CANCER GENE THERAPY – STATE-OF-THE-ART

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ABSTRACT

A number of gene therapy clinical trials are being carried out the world over. Gene therapy is being applied in (I) cancer diseases, involving the largest number of patients, (II) monogenic diseases, (III) infectious diseases, (IV) vascular diseases, (V) autoimmune diseases and others. In the last decade, several strategies of cancer gene therapy have emerged due to a rapid development of gene delivery systems, both viral (recombinant retroviruses, adenoviruses, AAVs, herpes viruses) and non-viral (liposomes, gene guns, electroporation). To date four main strategies of cancer gene therapy have been evaluated in clinical trials: (I) immunogene therapy, (II) suicide gene therapy, (III) antiangio-genic gene therapy, (IV) and administration of tumour suppressor genes.

These strategies mostly involve: malignant melanoma, prostate cancer, renal cell cancer, colon cancer, breast and ovarian cancers, lung cancers, neoplastic diseases of the blood and brain tumours.

At the Department of Cancer Immunology at the GreatPoland Cancer Center Gene Modified Tumour Vaccine has been tested in malignant melanoma patients for more than six years. Due to encouraging results from phase I and II of clinical trials a phase III was designed and will be started in 2003.

Key words: cancer gene therapy, viral vectors, immnotherapy, antiangiogeic therapy, suicide therapy.

Gene therapy is one of the fastest developing fields of medicine. Throughout the world about 500 clinical trials are being carried out in order to evaluate the efficiency of this treatment in different diseases. There are several fields of application of gene therapy: (I) cancer therapy, which involves the largest number of patients participating in clinical trials, (II) therapy of monogenic diseases, (III) infectious diseases, (IV) vascular diseases and others. Although the first clinical trial of gene therapy was started in 1992, currently, the majority of clinical protocols are still in phase I, phase I/II, and, as yet, there are only a few phase III studies.

There are four major strategies of cancer gene therapy: (I) immunogene therapy, (II) suicide gene therapy, (III) correction of tumour suppressor genes and (IV) anti-angiogenic gene therapy.

GENE DELIVERY SYSTEMS

One of the major challenges in gene therapy is the delivery of therapeutic gene(s) to target cells. Several gene transferring systems are used for this purpose and they can be divided into two groups: non-viral methods (comprising injection of naked DNA [1,2], "gene gun" [3,4] and liposomes [5,6] etc.) and viral methods, which include gene delivery systems constructed on the basis of different viruses. The viral gene delivery systems, currently used in clinical protocols involve retroviral vectors, recombinant adenoviruses and pox-viruses, adenoassociated vectors and herpes simplex vectors. Recombinant retroviral vectors were used in the first protocol of gene therapy, carried out in 1992 by Rosenberg at al [7]. The Molo-ney Murine Leukemia Virus (MoMLV)-based retroviral vectors are the most popular viral gene delivery vehicles used in clinical protocols so far (35.8%) [8,9]. They transduce dividing cells and integrate viral genome with the genome of host cells. Recombinant retroviruses are mostly used for the production of cancer vaccines. They introduce therapeutic genes into cancer cells *ex vivo,* thus creating a stable genetically modified cancer cell lines. Although most of gene therapy protocols use retroviral vectors, they are not the best tool to modify cancer cells *in vivo.*

Recombinant retroviruses cannot be grown to high titres, can transduce only dividing cells and are ineffective when administered *in vivo* because of rapid destruction by complement complex in serum [10]

Adenoviral vectors are used in more than 25% of clinical protocols [9]. They are becoming more and more popular because of some features they demonstrate: there is an established, relatively easy production system, adenoviruses can be produced in high titres, show high transduction efficiency and can be effectively administered *in vivo*. There are three generations of adenoviruses. The first generation is highly immunogenic [11]. The 1st generation adenoviral vectors lack only one adenoviral sequence – E1, thus the therapeutic gene is delivered to target cells along with the rest of viral genes. The remaining adenoviral sequences induce a strong humoral and cellular immune response directed against AdV-modified cells. In the second generation of adenoviral vectors some further adenoviral genes have been removed, but transduced cells still express a few products encoded by viral sequences [12]. The third generation of adenoviral vectors, so called "helper dependent – gutless vectors", has been constructed in Frank Graham's laboratory [13]. They have removed all viral genes, thus creating an extremely low immunogenic vector, which can deliver very large (up to 30 kb) therapeutic genes. Although the production of gutless adenoviral vectors is more complex than that of the first generation, the helper dependent system represents a very promising tool for gene therapy applications.

