Chinese Journal of Cancer

Review

Advancement and prospects of tumor gene therapy

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Abstract

Gene therapy is one of the most attractive fields in tumor therapy. In past decades, significant progress has been achieved. Various approaches, such as viral and non-viral vectors and physical methods, have been developed to make gene delivery safer and more efficient. Several therapeutic strategies have evolved, including gene-based (tumor suppressor genes, suicide genes, antiangiogenic genes, cytokine and oxidative stress-based genes) and RNA-based (antisense oligonucleotides and RNA interference) approaches. In addition, immune response-based strategies (dendritic cell– and T cell–based therapy) are also under investigation in tumor gene therapy. This review highlights the progress and recent developments in gene delivery systems, therapeutic strategies, and possible clinical directions for gene therapy.

Key words Gene therapy, RNA interference, antisense RNA, tumor suppressor gene, suicide gene, oxidative stress, dendritic cells, angiogenesis, viral vector, non-viral vector

Gene therapy involves the transfer of nucleic acids to somatic cells for the treatment of genetic disorders in humans. In this therapy, disease-causing genes are removed or replaced with normal or functional genes to fulfill the enzyme or protein requirement of the body [1]. The disease is expected to be eliminated after gene therapy.

The idea of gene therapy was introduced by Joshua Lederberg in 1963; however, research on human genetics did not accelerate until the 1980s. Subsequently, the first clinical study on gene transfer was conducted by Anderson *et al.* [2] in 1990. In that study, a 4-year-old girl with adenosine deaminase (ADA) deficiency was treated by transfecting the ADA gene into her white blood cells, resulting in considerable improvements in her immune system[2]. In the same year, gene therapy was also tested on patients suffering from melanoma, and the results showed that retroviral gene transfer was safe and practical[3]. Since 1989, more than 900 clinical trials on gene therapy have been approved worldwide. Among these trials, 70% are in the area of cancer gene therapy [3,4]. To date, substantive progress has been made in gene therapy, and this progress has benefited from advancements in the therapeutic strategies discussed next.

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Gene Delivery Systems

Although considerable strides have been made in developing gene-based therapeutic strategies, establishing efficient and safe gene delivery systems remains the main challenge in tumor gene therapy. The gene therapy vectors used in gene delivery systems can be categorized into two groups: viral and non-viral systems.

Viral systems

Viral vectors, which can transfer genetic materials into host cells, are biological systems derived from naturally evolved viruses. Viruses used in gene therapy have been modified to increase safety, enhance specific uptake, and improve efficiency. The viral vectors include retrovirus, adenovirus, Herpes simplex virus (HSV), adeno-associated virus (AAV), and poxvirus (vaccina virus)[5]. The most commonly used DNA viral vectors are based on adenoviruses and AAVs. The understanding of

viral vectors has increased greatly and their design and production have been improved. Several clinical trials have been performed for various viral vectors [6,7]. However, there are some drawbacks in terms of the safety and toxicity of these vectors and the size of the transfected genetic material. Therefore, great caution should be exercised when using viral vectors for the treatment of human diseases, and this topic should be investigated further.

Non-viral systems

The limitations associated with viral vectors have encouraged researchers to focus on non-viral systems. Many methods have been developed for the non-virus-mediated delivery of genetic materials, including non-viral vectors and physical approaches.

Non-viral vectors Non-viral vectors are safe, can be constructed and modified by simple methods, and exhibit high gene encapsulation ability[5]. Non-viral vectors include cationic polymers like polyethylenimine (PEI) and poly-L-lysine (PLL), cationic peptides, and cationic liposomes. Among these vectors, liposome is widely used in clinical trials for tumor therapy. In HLA-B7-negative melanoma patients, antitumor immunity is induced by injecting cationic liposomes containing HLA-B7- and β-2 microglobulin–encoding genes [6,8]. Further, patients with glioblastoma have been treated with cationic liposomes containing the β-interferon–encoding gene[9]. Hence, liposomes are considered safe for use in humans.

