REVIEW ARTICLE
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Cancer gene therapy

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ABSTRACT

Cancer gene therapy can be defined as transfer of nucleic acids into tumor or normal cells with aim to eradicate or reduce tumor mass by direct killing of cells, immunomodulation or correction of genetic errors, and reversion of malignant status. Initially started with lots of optimism and enthusiasm, cancer gene therapy has shown limited success in treatment of patients. This review highlights current limitations and almost endless possibilities of cancer gene therapy. The major difficulty in advancing gene therapy technology from the bench to the clinical practice is problem with gene delivery vehicles (so called vectors) needed to ferry genetic material into a cell. Despite few reports of therapeutic responses in some patients, there is still no proof of clinical efficacy of most cancer gene therapy approaches, primarily due to very low transduction and expression efficacy in vivo of available vectors. An „ideal" gene therapy vector should be administrated through a noninvasive route and should be targeted not only to primary tumor mass but also to disseminated tumor cells and micrometastases; it should also carry therapeutic gene with tumor-restricted, time-regulated, and sustained expression. Current strategies for combating the cancer with gene therapy can be divided into four basic concepts: (1) replacement of missing tumor suppressor gene and/or blocking of oncogenes or pro-inflammatory genes, (2) suicide gene strategies, (3) induction of immune-mediated destruction, and (4) inhibition of tumor angiogenesis. The advance in the clinical benefit of gene therapy will probably be first achieved with combining it with standard cancer treatment: chemotherapy, radiotherapy, and immunotherapy.

KEY WORDS: *Neoplasms; Gene Therapy; Genetic Vectors; Gene Targeting*

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INTRODUCTION

It is estimated that at least one in three will develop cancer and one in four men and one in five women will die from it (1). Relative lack of success of conventional approaches to cancer therapy (surgery, chemotherapy or radiation therapy) together with rapid progress in elucidation of molecular basis of cancer development has led to current initiatives to treat human cancer by genetic intervention, i.e. gene therapy. The idea of gene therapy is rather simple - introduce the gene (transgene) into patient's cells and its product should cure or slow down the progression of a disease. Cancer gene therapy is, mostly, based on utilization of safe viral delivery vehicles (vectors) for transfer therapeutic gene/s into cancer cells. Review on the research of amelioration of non-viral gene transfer strategies (2) is out of the scope of this article.

There are two main approaches: *in vivo* gene therapy, in which genes are delivered directly to target cells in the body and *ex vivo* gene therapy, in which the target cells are genetically modified outside the body and then reimplanted. General aim is to perform *in vivo* gene therapy against cancer and different ways to obtain this selectivity are developed. Once transferred therapeutic gene(s) may has(have) various impacts: reparation or compensation of aberration-mutation or loss of genetic materials in cancer cells (for instance, correction of defective tumor suppressor gene - p53), killing tumor cells directly, amelioration of tumor antigen presentation on surface of tumor cells or stimulation of the immune response against a tumor, inhibition of tumor vasculature formation (antiangiogenesis), generation of marked population of cells for tracing the origins of recurrent tumors, protection of vulnerable cell population against treatments such as chemotherapy, or even enhancement of effect of conventional therapies (such as radiotherapy).

So far, 656 cancer gene therapy clinical protocols (66.5 % of total number of gene therapy trials) are in different phase of evaluation worldwide (3). Unfortunately, successful delivery and efficient and targeted expression of therapeutic gene(s) into cancer cells are very difficult tasks to achieve. Next pages are dedicated to these problems.

DELIVERY

One of the major difficulties in advancing gene therapy technology from the laboratory to the clinic is problem with delivery. Presently, there are three main classes of clinically applicable viral gene delivery vehicles (replication-incompetent virus vectors, hybrid vectors and replication-competent viruses).

First class of vectors, replication- incompetent or replication-defective vectors, are genetically altered viruses that function simply like a shuttle to the cells with a single round of infection either integrating or transiently expressing the transgene without subsequent viral replication. Vectors based on murine C-oncoretroviruses were the first vectors in gene therapy and remain the most frequently used vectors today (261 gene therapy clinical trials or 26.4 % of total number) (4,3). Favorable feature of retroviral vectors is integration into cell genome, which provides long-term expression of therapeutic gene. However, retroviral system possesses several major drawbacks. These include inability to infect nondividing cells,

random integration of its genome with associated risk of insertional mutagenesis (2 leukemia cases in gene therapy trial of X-linked severe combined immunodeficiency [SCID-X]), problems with low titre production, limited capacity for therapeutic gene (maximum size of gene insert is 8 kb) and possibility of generation of new recombinant replication-competent retrovirus (RCR) (5,6).