The viral vectors currently used in gene therapy protocols are still not an ideal tool for novel approaches in cancer gene therapy such as genetic modification of stem cells. Popular recombinant viral vectors cannot deliver therapeutic genes to very early CD34+ progenitor cells and integrate their genome with a genome of non-dividing host cells. Lentiviral vectors represent universal solution to these problems [14]. They can be produced in a high titre, can transduce and integrate with the genome of non-dividing cells and efficiently deliver therapeutic genes to CD34+ cells. The lentiviral vectors are derived from HIV which belongs to the complex retroviruses. Unlike simple retroviruses (MoMLV), which contain only three structural genes, lentiviruses comprise nine (three structural - *gag*, *pol*, *env* and six regulatory - *Vif*, *Vpr*, *Vpu*, *Tat*, *Rev* and *Nef*) genes. Production of the most sophisticated (3rd generation) HIV-based viral vectors requires a quadruple transfection of 293 cells (three packaging plasmids and one transfer vector) [15]. Although there are still some safety concerns associated with the fact that the HIV is a deadly pathogen in humans, the last generation of lentiviral vectors will undoubtedly be soon applied in clinical trials of gene therapy.

CANCER IMMUNOGENE THERAPY

The first trial of cancer immunotherapy can be dated back to the 19th century, when Wiliam Coley used preparations derived from streptococcal cultures to treat tumours. In some of his patients the cancer burden diminished or even disappeared. Molecular biology and genetic engineering have created a new era in cancer immunotherapy. In the last two decades, multiple genes encoding immunostimulatory factors, MHC and costimulatory molecules have been synthesized, and now they are used in various gene therapy protocols. Tumour cells may be eliminated by the immune system by both cellular and humoral mechanisms [16,17, 18]. Cellular cytotoxicity is believed to play a major role in anticancer immunity. Specific cytotoxicity is mediated by a subpopulation of T cells possessing a T-cell receptor (TCR) consisting of alpha-beta chains. The alpha-beta cells comprise two subgroups – CD4+ and CD8+ cells, which recognize tumour antigens on tumour cells in a context of MHCII and MHCI molecules, respectively. Non-specific cytotoxicity is mediated by NK cells which recognize tumour cells that do not express MHC molecules. This kind of immune response is not antigen specific, but it does not require prior contact of immune effectory cells with target cells. Humoral anti-tumour immunity is mediated by antibodies produced by activated B-cells. Antibodies can eliminate tumour cells in various ways – by activating complement, by opso-

nization and antibody - dependent cellular cytotoxicity (ADCC) which is mediated by effectory cells, carrying Fc receptors. A key role in the induction of antigen specific immune response is played by professional antigen presenting cells (APCs) – dendritic cells (DC). DC behave as sentinels in the immune system. Immature DC are located in peripheral tissues and are characterized by a high phagocytic capacity, low expression of MHC and costimulatory molecules, and a low level of cytokine secretion. They capture and process various antigens, e.g. tumour antigens from apoptotic bodies or released from tumour cells destroyed in an antigen non-specific manner. Upon delivery of a 'danger signal' DC begin to mature. On the surface they upregulate MHC I and II molecules, costimulatory molecules (CD40, CD54, CD 80, CD86) and secrete cytokines. Maturing DC migrate towards draining lymph nodes, where they present tumour antigens in the context of MHC I or II molecules to CD8+ or CD4+ cells, respectively. Costimulatory molecules are necessary to provide a so called 'second signal', crucial for priming and activation of naïve T lymphocytes. IL-12 secreted by activated DCs acts on CD4+ cells and turns them into interferon-gamma (IFN-γ) producing Th1 cells. Such a polarized population of Th cells has been demonstrated to mediate a strong and long lasting antitumoural immune response. DCs are very effective in stimulating T cells, one DC can turn on 100 – 3,000 T cells [19].

