Hunter WD, Palmer CA, Feigenbaum F, Tornatore C, Tufaro F, Martuza RL. Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. *Gene Ther* 2000;7:1–4.
- [61] Rampling R, Cruickshank G, Papanastassiou V, Nicoll J, Hadley D, Brennan D, Petty R, MacLean A, Harland J, McKie E, Mabbs R, Brown M. Toxicity evaluation of replication-competent herpes simplex virus (ICP34.5 null mutant 1716) in patients with recurrent malignant glioma. *Gene Ther* 2000;7:859–866.
- [62] Das SK, Sarkar S, Dash R, Dent P, Wang XY, Sarkar D, Fisher PB. Cancer terminator viruses and approaches for enhancing therapeutic outcomes. *Adv Cancer Res* 2012;115:1–38.
- [63] Dorer DE, Nettelbeck DM. Targeting cancer by transcriptional control in cancer gene therapy and viral oncolysis. *Adv Drug Deliv Rev* 2009;61:554–71.
- [64] Osaki T, Tanio Y, Tachibana I, Hosoe S, Kumagai T, Kawase I, Oikawa S, Kishimoto T. Gene therapy for carcinoembryonic antigen-producing human lung cancer cells by cell type-specific expression of herpes simplex virus thymidine kinase gene. *Cancer Res* 1994;54:5258–5261.
- [65] Takahashi M, Sato T, Sagawa T, Lu Y, Sato Y, Iyama S, Yamada Y, Fukaura J, Takahashi S, Miyanishi K, Yamashita T, Sasaki K, Kogawa K, Hamada H, Kato J, Niitsu Y. E1B-55K-deleted adenovirus expressing E1A-13S by AFP-enhancer/promoter is capable of highly specific replication in AFP-producing hepatocellular carcinoma and eradication of established tumor. *Mol Ther* 2002;5:627–34.
- [66] Gupta VK, Park JO, Kurihara T, Koons A, Mauceri HJ, Jaskowiak NT, Kufe DW, Weichselbaum RR, Posner MC. Selective gene expression using a DF3/MUC1 promoter in a human esophageal adenocarcinoma model. *Gene Ther* 2003;10:206–12.
- [67] Pandha HS, Martin L-A, Rigg A, Hurst HC, Stamp GWH, Sikora K, Lemoine NR. Genetic prodrug activation therapy for breast cancer: a phase I clinical trial of c-erbB2 directed suicide gene expression. *J Clin Oncol* 1999;17:2180–2189.
- [68] Nishino K, Osaki T, Kumagai T, Kijima T, Tachibana I, Goto H, Arai T, Kimura H, Funakoshi T, Takeda Y, Tanio Y, Hayashi S. Adenovirus-mediated gene therapy spe-

cific for small cell lung cancer cells using a Myc-Max binding motif. *Int J Cancer* 2001;91:851–6.

- [69] Latham JP, Searle PF, Mautner V, James ND. Prostate specific antigen promoter/enhancer driven gene therapy for prostate cancer: construction and testing of a tissue specific adenovirus vector. *Cancer Res* 2000;60:334–341.
- [70] Vile RG, Nelson JA, Castleden S, Chong H, Hart IR. Systemic gene therapy of immune melanoma using tissue specific expression of the HSVtk gene involves an immune component. *Cancer Res* 1994;54:228–234.
- [71] Miyatake SI, Tani S, Feigenbaum F, Sundaresan P, Toda H, Narumi O, Kikuchi H, Hashimoto N, Hangai M, Martuza RL, Rabkin SD. Hepatoma-specific anti-tumor activity of an albumin enhancer/promoter regulated herpes simplex virus in vivo. *Gene Ther* 1999;6:564–572.
- [72] Kitazono M, Chuman Y, Aikou T, Fojo T. Adenovirus HSV-TK construct with thyroid-specific promoter: enhancement of activity and specificity with histone deacetylase inhibitors and agents modulating the camp pathway. *Int J Cancer* 2002;99:453–9.
- [73] McKie EA, Graham DI, Brown SM. Selective astrocytic transgene expression in vitro and in vivo from the GFAP promoter in a HSV RL1 null mutant vector-potential glioblastoma targeting. *Gene Ther* 1998;5:440–450.
- [74] Kubo H, Gardner TA, Wada Y, Koeneman KS, Gotoh A, Yang L, Kao C, Lim SD, Amin MB, Yang H, Black ME, Matsubara S, Nakagawa M, Gillenwater JY, Zhau HE, Chung LW. Phase I dose escalation clinical trial of adenovirus vector carrying osteocalcin promoter-driven herpes simplex virus thymidine kinase in localized and metastatic hormone-refractory prostate cancer. *Hum Gene Ther* 2003;14:227–241.
- [75] Li GC, He F, Shao X, Urano M, Shen L, Kim D, Borrelli M, Leibel SA, Gutin PH, Ling CC. Adenovirus-mediated heat-activated antisense Ku70 expression radiosensitizes tumor cells in vitro and in vivo. *Cancer Res* 2003; 63:3268–74.
- [76] Ruan H, Su H, Hu L, Lamborn KR, Kan YW, Deen DF. A hypoxia-regulated adeno-associated virus vector for cancer-specific gene therapy. *Neoplasia* 2001;3:255–63.
- [77] Walther W, Wendt J, Stein U. Employment of the *mdr1* promoter for the chemotherapy-inducible expression of therapeutic genes in cancer gene therapy. *Gene Ther* 1997; 4:544–52.
- [78] Walther W, Stein U, Fichtner I, Alexander M, Shoemaker RH, Schlag PM. *Mdr1* promoter-driven tumor necrosis factor- α expression for a chemotherapy-controllable combined in vivo gene therapy and chemotherapy of tumors. *Cancer Gene Ther* 2000 7:893–900.
- [79] Parr MJ, Manome Y, Tanaka T, Wen P, Kufe DW, Kaelin WG Jr, Fine HA. Tumor-selective transgene expression in vivo mediated by an E2F-responsive adenoviral vector. *Nat Med* 1997;3:1145–1149.