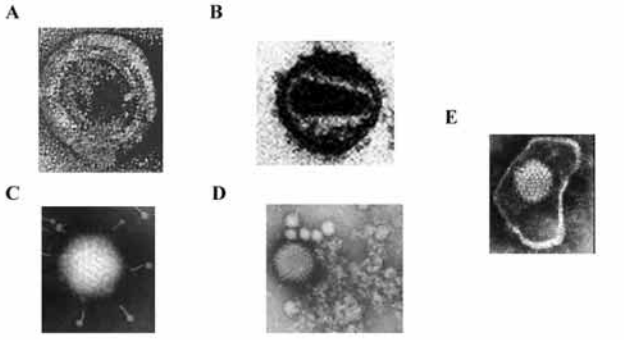

Figure 1. Various types of viruses used for creation of gene therapy vectors A - Moloney murine leukemia virus (Mo-MLV); B - Human immunodeficiency virus (HIV); C - Adenovirus (AV); D - Adeno-associated virus (AAV); E - Herpes simplex virus (HPV)

Concerning cancer gene therapy trials, great hopes were put into a phase III trial of retroviral delivery of the herpes simplex virus thymidine kinase (HSV-TK) gene to 248 patients with glioblastoma but it did not show any marked benefit (7). The failure of this clinical gene therapy protocol seems to be mainly due to the low tumor cell transduction rates observed (8). Another subgroup of retroviruses, lentiviruses, is considered as a promising gene delivery vehicle. They share all the standard properties of retroviruses and in addition they have unique ability of infecting both proliferating and quiescent cells (9). Despite all the safety safeguards that have been progressively introduced in human immunodeficiency virus (HIV) vectors, clinical acceptance of vectors derived from this pathogenic lentivirus is still subject to debate. As alternative, vectors from nonhuman lentiviruses, such as simian immunodeficiency virus, feline immunodeficiency virus and equine infectious anemia virus are designed (10).

Presently, adenoviral (Ad) vectors are the second most commonly used vectors in gene therapy trials (256 trials or 25.9 % of all trials) (3). They originate from Ad known for its low pathogenicity in humans, causing only mild symptoms associated with the common cold. Ad vectors are able to infect both dividing and nondividing cells and can be produce at high viral titres, which makes them attractive for gene therapy vectorology. The latest generation of helper-dependent, high capacity, gutted or gutless Ad vectors allows the introduction of up to 36 kb-long foreign DNA segment (11). However, transgene is transported to the host nucleus, but not inserted into host genome and its expression is short lived. Moreover, Ad particles stimulate strong immune reactions that clear the vector from the body, making long-term therapy impossible. To overcome these major drawbacks of Ad system, high doses and repeated administrations should be applied (12). Unfortunately, high doses given intravenously can produce lethal toxic reactions as revealed by phase I gene therapy clinical trial of ornithine transcarbamylase (OTC) deficiency at the University of Pennsylvania (USA) (13). However, efficient repeated administration of Ad vectors can be achieved as reported in several studies. For example, in a phase I/II trial for recurrent ovarian cancer where intraperitoneal readministration was used, transgene expression was measurable in 17 of 20 samples obtained after two or three cycles (14).

Recently, a non-pathogenic human parvovirus, adeno-associated virus (AAV), with ability to be stable maintained in both dividing and nondividing cells as integrated proviruses and to mediate long-term expression in variety of tissues, find potential application as replication-incompetent gene therapy vector. Site-specific integration on chromosome 19, as occurs with wild type AAV, will be a unique and valuable feature if incorporated into AAV vectors, further improving their safety. However, there are few limitations to the utility of this system, including small capacity for transgene (5 kb total) and the presence of immune responses to AAV capsid components and transgene products (15).

Finally, replication-incompetent vectors derived on herpesviruses should be mentioned. They are known as large capacity gene delivery vehicles (30 kb), which provide lifelong, latent infections with genomic material existing as a stable episome. Because of its tropism, herpes simplex virus type 1 (HSV-1) vectors are usually tested for gene transfer to the non-dividing cells of the nervous system (16). Second class of gene delivery vehicles, hybrid or chimeric vectors, combine the favorable properties of established viral vector systems (17). For example, hybrid between Ad and retrovirus, or HSV and AAV are developed (16). Third class of viral vectors, replication-competent, replication-selective, conditionally replicating, oncotropic or oncolytic viruses selectively target, replicate within, and destroy tumor cells by oncolysis, sparing surrounding normal tissue (18). Several types of replication competent vectors have been already tested in clinical trials, including conditionally replicative adenoviruses (CRAds), HSV, vaccinia virus, reovirus, poliovirus and Newcastle disease virus.