Gene immunotherapy of cancer uses different immunological elements in order to elucidate an effective antitumoural immune response. For example, genes encoding various cytokines *ex vivo* or *in vivo* (intratumourally) are introduced into cancer cells. Cancer cells secrete proteins encoded by genes introduced into the tumour microenvironment. Such immunostimulatory factors modulate the tumour microenvironment and provide a danger signal attracting and activating professional APCs. Some cytokines (IL-2, IL-6, IL-12, IFN-γ) have been thought to directly activate killer cells (CD8+, NK). However, according to the current research, it seems that they mainly act as danger signals for DCs [20]. Tumour cells expressing cyto-

kines such as IFN-γ or IL-12, not only strongly activate dendritic cells, but also induce a strong polarization of immune response towards the functional Th1 cells [21].

Since 1996 at the Department of Cancer Immunology USOMS, Great Poland Cancer Centre in Poznań, Poland, a genetically modified tumour vaccine (GMTV) has been tested in malignant melanoma patients. GMTV consisted of allogenic melanoma cells modified with genes encoding IL-6 and its agonistic soluble receptor (sIL-6R). For the last 5 years more than 220 patients have been enrolled in this study. More than 35% of clinical responses were observed. The encouraging results became a basis for the design of a phase III randomized clinical trial which will be initiated in the 2003 [22].

Another approach to cancer gene immunotherapy is turning of tumour cells into antigen presenting cells. Tumour antigens are presented in the context of MHC-I molecules by most tumour cells. However, tumour cells, lacking costimulatory molecules which provide a second signal necessary for proper activation of lymphocytes, induce an antigen-specific tolerance of these effectory cells. Introduction of genes encoding costimulatory molecules such as B7.1 or B7.2 was thought to revert the tumour induced tolerance and to elucidate a strong antitumour immune response mediated by CD8+ cells [23].

DCs as a key element in the induction of antitumoural immune response are promising targets for gene therapy. Introduction of genes encoding tumour antigens into DCs elucidates a strong antigen-specific immune response mediated by CD4+ and CD8+ cells [24]. However, this approach has several disadvantages, e.g. modification of DC *in vivo* is extremely difficult, and isolation and propagation of these cells *in vitro* is a very time-consuming process. Moreover, genetically modified and activated *ex vivo* DCs, after subcutaneous administration hardly migrate into draining lymph nodes, thus showing much lower than expected efficacy in the induction of anti-tumour response. This method requires that a large number of gene modified DCs be injected into patients.

SUICIDE THERAPY OF CANCER

This concept is based on a direct delivery of a gene encoding enzyme, which can convert a non-toxic prodrug into its toxic metabolite [25]. Gancyclovir – a nucleoside analogue is used in the treatment of patients infected by herpes viruses (HSV, VZV). Cells infected by a herpes virus produce thimidine kinase (TK) encoded by a viral genome, and, upon ganciclovir administration, become unable to divide and subsequently die [5,26]. In order to treat cancer patients, a cDNA encoding TK may be directly delivered to tumour cells by adenoviruses, lentiviruses, liposomes or by physical methods. A few days later, the patient receives intravenous administration of ganciclovir. Ganciclovir is transported with blood to all organs and tissues including tumours. This prodrug upon entry into a tumour cell, transduced with a vector encoding TK, becomes phosphorylated into its toxic metabolite (ganciclovir triphosphate), which, by inhibition of DNA synthesis induces cell's death. In a tumour mass, cancer cells are connected with each other via gaps junctions. In a process called 'bystander effect' phosphorylated form of gancyclovir may spread from one genetically modified cell to anothers, where it exerts the same therapeutic effect [27]. Thanks to the 'bystander effect' not all cancer cells in a tumour mass have to be modified with the TK gene in order to achieve a complete elimination of the tumour. In order to enhance the efficacy of suicide gene therapy one tries to combine it with immunotherapy. We have demonstrated that IL-6 and GM-CSF enhances the suicide effect of TK in a murine model of malignant melanoma [28]

Other genes used in suicide gene therapy trials are: Cytosine deaminase, which converts a prodrug – 5-Fluorocytosine into 5-Fluorouracil; cytochrome P-450 (converts Cyclophosphamide into Phosphoramide mustard) and nitroreductase.