- [80] Tsihlias J, Kapusta L, Slingerland J. The prognostic significance of altered cyclin-dependent kinase inhibitors in human cancer. *Annu Rev Med* 1999;50:401–423.
- [81] Naruse I, Hashiro H, Doboushi K, Minato K, Saito R, Mori M. Over-expression of p27 (kip1) induces growth arrest and apoptosis mediated by changes of Rb expression in lung cancer cell lines. *Int J Cancer* 2000;88:377–83.
- [82] Yang CT, You L, Yeh CC, Chang JWC, Zhang F, McCormick F, Jablons DM. Adenovirus-mediated p14 (ARF) gene transfer in human mesothelioma cells. *J Natl Cancer Inst* 2000;92:636–641.
- [83] Nettelbeck DM, Zwicker J, Lucibello FC, Gross C, Liu N, Brusselbach S, Muller R. Cell cycle regulated promoters for the targeting of tumor endothelium. *Adv Exp Med Biol* 1998;451:437–40.
- [84] Kimchi-Sarfaty C, Gottesman MM. SV40 pseudovirions as highly efficient vectors for gene transfer and their potential application in cancer therapy. *Curr Pharm Biotechnol* 2004;5:451–8.
- [85] Kursa M, Walker GF, Roessler V, Ogris M, Roedl W, Kircheis R, Wagner E. Novel shielded transferrin-polyethylene glycol-polyethynylemine/DNA complexes for systemic tumor-targeted gene transfer. *Bioconjug Chem* 2003;14 :222–231.
- [86] Liu X, Tian P, Yu Y, Yao M, Cao X, Gu J. Enhanced antitumor effect of EGF R targeted p21WAF-1 and GM-CSF gene transfer in the established murine hepatoma by peritumoral injection. *Cancer Gene Ther* 2002;9:100–108.
- [87] Suzuki M, Takayanagi A, Shimizu N. Recombinant single-chain antibodies with various oligopeptide tails for targeted gene delivery. *Gene Ther* 2003;10:781–88.
- [88] Davis ME. Non-viral gene delivery systems. *Curr Opin Biotechnol* 2002;13:128–131.
- [89] Neves C, Escriv V, Byk G, Schermann P, Wils P. Intracellular fate and nuclear targeting of plasmid DNA. *Cell Biol Toxicol* 1999;15:193–202.
- [90] Harriton-Gazal E, Feder R, Mor A, Graessmann A, Brack-Werner R, Jons D, Gilon C, Loyer A. Targeting of non-karyophilic cell-permeable peptides into the nuclei of intact cells by covalently attached nuclear localization signals. *Biochemistry* 2002;41:9208–9214.
- [91] Ogris M, Carlisle RC, Bettinger T, Seymour LW. Melittin enables efficient vesicular escape and enhanced nuclear access of non-viral gene delivery vectors. *J Biol Chem* 2001;276:47550–47555.
- [92] Logg R, Tai CK, Logg A, Anderson WF, Kasahara N. A uniquely stable replication-competent retrovirus vector achieves efficient gene delivery in vitro and in solid tumors. *Hum Gene Ther* 2001;12:921–932.
- [93] Martin F, Chowdhury S, Neil SJ, Chester KA, Gosset F-L, Collins MK. Targeted retroviral infection of tumor cells by receptor cooperation. *J Virol* 2003;77:2753–2756.