Shell nanoparticles, the newly described cationic core, offer more advantages than liposomes, namely, high gene transfection efficiency and concurrent delivery of drugs and genes to the same cells [10]. Another advantage of nanoparticle-based therapeutic strategies is that they simultaneously have enhanced efficacy and reduced adverse effects. This advantage can be attributed to properties such as their passive and active targeting[11]. Passive targeting allows effective localization of targets in tumor cells based on "enhanced permeability and retention (EPR)"[7]. Coating nanoparticles with targeting molecules, such as antibodies, peptides, nucleic acid aptamers, carbohydrates, and small molecules, can enhance the cellular uptake of nanoparticles. For example, using a Wistar rat model which had implanted with folate receptor-expressing C6 glioma cells, significant growth inhibition of C6 glioma xenografts was observed after treatment with nanoparticle/targeting molecule combinations, FA-PEG-PEI/pCD/5-FC and FA-PEG-PEI/pTRAIL [12]. Unlike other types of therapeutic agents, nanoparticles allow for custom design and property-tuning. Further, as more clinical data[13,14] becomes available and the optimal therapeutic properties of nanoparticles becomes clear, the nanoparticle-based approach will continue to improve.

Physical methods Naked DNA-based gene therapy is an attractive approach because it eliminates or reduces the disadvantages of viral vectors [15]. However, naked DNA is prone to tissue clearance and cannot be delivered effectively. Thus, physical approaches have been developed to facilitate plasmid DNA delivery *in vivo*.

Electroporation Electroporation (EP) is an efficient and simple method for the DNA delivery. This technique is based on the principle that applying electric pulses across the cell membrane creates a transmembrane potential difference, allowing transient membrane permeation and facilitating the insertion of DNA through the destabilized membrane[16].

EP is a safe and possible treatment approach *in vivo* and has been used to transfer genes into the cells of skeletal muscles, tumors, brain, liver, skin, and other tissues. Among these experiments, 38% are related to cancer treatment[16]. Moreover, genes related to immune response are mostly used in EP-mediated tumor treatment. To date, interleukin-12 (IL-12) and interferon-α (IFN-α) are the most successful cancer therapeutic genes delivered by EP in experimental models. Intratumor EP of IL-12 cDNA-encoding plasmid into melanomas has now reached clinical trials. In 2008, Daud *et al.*[17] confirmed that IL-12 delivery by *in vivo* EP was effective. Subsequently, other clinical trials for cancers expressing human epidermal growth factor receptor-2 (HER-2) and carcinoembryonic antigen (CEA)[18] have commenced. The true potential of this delivery approach will not be known until the results of the aforementioned and other clinical trials have been reported.

Ultrasound Although EP is more efficient, microbubble- enhanced ultrasound causes less damage and is less invasive [19]. As a therapeutic application, ultrasound can generally be used to deliver ultrasound energy directly to an object and to enhance the delivery of therapeutic drugs and genes[20].

In eukaryotic cells, the transcription of heat shock protein (HSP) is markedly enhanced after exposure to temperature conditions above those required for maximum growth [20,21]. The ability of HSP promoter to respond to high-intensity focused ultrasound (HIFU) has been demonstrated in various studies [20,22]. The results from those studies indicated that gene expression was increased at high temperatures after exposure to HIF [22,23]. This approach enhances the spatial targeting and the efficiency of gene delivery.

In addition, ultrasound can enhance gene delivery by altering vascular permeability in a method called sonoporation. Sonoporation has been applied in many tissues, including tumors[19], and has been used to deliver

oligonucleotides and small interfering RNA (siRNA)to tumors. In the treatment of prostatic tumors, microbubble and ultrasound have been applied to target siRNA to the androgen receptor[24]. Hecht *et al*.[25] reported that they have successfully administered an intratumoral endoscopic ultrasound injection of ONYX-015 and an intravenous injection of gemcitabine to patients with unresectable pancreatic carcinoma.

To develop more applications and improve the existing ones, an in-depth understanding of the ultrasound mechanisms discussed herein is required.

Other approaches There are several approaches for non-viral gene therapy in addition to those mentioned before. The hydrodynamic-based method affords efficient gene transfer and expression by rapid injection of a large volume of DNA solution through the tail vein of an animal. However, this technique may be harmful for the experimental animal[26]. Gene gun immunization through the skin is a reliable and reproducible method of DNA vaccine delivery. This method can induce immune response against both infectious disease-causing agents and cancer in animal models. DNA delivery using this approach requires 250–2500 times less DNA than the standard method of intramuscular delivery[27]. Further, the gene gun immunization is a highly efficient method of achieving antigen presentation.

