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Commend

GENE THERAPY RESEARCH IN CHINA

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Gene therapy was firstly proposed in China by professor Min Wu (Chinese Academy of Medical Science, Beijing) in 1974 as a potentially promising approach to disease treatment. Since 1980, many Chinese scientists have being made their efforts to investigate the somatic cell therapy and the basic researches on gene therapy. From 1990's, some important advances in gene therapy researches have been achieved in China. Many original papers on the gene therapy of cancer, inherited disease, and infectious disease have been published in the international journals. For example, the fibroblast-mediated gene therapy for hemophilia B was approved to enter clinical trials in China, and its research paper was published in Human Gene Therapy.[1] Up to now, there are many gene therapy research projects funded by the National Natural Science Foundation of China and National High Biotechnology Foundation. The National High Biotechnology Foundation has supported 10 gene therapy research projects conducted by laboratories in Beijing and Shanghai. These studies focused on development of better gene transfer vectors (e.g. modified AAV and Ad vectors) or efficient gene delivery systems (e.g. cell-targeting nonviral vectors), new approaches to gene therapy of diseases (e.g. vaccination with gene-modified antigen presenting cells, establishment of allo-vaccine bank, tissue-specific route of direct administration, induction of the differentiation of tumor cells by novel gene RA538). Basic researches on gene expression and mechanisms of pathogenesis of disease in animal models (e.g. and peripheral, artery disease) are being performed. Then, the established clinical protocols will be critically reviewed by Bureau of Drug Administration (BDA), Ministry of Public Health (MPH), China before being approved to enter clinical trials. On may 5, 1993, "Points to consider for the clinical research on human somatic cell therapy and gene therapy" was issued. The ethical and scientific review panels have been developed to inspect the application of gene therapy to human clinical trials. No one can do clinical trials of gene therapy in China unless reviewed and approved by BDA, MPH, China. According to the rapid advances in this area, "Points to consider for the clinical research on human somatic cell therapy and gene therapy" was revised in January, 1997, and two additional new documents were issued. One is "Guidance on application of gene therapy research on human subjects". The other is "Guidance on application of somatic cell therapy on human subjects". As to ethical issues, the only form of human gene therapy being considered today in China is somatic cell gene therapy. The BDA, MPH, China will not consider approving any germline gene therapy research recently. Up to now, two clinical trials of gene therapy have been approved in China. One is the "Treatment of Hemophilia B with autologous skin fibroblasts transduced with a human clotting Factor IX cDNA". (J.L Hsuch, Fudan University, Shanghai). The other is "Treatment of patients with malignant gliomas by *in situ* injection of Herpes Simples Thymidine Kinase vector producer cells and intravenous administration of Gancyclovir" (Jianren Gu, Shanghai Cancer Institute).

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Gene Therapy for Inherited Diseases

The concept of gene therapy originated from the observation that certain diseases are caused by inherited defect of a single functional gene. Theoretically, diseases caused by a known monogenic defect could be treated and potentially cured by replacement with corresponding functional gene. The first clinical trial of gene therapy in the world was performed on a patient with the deficiency of adenosine deaminase. In our country, the research of gene therapy for monogenic inherited diseases is focused on hemophilia and thalassemia. The hemophilia B gene therapy mediated by autologous fibroblasts has been approved to enter clinical trials.[1] and its basic researches on improving expression levels of clotting factor IX and utilizing novel carrier cells, such as myoblasts, are under investigation.[2] The basic research of thalassemia gene therapy is on going in the laboratory of Prof. Liu Depei (Chinese Academy of Medical Science, Beijing).[2,3]

Cancer Gene Therapy

The current strategies for cancer gene therapy include transducing suicide genes into tumor cells to confer drug sensitivity to tumor cells, the use of recombinant DNA constructs as immunotherapeutics, the use of recombinant DNA constructs to replace defective tumor suppresser or inactivate oncogens in tumor cells, transducing drug-resistant genes into bone marrow hematopoietic stem cells to protect them during chemotherapy.

The principle of suicide gene therapy of cancer is to transduce a drug-sensitivity gene into tumor cells whose product can convert a nontoxic prodrug administered systemically to a cytotoxic metabolite in tumor cells. This is one of the first strategies proposed for gene therapy of cancer patients. There are two different systems of suicide therapy. One is to use herpes simplex virus thymidine kinase (HSV-TK) gene and the prodrug gancyclovir (GCV), the other is to use E. coli cytosine deaminase (CD) gene and 5-fluorocytosine (5-FC). Suicide gene therapy exerts its therapeutic effects not only by its direct cytotoxic effect on suicide gene-expressing cells, but also by its "by-stander" effect on nontransduced cells. The mechanisms underlying by-stander effect include transfer toxic metabolite through gap junctions, phagocytosis of apoptotic vesicles and induction of antitumor immunity. The protocol that treatment of patients with malignant gliomas by *in situ* injection of HSV-TK vector producer cells and intravenous administration of gancyclovir[5] is under clinical evaluation, and it's the first clinical protocol for cancer gene therapy. Specific expression of suicide gene in tumor cells was explored by placing suicide gene under the control of tumor-specific promoter/enhancer, (e.g. AFP or CEA promoter). Combination of CD/5-FC suicide gene therapy with SCF or GM-CSF gene therapy was found to be capable of exhibiting synergistic therapeutic effects on preestablished hepatocellular carcinoma, which may result from the augmented antigen presentation by dendritic cells recruited by SCF or GM-CSF.