- [94] Kasahara N, Dozy AM, Kan YW. Tissue specific targeting of retroviral vectors through ligand-receptor interactions. *Science* 1994;266:1373–1376.
- [95] Khare PD, Liao S, Hirose Y, Kuroki M, Fujimura S, Yamauchi Y. Tumor growth suppression by a retroviral vector displaying scFv antibody to CEA and carrying the iNOS gene. *Anticancer Res* 2002;22:2443–2446.
- [96] Tai C-K, Logg CR, Park JH, Anderson WF, Press MF, Kasahara N. Antibody-mediated targeting of replication competent retroviral vectors. *Hum Gene Ther* 2003;14:789–802.
- [97] Bergelson JM, Cunningham JA, Droguett G, Kurt-Jones EA, Krithivas A, Hong JS, Horwitz MS, Crowell RL, Finberg RW. Isolation of a common receptor for coxsackie B viruses and adenoviruses 2 and 5. *Science* 1997;275:1320–1323.
- [98] Wickham TJ, Tzeng E, Shears LL, Roelvink PW, Li Y, Lee GM, Brough DE, Lizonova A, Kovesdi I. Increased in vitro and in vivo gene transfer by adenovirus vectors containing chimeric fiber proteins. *J Virol* 1997;71:8221–8229.
- [99] Haisma HJ, Grill J, Curiel DT, Hoogeland S, van Beusechem VW, Pinedo HM, Gerritsen WR. Targeting of adenoviral vectors through a bispecific single-chain antibody. *Cancer Gene Ther* 2000;7:901–904.
- [100] Grill J, van Beusechem VW, Van Der Valk P, Dirven CM, Leonhart A, Pherai DS, Haisma HJ, Pinedo HM, Curiel DT, Gerritsen WR. Combined targeting of adenoviruses to integrins and epidermal growth factor receptors increases gene transfer into primary glioma cells and spheroids. *Clin Cancer Res* 2001;7:641–650.
- [101] Hong SS, Galaup A, Peytavi N, Chazal N, Boulanger P. Enhancement of adenovirus-mediated gene delivery by use of an oligopeptide with dual binding specificity. *Hum Gene Ther* 1999;10:2577–2586.
- [102] Bartlet JS, Kleinschmidt J, Boucher RC, Samulski RJ. Targeted adenoassociated virus vector transduction of non-permissive cells mediated by a bispecific (ab-g) antibody. *Nat Biotechnol* 1999;17:181–186.
- [103] Perabo L, Büning H, Kofler DM, Ried MU, Girod A, Wendtner CM, Enssle J, Hallek M. In vitro selection of viral vectors with modified tropism: the adenoassociated virus display. *Mol Ther* 2003;8:151–157.
- [104] Lane DP, Cheok CF, Lain S. p53-based cancer therapy. *Cold Spring Harb Perspect Biol* 2010;2:a001222.
- [105] Tassone P, Old M, Teknos TN, Pan Q. p53-based therapeutics for head and neck squamous cell carcinoma. *Oral Oncol* 2013;49:733–7.
- [106] Beckta JM, Ahmad SF, Yang H, Valerie K. Revisiting p53 for cancer-specific chemo- and radiotherapy: ten years after. *Cell Cycle* 2014;13:710–3.

- [107] Issaeva N, Friedler A, Bozko P, Wiman KG, Fersht AR, Selivanova G. Rescue of mutants of the tumor suppressor p53 in cancer cells by a designed peptide. *Proc Natl Acad Sci U S A* 2003;100:13303–7.
- [108] Oshima Y, Sasaki Y, Negishi H, Idogawa M, Toyota M, Yamashita T, Wada T, Nagoya S, Kawaguchi S, Yamashita T, Tokino T. Antitumor effect of adenovirus-mediated p53 family gene transfer on osteosarcoma cell lines. *Cancer Biol Ther* 2007;6:1058–66.
- [109] Das S, Nama S, Antony S, Somasundaram K. p73 β -expressing recombinant adenovirus: a potential anticancer agent. *Cancer Gene Ther* 2005;12:417–426.
- [110] Knudsen KE, Weber E, Arden KC, Cavenee WK, Feramisco JR, Knudsen ES. The retinoblastoma tumor suppressor inhibits cellular proliferation through two distinct mechanisms: inhibition of cell cycle progression and induction of cell death. *Oncogene* 1999;18: 5239–45.
- [111] Liu F, Li Q, Zhang P, Chen F, Cheng Y. Role of adenovirus-mediated retinoblastoma 94 in the treatment of human non-small cell lung cancer. *Mol Med Rep* 2015;11:3349–3353.
- [112] Macnab SA, Turrell SJ, Carr IM, Markham AF, Coletta PL, Whitehouse A. Herpesvirus saimiri-mediated delivery of the adenomatous polyposis coli tumour suppressor gene reduces proliferation of colorectal cancer cells. *Int J Oncol* 2011; 39:1173–1181.
- [113] Tait DL, Jensen RA, Holt JT, Johnson DH, Gralow J, King MC. Gene therapy for breast and ovarian cancer with BRCA1. *Breast Dis* 1998;10:89–98.
- [114] Farooqi AA, Rehman ZU, Muntane J. Antisense therapeutics in oncology: current status. *Onco Targets Ther* 2014;3:2035–42.
- [115] Bedikian AY, Garbe C, Conry R, Lebbe C, Grob JJ; Genasense Melanoma Study Group. Dacarbazine with or without oblimersen (a Bcl-2 antisense oligonucleotide) in chemotherapy-naïve patients with advanced melanoma and low-normal serum lactate dehydrogenase: ‘The AGENDA trial’. *Melanoma Res* 2014;24:237–243.
- [116] O’Brien S, Moore JO, Boyd TE, Larratt LM, Skotnicki A, Koziner B, Chanan-Khan AA, Seymour JF, Bociek RG, Pavletic S, Rai KR. Randomized phase III trial of fludarabine plus cyclophosphamide with or without oblimersen sodium (Bcl-2 antisense) in patients with relapsed or refractory chronic lymphocytic leukemia. *J Clin Oncol* 2008;26:820.
- [117] Chanan-Khan AA, Niesvizky R, Hohl RJ, Zimmerman TM, Christiansen NP, Schiller GJ, Callander N, Lister J, Oken M, Jagannath S. Phase III randomised study of dexamethasone with or without oblimersen sodium for patients with advanced multiple myeloma. *Leuk Lymphoma* 2009;50:559–65.