Minicircle DNA systems in gene therapy Minicircle DNA (mcDNA) is a novel form of supercoiled DNA containing only the therapeutic gene expression cassette and not the bacterial backbone genome[28]. It is generated by site-specific recombination in *E.coli*[29,30]. mcDNA is superior to conventional plasmid because the former exhibits improved gene expression efficiency and prolongs the time span in transfected cells[28,29,31]. Darquet *et al*.[32] reported that the *in vivo* injection of mcDNA leads to 13- to 50-fold increase in reporter gene expression in skeletal muscle and human carcinoma grafts in nude mice compared to the injection of an equal amount of parental plasmid. Further, Chen *et al*.[29] reported that the expression of a transgene in mcDNA-transfected mouse liver was 45 to 560 folds greater than in standard DNA-transfected mouse liver. Wu *et al*.[33] achieved a remarkable anti-nasopharyngeal carcinoma effect by performing mcDNA-mediated IFN-γ gene transfer in nude mouse. Further, mcDNA is a safe vehicle to achieve the deletion of antibiotic resistance genes.

Gene-Based Therapeutic Strategies

Due to the complex nature of cancers, a variety of gene-based therapeutic strategies have been used in tumor gene therapy.

Tumor suppressor gene therapy

A mutation or deficiency in tumor suppressor genes (TSGs) is critical for the multi-step development and progression of human malignancies. TSGs include *p53*, *PTEN*, *Rb*, *BRCA1/BRCA2*, *DPC4*, *VHL*, and *M6P/IGF2* as well as the newly discovered *SDH5*. Among these genes, the prevalence of *p53* is high in human tumors, and it is one of the most studied TSGs. In 2003, drug license, production approval, and good manufacturing practice certificate were successfully obtained from the China State Food & Drug Administration[34] for recombinant human Ad-p53, and it became the world's first commercialized gene therapeutic product to receive approval. In addition to the treatment of tumors with null or mutant genotype, p53 gene therapy has been proved to be effective against tumors with wild-type p53, especially when used in combination with chemotherapy or radiotherapy[35,36].

Suicide gene therapy

Suicide gene therapy is also termed as gene-directed enzyme pro-drug therapy (GDEPT)[37], and this approach has attracted increasing attention. GDEPT has potential advantages over conventional therapy in that it involves specific activation of target cells and has an expanded killing effect called the "bystander effect." The GDEPT approach encompasses several therapeutic systems, such as VZV-tk/Ara-M, NTR/CB1954[38], CPG2/ CMDA[39], PNP/6-MeP-dR[40], HSV-TK/GCV, and CD/5FCB[7,10]. HSV-TK/GCV and CD/5FCB are the best characterized systems[37], and clinical trials using HSV-TK are currently in the phase III stage. Despite its advantages, suicide gene therapy exhibits limited efficiency due to low targeting potential, suicide gene expression, catalytic activity of the enzyme product, and killing effects. In view of these shortcomings, several efforts are directed toward modifying suicide genes, screening for new pro-drugs, combining GDEPT with traditional therapies, and improving tumor targeting[41,42]. For example, Dilber *et al*.[43] designed a fusion protein comprising HSV-TK and the HSV-1 tegument protein VP22. This fusion protein is more efficient than HSV-TK alone[43]. Further studies will help determine if this method can be applied for clinical purposes.

Antiangiogenic gene therapy

Angiogenesis is an important process that supports the growth of solid tumors; therefore, inhibiting angiogenesis might accordingly arrest tumor growth. Several negative regulators of angiogenesis, such as angiostatin, endostatin, vasostatin, modulators of

vascular endothelial growth factor activity (sFLT-1), and cytokines/chemokines with marked anti-endothelial activity (IL-12, IFN-α, CXCL10, and others)[44], have been used in tumor therapy. We have constructed a replication-defective adenovirus carrying human endostatin gene (E10A) and have started evaluating its effects on solid tumors in phase II clinical trials[45]. The results of this study showed that our construct had promising therapeutic effects.

Cytokine-based gene therapy

Cytokine-based therapy is an attractive approach to modulate and enhance the immune response to tumors. The cytokines investigated include IL-2, IL-1, IL-12, IFN-α, GM-CSF, IL-4, and IFN-γ. Among them, GM-CSF and IL-12 are the most widely studied[46]. A phase I study designed to determine the use of IL-12 plasmid/lipopolymer complexes in treating recurrent ovarian cancer revealed that this approach is generally safe and well tolerated[47]. Similarly, a phase I clinical trial was conducted to determine the safety of adenovirus vector-mediated delivery of the IL-12 gene for recurrent or persistent prostate cancer[48]. Recent research suggests that combination immunotherapy may possess remarkable potential for clinical applications, although more tests are warranted to confirm the efficacy of this treatment.