CRAds are adenoviruses modified to replicate in human cells and lyse them if a specific genetic defect is present in the cells. For example, ONYX-015, also known as CI-1042 and dl1520 (Onyx Pharmaceuticals, Pfizer Corp, USA) is an oncolytic adenovirus with an E1B-55kD gene deletion, which replicate in and lyse p53-negative tumor cells (19). p53 is deleted or mutated in >50 % of all human cancers (20). ONYX-015 was the first replication-selective viral agent tested in human (19). Phases I and II clinical trials with ONYX-015 as a single antitumor agent in patients with recurrent, refractory squamous cell carcinoma of the head and neck have shown durable responses and clinical benefit in 14% to 21% of these end-stage patients (21). In combination with chemotherapy (cisplatin and 5-fluorouracil [5-FU]), however, encouraging antitumoral activity has been demonstrated. Objective response (i.e. at least a 50% reduction in tumor size) was detected in 19 cases (or 63% of patients), with 8 complete responses (i.e. complete disappearance of measurable tumor) (22). Nowadays, ONYX-015 is under evaluation in phase III study in patients with head and neck cancer performed by Onyx Pharmaceuticals and Pfizer. In last 3 years, ONYX-015 was tested as monotherapy and combination therapy with chemotherapy in phases I and II trials in treatment of colorectal, hepatobiliary, hepatocellular, ovarian and pancreatic carcinomas, liver metastasis from gastrointestinal malignancies and lung metastasis (23).

Multimutated, conditionally replicating HSV-1 viruses (G207, 1716) are preferentially used for treatment of brain tumors (24). Enhancement of antitumor activity was observed when these vectors were used in combination with traditional therapy such as radiotherapy and chemotherapy (25, 26). Also, oncolytic HSV-1 vectors expressing suicide genes or immunostimulatory genes have been constructed to maximize tumor destruction through multimodal therapeutic mechanisms (27, 28).

TARGETING

Systemically targeted vectors are the highest goal to persuade in gene therapy. Gene delivery systems with ability to target tumor cells widely throughout the body of a patient would simultaneously increase real titres and efficacy and decrease potential toxicity. Injection of vectors into bloodstream for the treatment of cancer requires not only that the vectors be targeted to infect only tumor cells, but, also, that they be protected from degradation, sequestration or immune attack for long period of time, so that they can reach the appropriate destination (both primary tumor and distant metastasis) and penetrate into the tumor from the bloodstream before carrying out targeted infection.

Generally, two different principles of targeted gene-transfer to cells of interest exist (29). The first one, targeted delivery or cellular targeting is achieved by modification of viral envelopes and capsids (chimeric envelops, pseudotyping, molecular conjugation with specific antibodies or ligands, etc.), which restrained their interaction with a specific cell surface receptor (29-33). The second principle, so called targeted expression or transcriptional targeting restricts the expression of the therapeutic gene to appropriate cells, by plac-

ing therapeutic gene under control of tissue - specific promoters and enhancers. In the terms of cancer gene therapy, transcription targeting means to drive the expression of therapeutic gene by tumor-specific control elements (melanoma inhibitory activity [MIA] promoter, tyrosinase promoter and tyrosinase enhancer element for melanoma, cyclooxygenase 2 [cox-2] gene promoter and L-plastin promoter for ovarian cancer, etc.) (29, 34-36). Another way to achieve desirable safety of cancer gene therapy vectors is to obtain exogenous control of transgene expression and to raise or lower level of therapeutic protein according to therapeutic need (37). There are several types of inducible systems: pharmacological regulated systems (controlled by exogenic administration of a small molecule drug, such as antibiotic tetracycline), physiological regulated systems (sensitive to physiological signals, such as glucose deprivation and chronic hypoxia, via promoter of glucose-regulated proteins (GRP78) and hypoxia response element (HRE)/hypoxia-inducible factor (HIF) system), radiation - inducible systems (application of radiation-inducible promoters such as early growth response 1 [Egr-1] and WAF-1 promoter) etc. (29).

THERAPEUTIC GENES

Choice of therapeutic gene is crucially important in order to compensate above-mentioned deficiencies of current available vectors. It is well known fact that cancer development and its progression from benign to more malignant phenotypes involve numerous molecular genetic changes. The genes affected by these alterations are considered to be those responsible for cell cycling, apoptosis, signal transduction and angiogenesis. It is obvious that genes classified as oncogenes and tumor suppressor genes, together with genes related to DNA replication and repair should be used as therapeutic transgenes in cancer gene therapy. Their complementary DNA (cDNA) is being introduced into tumor cells in sense or antisense orientation with the purpose of leading to genetic recovery.

At least 20 clinical trials of p53 gene replacement with limited success have been performed (23). The most promising are: INGN 201 or ADVEXIN (Ad5CMV-p53 vector, Introgen Therapeutics, USA) and SCH 58500 (rAd-p53, Canji) currently involved in phase III gene therapy trial in patients with refractory head and neck cancer and stage III ovarian cancer, respectively (38, 39).