- [118] Sun HX, He HW, Zhang SH, Liu TG, Ren KH, He QY, Shao RG. Suppression of N-Ras by shRNA-expressing plasmid increases sensitivity of HepG2 cells to vincristine-induced growth inhibition. *Cancer Gene Ther* 2009;16:693-702.
- [119] Altieri DC. 2008. Survivin, cancer networks and pathway-directed drug discovery. *Nat Rev Cancer* 2008;8:61-70.
- [120] Talbot DC, Ranson M, Davies J, Lahn M, Callies S, André V, Kadam S, Burgess M, Slapak C, Olsen AL, McHugh PJ, de Bono JS, Matthews J, Saleem A, Price P. Tumor survivin is downregulated by the antisense oligonucleotide LY2181308: a proof-of-concept, first-in-human dose study. *Clin Cancer Res* 2010;16:6150-8.
- [121] Vassaux G, Martin-Duque P. Use of suicide genes for cancer gene therapy: study of the different approaches. *Expert Opin Biol Ther* 2004;4:519-530.
- [122] Tomicic MT, Thust R, Kaina B. Ganciclovir-induced apoptosis in HSV-1 thymidine kinase expressing cells: critical role of DNA breaks, Bcl-2 decline and caspase-9 activation. *Oncogene* 2002;21:2141-2153.
- [123] Liu Y, Ye T, Maynard J, Akbulut H, Deisseroth A. Engineering conditionally replication-competent adenoviral vectors carrying the cytosine deaminase gene increases the infectivity and therapeutic effect for breast cancer gene therapy. *Cancer Gene Ther* 2006;13:346-56.
- [124] Zhang L, Akbulut H, Tang Y, Peng X, Pizzorno G, Sapi E, Manegold S, Deisseroth A. Adenoviral vectors with E1A regulated by tumor-specific promoters are selectively cytolytic for breast cancer and melanoma. *Mol Ther* 2002;6:386-93.
- [125] Akbulut H, Tang Y, Maynard J, Zhang L, Pizzorno G, Deisseroth A. Vector targeting makes 5-fluorouracil chemotherapy less toxic and more effective in animal models of epithelial neoplasms. *Clin Cancer Res* 2004;10:7738-7746.
- [126] Prados J, Melguizo C, Rama A, Ortiz R, Boulaiz H, Rodriguez-Serrano F, Caba O, Rodriguez-Herza JJ, Ramos JL, Aranega A. Combined therapy using suicide gef gene and paclitaxel enhances growth inhibition of multicellular tumour spheroids of A-549 human lung cancer cells. *Int J Oncol* 2008;33:121-127.
- [127] Liao Z, Huang C, Zhou F, Xiong J, Bao J, Zhang H, Sun W, Xie C, Zhou Y. Radiation enhances suicide gene therapy in radioresistant laryngeal squamous cell carcinoma via activation of a tumor-specific promoter. *Cancer Lett* 2009;283:20-8.
- [128] Matthews T, Boehme R. Antiviral activity and mechanism of action of ganciclovir. *Rev Infect Dis* 1998;10:490-4.
- [129] Garcia-Rodríguez L, Abate-Daga D, Rojas A, González JR, Fillat C. E-cadherin contributes to the bystander effect of TK/GCV suicide therapy and enhances its antitumoral activity in pancreatic cancer models. *Gene Ther* 2011;18:73-8.
- [130] Sangro B, Mazzolini G, Ruiz M, Ruiz J, Quiroga J, Herrero I, Qian C, Benito A, Larrache J, Olagüe C, Boan J, Peñuelas I, Sádaba B, Prieto J. A phase I clinical trial of thy-

midine kinase-based gene therapy in advanced hepatocellular carcinoma. *Cancer Gene Ther* 2010;17:837–43.