Oxidative stress-based gene therapy

Oxidative stress, namely over-production of reactive oxygen species (ROS), has several pro-tumorigenic effects, such as increasing DNA mutation rate and inducing DNA damage, genome instability, and cell proliferation. Conversely, it can also be developed to kill tumors by delivering excessive oxidative stress into tumor cells or by disrupting the antioxidative defense systems of tumor cells[49-51]. One approach to achieve this killing effect is to deliver ROS-generating enzymes, such as glucose oxidase or xanthine oxidase, to tumor tissues directly. Stegman *et al.*[52] transferred the gene encoding α-amino acid oxidase (DAAO) into glioma cells and showed that the DAAO/D-amino system could be appropriate to treat malignant brain tumors. Another approach is to impair or inhibit the molecules that prevent oxidative stress in tumor cells. This strategy was confirmed to work in many experimental solid tumors using ZnPP, a potent inhibitor of the ROS-defensive enzyme heme oxygenase[53]. Although oxidative stress-based gene therapy is promising, it should be noted that high levels of oxidative stress are cytotoxic, resulting in decreased cell proliferation and increased apoptotic/necrotic cell death, whereas low or intermediate levels of oxidative stress are most effective in promoting cancer development[51]. Thereby, the level of oxidative stress should be optimized to achieve the better treatment and less side effects.

Therapeutic RNA-Based Strategies

Abnormally high expression of some genes, such as Ras, c-myc, epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), and cyclin-dependent kinase (CDK), may lead to tumor occurrence and development. In recent years, cancer therapy has involved approaches to silence or modulate the expression of the aforementioned genes. Several therapeutic strategies have been developed to manipulate the functions of these genes, and among them, RNA interference (RNAi) and antisense oligonucleotides (ASO) are the two most powerful approaches. Modified RNA molecules and ASOs are designed to bind target RNAs by well-characterized Watson-Crick base pairing and to modulate their function by suppressing protein expression or directly degrading mRNAs[54]. Herein, we will review the potential and challenges of these molecules in therapeutic applications.

RNAi-based therapy

Small interfering RNAs (siRNAs) are small (21–25 nt) dsRNAs that are mainly involved in guiding mRNA degradation. RNAi-based gene therapy encompasses two approaches: plasmid- or viral vector–mediated delivery of short hairpin RNA (shRNA) precursors[55] and direct delivery of small dsRNAs (siRNAs or siRNA precursors) to target cells. The latter is more suitable for cancer therapy and can be applied for clinical purposes. RNAi-based gene therapy has been used to treat wet age-related macular degeneration and respiratory syncytial virus infection. Meanwhile, the application of RNAi-based gene therapy for cancer is in the preclinical stage. Although RNAi-based therapy has been confirmed efficacious, improvements are required and a number of challenges must be addressed to realize its full potential[56-58].

ASO-based therapy

ASOs are single-stranded DNA and RNA molecules (13–25 nt) that are precisely complementary to a particular mRNA. ASO-based therapy has been extensively investigated over the past two decades owing to its conceptual simplicity, ease of design, and low cost[59,60]. Recent studies showed that cancer cells treated with specific ASOs showed a marked decrease in tumorigenic p73 transcript and protein. However, this

approach did not destroy the wild-type p73. ASOs rescued cells from apoptosis inhibition and decreased tumor cell proliferation[59].

Although remarkable progress has been made in ASO-based therapy, there are several drawbacks to this type of gene therapy. First, ASOs can induce immune response in a sequence-dependent or -independent manner[61]. Many efforts have been made to avoid the activation of innate cellular immunity for safe application of RNAi in a clinical setting. For example, chemical modifications can be made to RNAs so not to induce type I IFN production[57]. Second, off-target effects (OTEs) are known to arise from both sequence-dependent and -independent processes[55,62]. Dykxhoorn *et al*.[51] demonstrated that an RNA-containing sequence similar to mRNA 3'-untranslated regions (UTRs) might be prone to cause OTEs. However, such OTEs do not occur if the sequence is complementary to the open reading frame of mRNA transcripts[63,64]. Therefore, detrimental OTEs can be reduced by ensuring that the siRNA sequence does not match the sequence of an mRNA's 3'-UTRs. Chemical modification is also a promising approach to reduce potential OTEs[55]. Thus far, sequence-mediated OTEs have not been reported in preclinical or early clinical trials of siRNAs[57].