ADMINISTRATION OF TUMOUR SUPPRESSOR GENES

The essence of a neoplastic transformation of healthy cells is a mutation within two classes of genes: protooncogenes and suppressor genes. Proteins encoded by mutated protooncogenes deliver a signal to the nucleus and subsequently induce cellular divisions. On the other hand, proteins encoded by mutated suppressor genes are unable to inhibit protooncogene-induced proliferation. Such a transformed cell dynamically proliferates and forms a tumour. Several researchers had demonstrated that administration of intact suppressor genes into cancer cells reverts their neoplastic properties and induces tumour regression. However, clinical outcome of such therapies is not satisfactory, mostly because of low transduction efficiency achieved with currently available gene delivery systems. In order to eliminate all cancer cells, each of them must be modified with an intact suppressor gene. In case there is one unmodified cell left, it may further proliferate and form new tumours. However, Xu et al. demonstrated that liposome-mediated transfer of the p53 gene to mammary tumours in mice led to less than 5% tumour cell transfection, but was associated with a strong regression of tumours. It was shown that relatively low expression of p53 gene within the tumour mass induced a strong reduction in the number of blood vessels in the treated tumours [29]. Other suppressor genes used in cancer gene therapy are: BRCA1 in ovarian cancer, prostate cancer and breast cancer carried out by Holt JT et al. [30,31], and p16 in pancreatic and prostate cancer Vieweg J. et al [32,33].

Another approach to suppressor gene therapy of cancer is the application of mutated adenovirus. The early adenoviral E1b sequence is responsible for turning off a p53-mediated apoptosis upon entry of an adenovirus into a cell. Apoptotic death of infected cells, prevents from viral replication. ∆E1b adenovirus administered to cancer patients may replicate only in cancer cells. which lack the functional p53 gene. Viruses may replicate in them, elucidate a cytopathic effect, and subsequently infect and destroy the rest of cancer cells [34,35].

ANTIANGIOGENIC GENE THERAPY

Tumours require efficient blood supply in order to grow. Inhibition of angiogenesis

is a promising strategy for treating cancer patients. Although numerous endogenous angiogenesis inhibitors have been discovered, the clinical evaluation of these agents has been hindered by high dose requirements, manufacturing constraints, and relative instability of the corresponding recombinant proteins. Antiangiogenic therapy is directed specifically against microvascular endothelial cells that have been recruited into the tumour bed. Specific antiangiogenic therapy has little or no toxicity, does not require that the therapeutic agent enters any tumour cells nor cross the blood-brain barrier. It controls tumour growth independently of tumour cell type, and does not induce acquired drug resistance [36]. The delivery of genes encoding antiangiogenic proteins seems to be a promising approach, avoiding obstacles associated with systemic administration of such drugs. Therapeutic genes enoding antiangiogenic agents can be delivered into patients by different systems e.g. recombinant adenoviruses [37] or liposomes [38]. Antiangiogenic gene therapy may be performed as a systemic or local treatment. Scientists still argue over the best way of administration. Local (intratumoural) administration is associated with a strong "bystander effect which augments the angiostatic activity of introduced genes" [29] and should not be associated with potential side effects of systemic therapy. On the other hand, systemic administration of genes encoding antiangiogenic factors, makes possible a long-lasting elevation of, e.g. endostatin levels in blood [40]. Thus could it be a proper treatment (i) after surgery or radiotherapy to prevent recurrence of distant metastases, (ii) in combination with chemotherapy (iii) in combination with vaccine therapy or immunotherapy, or (iv) in combination with other types of gene therapy, for example, delivery of tumour suppressor genes.

Antiangiogenic therapy may be divided into two strategies. Direct antiangiogenic therapy targets endothelial cells (application of angiostatin or endostatin), whereas indirect therapy interferes with a tumour-derived angiogenic factor or the receptor for it (administration of gene encoding a truncated form of native soluble FLT-1 (endothelium-specific receptor tyrosine kinase), which is a receptor for VEGF) [41].

THE FUTURE PROSPECTS

Although a dramatic progress in cancer treatment took place in the last century, the effects of therapies are still not satisfactory. However, a dynamic development of several approaches to cancer gene therapy over the last two decades seems to signal a future breakthrough in oncology. Novel, much more effective gene delivery systems will soon be used in clinical trials. Lentiviral vectors, which are derived from a deadly virus, despite some temporary safety concerns will surely enter oncological clinics. Studies carried out in animal models will further modify current clinical protocols. Probably, several cancer gene therapy strategies will have to be combined in order to make the 'escape' of cancer cells from one particular treatment impossible. New tumour-associated antigens, construction of 'designer' cytokines and a better understanding of immunological processes will undoubtedly contribute to greater advancement in immunogene therapy. New discoveries in molecular biology will also provide new concepts for novel approaches in gene therapy of malignancies.

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