- [131] Zhang J, Wei F, Wang H, Li H, Qiu M, Ren P, Chen X, Huang Q. Potent anti-tumor activity of telomerase-dependent and HSVTK armed oncolytic adenovirus for non-small cell lung cancer in vitro and in vivo. *J Exp Clin Cancer Res* 2010;29:52.
- [132] Miletic H, Fischer YH, Girolou T, Rueger MA, Winkeler A, Li H, Himmelreich U, Stenzel W, Jacobs AH, von Laer D. Normal brain cells contribute to the bystander effect in suicide gene therapy of malignant glioma. *Clin Cancer Res* 2007;13:6761–6768.
- [133] Blumenthal M, Skelton D, Pepper KA, Jahn T, Methangkool E, Kohn DB. Effective suicide gene therapy for leukemia in a model of insertional oncogenesis in mice. *Mol Ther* 2007;15:183–192.
- [134] Beltinger C, Fulda S, Kammertoens T, Meyer E, Uckert W, Debatin KM. Herpes simplex virus thymidine kinase/ganciclovir induced apoptosis involves ligand independent death receptor aggregation and activation of caspases. *Proc Natl Acad Sci U S A* 1999;96:8699–704.
- [135] Gadi VK, Alexander SD, Kudlow JE, Allan P, Parker WB, Sorscher EJ. In vivo sensitization of ovarian tumors to chemotherapy by expression of E. coli purine nucleoside phosphorylase in a small fraction of cells. *Gene Ther* 2000;20:1738–1743.
- [136] Karle P, Renner M, Salmons B, Günzburg WH. Necrotic, rather than apoptotic, cell death caused by cytochrome P450-activated ifosfamide. *Cancer Gene Ther* 2001;8:220–30.
- [137] Hamstra DA, Pagé M, Maybaum J, Rehemtulla A. Expression of endogenously activated secreted or cell surface carboxypeptidase A sensitizes tumor cells to methotrexate- α -peptide prodrugs. *Cancer Res* 2000;60:657–665.
- [138] Ambade AV, Joshi GV, Mulherkar R. Effect of suicide gene therapy in combination with immunotherapy on antitumour immune response & tumour regression in a xenograft mouse model for head & neck squamous cell carcinoma. *Indian J Med Res* 2010;312:415–422.
- [139] Cao X, Huang X, Ju DW, Zhang W, Hamada H, Wang J. Enhanced antitumoral effect of adenovirus-mediated cytosine deaminase gene therapy by induction of antigen-presenting cells through stem cell factor/granulocyte-macrophage colony-stimulating factor gene transfer. *Cancer Gene Ther* 2000;7:177–186.
- [140] Khatri A, Husaini Y, Ow K, Chapman J, Russell PJ. Cytosine deaminase-uracil phosphoribosyltransferase and interleukin (IL)-12 and IL-18: a multimodal anticancer interface marked by specific modulation in serum cytokines. *Clin Cancer Res* 2009;15:2323–2334.
- [141] Chen CT, Yamaguchi H, Lee HJ, Du Y, Lee HH, Xia W, Yu WH, Hsu JL, Yen CJ, Sun HL, Wang Y, Yeh ET, Hortobagyi GN, Hung MC. Dual targeting of tumor angiogen-

esis and chemotherapy by endostatin–cytosine deaminase– uracil phosphoribosyl-transferase. *Mol Cancer Ther* 2011;10:1327–1336.

- [142] Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing the T cell response. *Nat Rev Immunol* 2012;12:269–281.
- [143] Kaufman HL, Bines SD. OPTIM trial: a Phase III trial of an oncolytic herpes virus encoding GM-CSF for unresectable stage III or IV melanoma. *Future Oncol* 2010;6:941–9.
- [144] Goshima F, Esaki S, Luo C, Kamakura M, Kimura H, Nishiyama Y. Oncolytic viral therapy with a combination of HF10, a herpes simplex virus type 1 variant and granulocyte-macrophage colony-stimulating factor for murine ovarian cancer. *Int J Cancer* 2014;134:2865–77.
- [145] Goins WF, Huang S, Cohen JB, Glorioso JC. Engineering HSV-1 vectors for gene therapy. *Methods Mol Biol* 2014;1144:63–79.
- [146] Lim F, Khalique H, Ventosa M, Baldo A. Biosafety of gene therapy vectors derived from herpes simplex virus type 1. *Curr Gene Ther* 2013;13:478–91.
- [147] Bischoff JR, Kirn DH, Williams A, Heise C, Horn S, Muna M, Ng L, Nye JA, Sampson-Johannes A, Fatta-Kammertoens Ty A, McCormick F. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science* 1996;274:373–376.
- [148] Hay JG, Shapiro N, Sauthoff H, Heitner S, Phupakdi W, Rom WN. Targeting the replication of adenoviral gene therapy vectors to lung cancer cells: the importance of the adenoviral E1b-55kD gene. *Hum Gene Ther* 1999;10:579–590.
- [149] Rothman T, Hungstermann A, Whitaker NJ, Scheffner M, zur Hausen H. Replication of ONYX-015 a potential anticancer adenovirus, is independent of p53 status in tumor cells. *J Virol* 1998;72:9470–9478.
- [150] Liu S, Chen P, Hu M, Tao Y, Chen L, Liu H, Wang J, Luo J, Gao G. Randomized, controlled phase II study of post-surgery radiotherapy combined with recombinant adenoviral human p53 gene therapy in treatment of oral cancer. *Cancer Gene Ther* 2013;20:375–8.
- [151] Khuri FR, Nemunaitis J, Ganly I, Arseneau J, Tannock IF, Romel L, Gore M, Ironside J, MacDougall RH, Heise C, Randlev B, Gillenwater AM, Bruso P, Kaye SB, Hong WK, Kirn DH. A controlled trial of intratumoral ONYX-015, a selectively replicating adenovirus in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nat Med* 2000;6:879–885.
- [152] Ganly I, Kirn D, Eckhardt G, Rodriguez GI, Soutar DS, Otto R, Robertson AG, Park O, Gulley ML, Heise C, Von Hoff DD, Kaye SB. A phase I study of ONYX-015, an E1B attenuated adenovirus, administered intratumorally to patient with recurrent head and neck cancer. *Clin Cancer Res* 2000;6:798–806.