Cancer immunogene therapy is the most active area in gene therapy, and accounts for most of the currently approved clinical protocols of cancer gene therapy in the world.[6] The major advantage of this approach is the potential to generate effective systemic antitumor immunity.

Different approaches have been evaluated to deliver target gene into body. T cells(i.e. TIL or LAK) can be genetically modified with cytokine gene such as TNF-α and adoptively transfused into cancer patients. But the transfused T cells are poorly targeted into tumor tissues. An promising approach is to transduce cytokine gene or other immunoregulatory molecule gene into tumor cells to generate novel tumor vaccine. Various tumor vaccines genetically modified with cytokine gene (i.e. IL-2, IL-3, IL-4, IL-6, IFN-γ, GM-CSF, etc) have been evaluated in different tumor models. Combination of IL-2 gene-modified tumor vaccine with IL-4 or IL-6 gene modified tumor vaccine could induce antitumor immunity more potently.[7] Enhanced antitumor effects were demonstrated when cytokine gene-modified tumor vaccines were combined with adoptive chemoimmunotherapy AK/IL-2/DOX.[9,10] Trans-fecting B7 or MHC genes into tumor cells are capable of improving tumor immunogenicity. Recently, it's shown that tumor cells transduced with allo-MHC II gene could induce effective antitumor effects.[11] Another alternate approach is to inject cytokine gene-modified fibroblasts. Intrasplenic transplantation of genetically modified hepatocytes or fetal liver cells could be a promising approach to treating liver cancer. It has been demonstrated that the hepatocytes intrasplenically could preferentially reside in liver,[12] and express target gene. Intrasplenic

trasplantation of IL-2 gene-modified fetal liver cells could inhibit the preestablished metastatic liver carcinoma in animal model.[13]

Recently, cytokine gene transfer into antigen-presenting cells (i.e. dendritic cells and macrophages) represents a novel approach to cancer immunogene therapy and is receiving great attention. Antigen presentation is critical for the induction of antitumor immune responses. The gene transfer efficiency of dendritic cells and macrophages by adenovirus vector was evaluated. It has been demonstrated that GM-CSF gene-modified dendritic cells (DC) acquired more potent costimulatory activity, and capable of inducing protective and therapeutic antitumor immunity more potently after pulsed with tumor antigen or fused with tumor cells.[14,15] Macrophages genetically engineered with GM-CSF, M-CSF or IFN-γ possess more potent cytotoxicity against tumor cells and exhibit more potent therapeutic effects after pulsed with tumor antigen.[16] To apply this approach to clinical trials, we have established the methods to generate large numbers of human dendritic cells and macrophages, and constructed replication-defective recombinant adenovirus vector harboring human GM-CSF, or IL-12. To improve the preferential chemotaxis of DC on T cells, we genetically modified DC with lymphotactin(Lptn) gene. It was found that Lptn gene-modified DC pulsed with tumor peptide could induce protective and therapeutic antitumor immunity more potently.

The approach to *in vivo* gene delivery seems to be promising in immunogene therapy. Liposome can efficiently mediate *in vivo* cytokine gene transfer (e.g. mIL-2, hIL-6, hG-CSF genes), consequently induce effective antitumor immunity. Vaccinia virus and adenovirus harboring cytokine genes (e.g. hIL-2, mGM-CSF, hTNF-α, mIFN-γ gene) are capable of delivering target genes into tumor cells for therapeutic purpose.[17,18]

Replacement of tumor suppresser gene is another promising strategy for cancer gene therapy. Transducing tumor suppresser genes(i.e. RATS1, p53, RA538, p16) into tumor cells via retroviral vector or adenovirus vector could induce apoptosis of tumor cells.[19,20] Direct administration of recombinant adenoviruses of tumor suppresser genes into tumor tissue seems to be more attractive and applicable in clinical situation.