Other tumor suppressor genes, such as retinoblastoma (Rb), PTEN (phosphatase, tenesin homologue), mda-7 (melanoma differentiation associated gene-7) and OPCML (opioid binding protein/cell adhesion molecule-like gene) are under evaluation, too (23). The latest microarray technologies will reveal potential targets for future gene therapy. One should keep in mind: restoring tumor suppressor gene function may be insufficient and combination treatments, such as multiple genes or chemotherapy may be required.

Intuitively, simple eradication of tumor cells seems the best and the safest solution. The self-renewing potential of malignant tumors dictates that tumor cells should be cleared as efficiently as possible rather than genetically corrected. There is still no clear consensus on tumor-clearing approach using gene transfer. The most frequently used genes are those designed to kill cells directly (suicide genes) or indirectly through induction of immune-mediated destruction (immunogenic antigens or cytokines).

The first one, suicide genes, can be subdivided into two types. Protein product of the first type directs tumor cell killing directly (toxin gene therapy). For example, potent cytotoxic diphtheria toxin A (DTA) gene, driven by tumor-specific promoter, efficiently and specifically eradicates tumor cells (40). Second type of suicide genes encodes an enzyme capable of converting an inactive prodrug into a cytotoxic drug (gene-directed enzyme/ prodrug therapy [GDEPT]). Only cells bearing the suicide gene will be killed upon the subsequent prodrug treatment. The most widely used enzyme/prodrug system is herpes simplex virus thymidine kinase/ganciclovir (HSV-TK/GCV) (41).

Indirect killing of tumor cells by induction of anti-tumor immune response in patient (immuno-gene therapy) can be achieved in different ways. One way to stimulate immune system is in vivo transfer of one or more cytokine genes (interleukines [IL-2, IL-4, IL-6, IL-7, IL-12], tumor necrosis factor α(TNFα), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon γ(INFγ), etc.) into tumor cells or *ex vivo* transfer in autologous fibroblasts followed by injection in situ (42). Another way is to deliver tumor antigens into suitably activated dendritic cells that will generate antitumor immunity.

An alternative approach for eradication of tumor cells is anti-angiogenic gene therapy. One of the most notable distinguishing features of tumor growth and progression is the absolute requirement for expanded blood supply provided through the sprouting of new capillaries from pre-existing blood vessels (angiogenesis). Therefore, blocking the process of tumor neoangiogenesis is a promising strategy to arrest tumor growth. It involves various targets at the interface between the malignant population and the supporting stroma. For instance, the migration of tumor endothelium can be inhibited by interfering with matrix metalloproteinases (MMPs) and their unique ability to degrade extracellular matrix (EMC) (43). Another target can be a critical mediator of tumor vascularization known as vascular endothelial growth factor (VEGF). VEGF is also a key factor produced by solid tumors to inhibit recognition and destruction of tumor cells by immune system. Therefore, the inhibition of VEGF activity by specific single-chain antibody (scFv) will be beneficial not only for tumor growth inhibition and metastasis prevention, but also might improve immunotherapy (44). In addition, genes for naturally circulating factors capable of suppressing angiogenesis, such as angiostatin, endostatin and vasostatin, can be delivered and overexpressed in tumor cells (45-47).

CONCLUDING REMARKS

The latest approach in treatment of cancer is gene therapy. Although there is no yet marketed cancer gene therapy, considerably progress has been made in defining strategies and targets for gene treatment of cancer. In spite selectivity and efficacy demonstrated in experimental systems and in clinical trials, cancer gene therapy still has few problems to solve before it becomes routinely adopted in clinic. The main challenge is the improvement of gene delivery. The "magic" vector should be administered through a noninvasive route, protected from degradation and immune attack and safe for recipient and environment. Moreover, it should hit only desired cells within the target tissue and then allow the expression of therapeutic amounts of the transgene product with desired regulation for a defined length of time. Finally, it is crucially important that gene therapeutic action should be effective not only against primary tumor mass, but also towards distant sites of disseminated tumor cells and micrometastases, as well.

Clinical trials have produced a substantial amount of data and have contributed to the continuous improvement of vector systems, delivery methods and clinical protocols. Gene therapy has largely been tolerated with minimal toxicity in the most of the trials.

Conditionally replicating viruses (CRVs) offer the promise of a powerful weapon in our clinical arsenal against cancer. Therapy with oncolytic viruses seems to hold more promise in early clinical trials than gene therapy with non-replicating virus vectors. However, further major advancements in virus designs, application modalities and understanding of the host's immune system with the virus are clearly needed before oncolytic virus therapy can be introduced into clinical practice. Gene therapy for cancer may be most successful when combined with standard antitumor therapies (chemotherapy and radiotherapy) and may significantly enhance current treatment strategies. This is confirmed with different oncolytic viruses and some of these enter phase III clinical trials.

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