- [153] Vasey PA, Schulman LN, Campos S, Davis J, Gore M, Johnston S, Kirn DH, O'Neill V, Siddiqui N, Seiden MV, Kaye SB. Phase I trial of intraperitoneal injection of the E1B-55kd-gene deleted adenovirus ONYX-015 (dl1520) given on days 1 through 5 every 3 weeks in patients with recurrent/refractory epithelial ovarian cancer. *J Clin Oncol* 2002;20:1562–1569.
- [154] Fueyo J, Gomez-Manzano C, Alemany R, Lee P, McDonnell T, Mitlianghel P, Shi YX, Levin VA, Yung WK, Kyritsis AP. A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect in vivo. *Oncogene* 1999;19:1–11.
- [155] Heise C, Hermiston T, Johnson L, Brooks G, Sampson-Johannes A, Williams A, Hawkins L, Kirn D. An adenovirus E1A mutant that demonstrates potent and selective systemic anti-tumoral efficacy. *Nat Med* 2000;6:1134–1139.
- [156] Rodriguez R, Schuer ER, Yeong L, Henderson GA, Simons JW, Henderson DR. Prostate attenuated replication competent adenovirus (ARCA) CN706 a selective cytotoxic for prostate specific antigen positive prostate cancer cells. *Cancer Res* 1997;57:2559–2563.
- [157] Hallenbeck PL, Chang YN, Hay C, Golightly D, Stewart D, Lin J, Phipps S, Chiang YL. A novel tumor-specific replication restricted adenoviral vector for gene therapy of hepatocellular carcinoma. *Hum Gene Ther* 1999;10:1721–1733.
- [158] Brunori M, Malerba M, Kashiwasaki H, Iggo R. Replicating adenoviruses that target tumors with constitutive activation of the wnt signalling pathway. *J Virol* 2001;75:2857–2865.
- [159] Kurihara T, Brough DE, Komesdi I, Kufe DW. Selectivity of a replication-competent adenovirus for human breast carcinoma cells expressing the MUC1 antigen. *J Clin Invest* 2000;106:763–771.
- [160] Wildner O, Moris JC. Therapy of peritoneal carcinomatosis from colon cancer with oncolytic adenoviruses. *J Gene Med* 2000;2:353–360.
- [161] Chung I, Deisseroth AB. Recombinant adenoviral vector containing tumor-specific L-plastin promoter fused to cytosine deaminase gene as a transcription unit: generation and functional test. *Arch Pharm Res*. 2004;27:633–639.
- [162] Akbulut H, Zhang L, Tang Y, Deisseroth A. The cytotoxic effect of replication competent adenoviral vectors carrying L-plastin promoter regulated E1A and cytosine deaminase genes in cancers of the breast, ovary and colon. *Cancer Gene Ther* 2003;10:388–395.
- [163] Akbulut H, Tang Y, Deisseroth A. Vector vaccination and vector targeted chemotherapy in solid tumors. *J BUON* 2009;14:141–146.
- [164] Hashiro G, Loh PC, Jav ST. The preferential cytotoxicity of reovirus for certain transformed cell lines. *Arch Virol* 1977;75:3335–3342.

- [165] Maxwell IH, Terrel KL, Maxwell F. Autonomous parvovirus vectors. *Methods* 2002;28:168–181.
- [166] Strong JE, Coffey MC, Tang D, Sabinin P, Lee PWK. The molecular basis of viral oncolysis: usurpation of the RAS signaling pathway by rheovirus. *EMBO J* 1998;12:3351–3362.
- [167] Stojdl DF, Lichty B, Knowles S, Marius R, Atkins H, Sonenberg N, Bell JC. Exploiting tumor-specific defects in the interferon pathway with a previously unknown oncolytic virus. *Nat Med* 2000;6:821–825.
- [168] Malerba M, Daeffler L, Rommelaere J, Iggo D. Replicating parvoviruses that target colon cancer cells. *J Virol* 2003;77:6683–6691.
- [169] Phangso A, Lorence RM, Reichard KW, Peoples ME, Walter RJ. New castle disease virus therapy of human tumor xenografts: anti-tumor effects of local or systemic administration. *Cancer Lett* 2001;172:27–36.
- [170] Pecora AL, Rizvi N, Cohen GI, Menopol NJ, Sterman D, Marshal JL, Goldberg S, Gross P, O'Neil JD, Groene WS, Roberts MS, Rabin H, Bamat MK, Lorence RM. Phase I trial of intravenous administration of PV701, an oncolytic virus, in patients with solid tumors. *J Clin Oncol* 2002;20:2251–2266.
- [171] Kuo L, Godeke GJ, Ramsanman MJB, Masters PS, Rottier PJM. Retargeting of coronavirus by substitution of the spike glycoprotein ectodomain: crossing the host cell species barrier. *J Virol* 2000;74:1393–1406.
- [172] Ortego J, Escors D, Laude H, Enjuanes L. Generation of a replication competent, propagation-deficient virus vector based on the transmissible gastroenteritis coronavirus genome. *J Virol* 2002;76:11518–11529.
- [173] Kong HL, Hecht D, Song W, Kovesdi I, Hackett NR, Yayon A, Crystal RG. Regional suppression of tumor growth by in vivo transfer of a cDNA encoding a secreted form of the extracellular domain of the flt-1 vascular endothelial growth factor receptor. *Hum Gene Ther* 1998;10:823–833.
- [174] Takayama K, Ueno H, Nakanishi Y, Sakamoto T, Inoue K, Shimizu K, Ohashi H, Hara N. Suppression of tumor angiogenesis and growth by gene transfer of a soluble form of vascular endothelial growth factor receptor into a remote organ. *Cancer Res* 2000;60:2169–2177.
- [175] Szary J, Szala S. Intra-tumoral administration of naked plasmid DNA encoding mouse endostatin inhibits renal carcinoma growth. *Int J Cancer* 2001; 91:835–839.
- [176] Tai KF, Chen PJ, Chen DS, Hwang LH. Concurrent delivery of GM-CSF and endostatin genes by a single adenoviral vector provides a synergistic effect on the treatment of orthotopic liver tumors. *J Gene Med* 2003;5:386–398.