Gene Therapy for Other Diseases

Besides inherited diseases and cancer, gene therapy is also an attractive strategy for treatment of other diseases. Among them, the most promising protocols are gene therapy of peripheral artery disease with direct intramuscular injection of VEGF recombinant DNA constructs,[21] gene therapy of Parkinson's disease. Besides, there are some potential projects under investigation, including gene therapy of HBV with the antisense phosphorothiate oliodeoxynucleotides targeted to HBV genome,[22] and treatment of thrombotic diseases via delivery gene therapeutics of micro-urokinase,[23] and prevention of intimal hyperplasia of vascular anastomotic site with pro-urokinase.[24]

Gene Transfer into Hematopoietic Stem Cells

Hematopoietic stem cells receive great attention in gene transfer and gene therapy. Current protocols are attempting to protect bone marrow during large-dose chemotherapy by transducing the multiple-drug resistance gene (MDR1) into normal bone marrow of blood-derived hematopoietic stem cells. The MDR1 gene produces P-glycoprotein, which functions as a cellular efflux pump and may be responsible for the resistance of tumor cells to various hydrophobic cytotoxic drugs. Transducing MDR1 gene into hematopoietic cells could render them resistant to systemically administered chemotherapeutics. The potential advantage of this approach is that it may allow for high-dose chemotherapy with less toxicity and more potent efficacy. This approach is under preclinical evaluation in China. Transfering hematopoietic growth factor genes into hematopoietic stem cells will accelerate hematopoietic recovery from hematopoietic suppression induced by chemotherapy, radiotherapy.[25]

Gene Expression Vector Design

Expression vector is critical for gene delivery and expression, but all the exiting vectors have their limitations. Therefore, vector design is very important for future research. Retroviral vector can integrate into dividing cells and express exogenous gene stablely, is utilized in the majority of currently approved gene transfer and gene therapy clinical protocols. But retrovirus vector has its own limitations, such as low transfer efficiency, incapability of gene transfer into nondividing cells, limited inserted capacity,

limited virus titers, etc. Consequently, more versatile vectors are needed for the successful application of gene therapy. Adenovirus vector can mediate gene transfer efficiently regardless of the cellular mitotic status, and is increasingly being used in gene transfer and gene therapy. But adenovirus vector is high immunogeneic, which means that it is necessary to reduce its immunogenicity by genetic modification to make it more applicable. Vaccinia virus can accommodate large inserts, and also receives recent attention for the use in the delivery of genes for therapeutic purpose.[17,18] Adenovirus-associated virus (AAV) is capable of infecting both dividing cells and nondividing cells efficiently, and safely integrating at a specific location in human chromosome.[19] So AAV vector is potentially attractive in gene therapy. HSV capable of establishing latent infection in brain has received intrigue in their potential application in gene transfer into neurons and brain tumors. The defective plasmid-derived HSV vector pHSVL has been constructed and demonstrated to be capable of mediating gene transfer into Vero cells and motoneurons *in vitro*.[26] Nonviral expression vector can be *in vivo* delivered directly and mediate gene transfer efficiently. Myoblasts are potentially effective target cells for *in vivo* delivery of plasmid vector.

The specificity of therapeutic gene expression is one of the most concerned aspects in vector design, which could be achieved by conferring targeting capability to vector or using tissue-specific promoter, and optimizing the gene delivery route. Attachment of certain ligands to viral capsid or envelope either chemically or with antibody or via genetic manipulation is a feasible and effective strategy to conferring targeting capability to viral vectors. In a recent report, an artificial virus-like DNA-protein complex was generated by conjunction of an autonomously replicating Epstein-Bar virus-based vector with galactosylated histone, and demonstrated to be capable of mediating gene trasfer into hepatocytes.[27] The use of tissue-specific promoter, such as carcinoembryonic antigen (CEA) promoter, alpha-fetoprotein promoter (AFP), albumin promoter has been demonstrated to successfully generate specific expression vector.[28] Gene expression control and modulation is another important issue in gene therapy. Inducible promoter such as early growth response gene (Egr-1) promoter has been shown to be capable of inducing the expression of target gene in tumor cells by irradiation.[29]

Summary

Gene therapy, a novel strategy for disease treatment, is potentially promising in clinical application. It can be used to augment the exiting therapeutic approaches, such as immunotherapy and chemotherapy. When gene therapy is combined with other therapeutics, additive and synergistic effects may be achieved. Rapid progress of gene therapy has been made in China, and it is predictable that increasingly more clinical protocols will be submitted to BDA, MPH. Critical review by BDA, MPH is necessary prior to any clinical trials. Anyway, gene therapy is novel strategy for disease treatment and undoubtedly has some limitations. Consequently, it is imperative to avoid unrealistic expectation for this emerging approach. Since failure to meet unrealistic expectations in patients with highly advanced disease may discourage further development. It is encouraged to find novel efficacious gene for gene therapy. Vector design is critical for future application of gene therapy. The goal of vector design is to increase the precision of targeting, reduce toxicity, and improve the efficiency of gene expression and the therapeutic effects. Although much research needed to be done, the specific targeting of effective gene may render gene therapy more promising for future application.

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