Immune Cell–Based Gene Therapy

Immune-based gene therapy can be used to treat tumors by enhancing antitumor immune response. Advances in immunology have led to the development of many novel immune therapies involving gene-modified dendritic cells (DCs), gene-modified T cells, and others.

DC-based cancer gene therapy

DCs can be pulsed with tumor-associated antigen through viral vectors, non-viral vectors, cDNA, or mRNA. Therefore, modified DCs can present specific tumor-associated antigens[65]. Kyte *et al*.[66] transfected DCs with allogenic mRNA from whole tumors, and administered the DCs to 19 prostate cancer patients who had completed vaccination. T-cell vaccine responses were observed in 12 patients, and stable or decreased prostate-specific antigen levels were observed in 11 patients[66]. Furthermore, DCs can be engineered to express co-stimulatory molecules and adhesion molecules or to down-regulate negative modulators, thereby increasing their T cell-activating ability. The co-stimulatory molecules include CD40 ligand (CD40L, CD154), CD70, OX40 ligand, and the adhesion molecule CD54, while the negative regulators include SOCS1. Schmitz *et al*.[67] transferred the gene encoding CD40L into pancreatic tumor cell-DC hybrids and observed that the efficiency of antitumoral response was elevated in an *in vivo* mouse model . Furthermore, DCs have been engineered to express other molecules, including cytokines, chemokines, and homing molecules[68,69]. Some trials showed encouraging results such as good immune responses and safety of the use of DCs; however, the clinical efficacy of this method was limited.

T cell–based cancer gene therapy

Because endogenous T cells lack an effective repertoire against tumor antigens, they can be modified to express tumor-specific T-cell receptor (TCR) genes. Some groups have engineered T cells to express natural αβTCR. These modified T cells can recognize tumor-specific antigens in addition to the functions of endogenous TCRs[70]. Johnson *et al*.[71] modified T cells using gp100-specific TCR/TCR, and the gene-modified T cells were detected for at least 1 month after treatment in patients with metastatic melanoma. In another approach, T cells can be transduced to express chimeric tumor antigen-specific receptors that contain a signal-chain antibody. These chimeric receptors, called T bodies, target surface antigens in an MHC-independent manner[72]. Haynes *et al*.[73] studied the effect of T cells expressing scFv-z chimeric receptors on the growth of human colon carcinoma in a severe combined immunodeficiency (SCID)/SCID mouse model or the growth of colon adenocarcinoma in syngeneic C57BL/6 mouse model. An efficient retroviral gene delivery system was used in their study and high and equivalent expression of scFv-z and scFv-g receptors was achieved in T cells[73]. Moreover, CCR7 was transferred into T cells to alter their homing[74]; cytokine-encoding genes, including IL-4, IL-10, and IL-12, were transferred into T cells to enhance antitumor immunity[46]; and a chimeric GM-CSF-IL-12 receptor was transferred into T cells to increase their circulating half-life[75].

The data from preclinical and clinical trials suggest the feasibility of T cell–based cancer gene therapy, but there are still many challenges[76]. Similar to other strategies, T cell–based cancer gene therapy exhibits limited clinical efficacy although immune responses were obtained during clinical trials. Furthermore, some studies suggest that preferable antigen receptors must be carefully selected to avoid the development of lethal autoimmune responses[77]. However, the true potential of these strategies will remain uncertain until the results of the previously mentioned and new clinical trials are reported.

Conclusion

Although a wide variety of tumor gene therapies have been investigated, the clinical applications of these strategies have not progressed sufficiently. Therefore,

rigorous and innovative research efforts are required to exploit the full potential of gene therapy. Owing to its principles and advantages, gene therapy is expected to be a routine clinical practice in the coming years.

Acknowledgment

This work was supported by Foundation from Knowl edge Innovation Program in CAS (No. KSCX1-YW-R-10); National Natural Science Foundation of China (No. 30973448).

Received: 2010-02-20; revised: 2010-03-15; accepted: 2010-04-15.

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