- [177] Ma HI, Lin SZ, Chiang YH, Li J, Chen SL, Tsao YP, Xiao X. Intratumoral gene therapy of malignant brain tumor in a rat model with angiostatin delivered by adeno-associated viral (AAV) vector. *Gene Ther* 2002; 9:2–11.
- [178] Jin RJ, Kwak C, Lee SG, Lee CH, Soo CG, Park MS, Lee E, Lee SE.. The application of an anti-angiogenic gene (thrombospondin-1) in the treatment of human prostate cancer xenografts. *Cancer Gene Ther* 2000;7:1537–1542.
- [179] Tanaka T, Manome Y, Wen P, Kufe DW, Fine HA. Viral vector-mediated transduction of a modified platelet factor 4 cDNA inhibits angiogenesis and tumor growth. *Nat Med* 1997;3:437–442.
- [180] Maemondo M, Narumi K, Saijo Y, Usui K, Tahara M, Tazawa R, K, Matsumoto K, Nakamura T, Nukiwa T. Targeting angiogenesis and HGF function using an adeno-viral vector expressing the HGF antagonist NK4 for cancer therapy. *Mol Ther* 2002;5:177–185.
- [181] Griscelli F, Li H, Cheong C, Opolon P, Bennaceur-Griscelli A, Vassal G, Soria J, Soria C, Lu H, Perricaudet M, Yeh P. Combined effects of radiotherapy and angiostatin gene therapy in glioma tumor model. *Proc Natl Acad Sci U S A* 2000; 97:6698–6703.
- [182] Varda-Bloom N, Shaish A, Gonen A, Levanon K, Greenbereger S, Ferber S, Levkovitz H, Castel D, Goldberg I, Afek A, Kopolovitch Y, Harats D. Tissue specific gene therapy directed to tumor angiogenesis. *Gene Ther* 2001;8:819–827.
- [183] Kaliberov SA, Buchsbaum DJ, Gillespie GY, Curiel DT, Arafat WO, Carpenter M, Stackhouse MA. Adenovirus-mediated transfer of BAX driven by the vascular endothelial growth factor promoter induces apoptosis in lung cancer cells. *Mol Ther* 2002;6:190–198.
- [184] Savontaus MJ, Sauter BV, Huang TG, Woo SL. Transcriptional targeting of conditionally replicating adenovirus to dividing endothelial cells. *Gene Ther* 2002;9:972–979.
- [185] Nör JE, Hu Y, Song W, Spencer DM, Núñez G. Ablation of microvessels in vivo upon dimerization of Caspase-9. *Gene Ther* 2002; 9:444–451.
- [186] Nguyen JT, Wu P, Clouse ME, Hlatky L, Terwilliger EF. Adeno-associated virus-mediated delivery of antiangiogenic factors as an antitumor strategy. *Cancer Res* 1998;58:5673–5677.
- [187] Li H, Lu H, Griscelli F, Opolon P, Sun LQ, Ragot T, Y, Belin D, Soria J, Soria C, Perricaudet M, Yeh P. Adenovirus-mediated delivery of a uPA/uPAR antagonist suppresses angiogenesis dependent tumor growth and dissemination in mice. *Gene Ther* 1998;5:1105–1113.
- [188] Dranoff G. GM-CSF-based cancer vaccines. *Immunol Rev* 2002; 188: 147–154.

- [189] Shashkova EV, Spencer JF, Wold WS, Doronin K. Targeting interferon-alpha increases antitumor efficacy and reduces hepatotoxicity of E1A-mutated spread-enhanced oncolytic adenovirus. *Mol Ther* 2007; 15: 598–607.
- [190] Choi IK, Lee JS, Zhang SN, Park J, Sonn CH, Lee KM, Yun CO. Oncolytic adenovirus co-expressing IL-12 and IL-18 improves tumor-specific immunity via differentiation of T cells expressing IL-12Rbeta2 or IL-18Ralpha. *Gene Ther* 2011; 18: 898–909.
- [191] Jinushi M, Tahara H. Cytokine gene-mediated immunotherapy: current status and future perspectives. *Cancer Sci* 2009; 100: 1389–1396.
- [192] Choi KJ, Zhang SN, Choi IK, Kim JS, Yun CO. Strengthening of antitumor immune memory and prevention of thymic atrophy mediated by adenovirus expressing IL-12 and GM-CSF. *Gene Ther* 2012; 19: 711–723.
- [193] Pesonen S, Diaconu I, Cerullo V, Escutenaire S, Raki M, Kangasniemi L, Nokisalmi P, Dotti G, Guse K, Laasonen L, Partanen K, Karli E, Haavisto E, Oksanen M, Karioja-Kallio A, Hannuksela P, Holm SL, Kauppinen S, Joensuu T, Kanerva A, Hemminki A. Integrin targeted oncolytic adenoviruses Ad5-D24-RGD and Ad5-RGD-D24-GMCSF for treatment of patients with advanced chemotherapy refractory solid tumors. *Int J Cancer*. 2012;130:1937–1947.
- [194] Lapteva N, Aldrich M, Weksberg D, Rollins L, Goltsova T, Chen SY, Huang XF. Targeting the intratumoral dendritic cells by the oncolytic adenoviral vaccine expressing RANTES elicits potent antitumor immunity. *J Immunother* 2009; 32:145–156.
- [195] Charles LG, Xie YC, Restifo NP, Roessler B, Sanda MG. Antitumor efficacy of tumor-antigen-encoding recombinant poxvirus immunization in Dunning rat prostate cancer: implications for clinical genetic vaccine development. *World J Urol*. 2000;18:136–142.
- [196] Deisseroth A, Tang Y, Zhang L, Akbulut H, Habib N. TAA/ecdCD40L adenoviral prime-protein boost vaccine for cancer and infectious diseases. *Cancer Gene Ther*. 2013;20[2]:65–69.
- [197] Tang Y, Akbulut H, Maynard J, Petersen L, Fang X, Zhang WW, Xia X, Koziol J, Linton PJ, Deisseroth A. Vector prime/protein boost vaccine that overcomes defects acquired during aging and cancer. *J Immunol* 2006;177: 5697–56707.
- [198] Akbulut H, Tang Y, Akbulut KG, Maynard J, Deisseroth A. Addition of adenoviral vector targeting of chemotherapy to the MUC-1/ecdCD40L VPPP vector prime protein boost vaccine prolongs survival of mice carrying growing subcutaneous deposits of Lewis lung cancer cells. *Gene Ther*. 2010;17[11]:1333–1340.
- [199] Rochlitz C, Figlin R, Squiban P, Salzberg M, Pless M, Herrmann R. Phase I immunotherapy with a modified vaccinia virus (MVA) expressing human MUC1 as antigen-specific immunotherapy in patients with MUC1-positive advanced cancer. *J Gene Med* 2003;5:690–699.

- [200] Von Mehren M, Arlen P, Tsang KY, Rogatko A, Meropol N, Cooper HS. Pilot study of a dual gene recombinant avipox vaccine containing both carcinoembryonic antigen (CEA) and B7.1 transgenes in patients with recurrent CEA-expressing adenocarcinomas. *Clin Cancer Res* 2000;6:2219–2228.
- [201] Nagai E, Ogawa T, Kielian T, Ikubo A, Suzuki T. Irradiated tumor cells adenovirally engineered to secrete granulocyte/macrophage-colony-stimulating factor establish antitumor immunity and eliminate pre-existing tumors in syngeneic mice. *Cancer Immunol Immunother* 1998;47:72–80.
- [202] Ginn SL, Alexander IE, Edelstein ML, Abedi MR, Wixon J. Gene therapy clinical trials worldwide to 2012—an update. *J Gene Med* 2013;15:65–77.
- [203] Nemunaitis J. Vaccines in cancer: GVAX, a GM-CSF gene vaccine. *Expert Rev Vaccines* 2005;4:259–274.
- [204] Nemunaitis J, Jahan T, Ross H, Sterman D, Richards D, Fox B. Phase 1/2 trial of autologous tumor mixed with an allogeneic GVAX vaccine in advanced-stage non-small-cell lung cancer. *Cancer Gene Ther* 2006;13:555–562.
- [205] Akbulut H, Tang Y, Akbulut KG, Maynard J, Zhang L, Deisseroth A. Antitumor immune response induced by i.t. injection of vector-activated dendritic cells and chemotherapy suppresses metastatic breast cancer. *Mol Cancer Ther.* 2006;5:1975–1985.
- [206] Breckpot K, Dullaers M, Bonehill A, van Meirvenne S, Heirman C, de Greef C. Lentivirally transduced dendritic cells as a tool for cancer immunotherapy. *J Gene Med* 2003;5:654–667.
- [207] Di Lorenzo G, Buonerba C and Kantoff PW. Immunotherapy for the treatment of prostate cancer. *Nat Rev Clin Oncol* 2011;8: 551–561.
- [208] Barrett DM, Singh N, David L. Porter DL, Grupp SA, Carl H. Chimeric antigen receptor therapy for cancer. *Annu Rev Med* 2014;65: 333–347.
- [209] Sun M, Shi H, Liu C, Liu J, LiuX, Sun Y. Construction and evaluation of a novel humanized HER2-specific chimeric receptor. *Breast Cancer Res* 2014;16:R61.
- [210] Jensen MC, Riddell SR. Design and implementation of adoptive therapy with chimeric antigen receptor-modified T cells *Immunol Rev.* 2014 J;257:127–144.
- [211] Shi H, Sun M, Liu L, Wang Z. Chimeric antigen receptor for adoptive immunotherapy of cancer: latest research and future prospects. *Mol Cancer* 2014;13:219.