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Recent Progresses in Brain Gene Therapy

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ABSTRACT

Gene therapy consists of the introduction of genetic material into cells for a therapeutic purpose. The understanding of the basics of the molecular and cellular mechanisms in disease treatments has resulted in the development of high-tech gene transfer materials with improved therapeutic efficacy. Based on the results of more than 2000 clinical trials to date, gene therapy is going to be included in standard treatment approaches in some specific diseases. Also, gene therapy has been highly improved in central and peripheral nervous system diseases. This review tried to focus on techniques and approaches in brain tumors and nervous system's gene therapy strategies and will discuss about associated problems and potential future in management of inherited or acquired neurological disorders.

Keywords: Gene therapy; Brain; Treatment; Nervous system

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INTRODUCTION

Gene therapy is the process by which genetic material is transferred into somatic cells to bring about a therapeutic effect. A better term would be gene transfer. The realization that multiple genetic alterations occur during the process of tumor formation has directed attention to the use of gene transfer as a therapeutic option. Any transfer of genetic material that can modulate gene expression in the host's cell for a therapeutic effect can be used for gene therapy [1]. The first attempt for medical transfer of foreign genes into human not counting organ transplantation to treat beta thalassemia, was conducted by Martin Cline on July 1980 [2]. The first approved gene therapy clinical trial was carried out in a 4-year-old girl suffering from adenosine deaminase (ADA) deficiency on September 1990. William French Anderson utilized a modified retrovirus to transfer normal ADA genes to T lymphocytes of the patient [3]. Gendicine, an adenovirus-p53 based gene therapeutic, was the first commercially available gene drug which was introduced in China on October 2003, approved for head and neck squamous cell carcinoma [4]. After that, by December 2004, 667 clinical gene therapy protocols had been submitted for review to the Recombinant DNA Advisory Committee (RAC) of National Institute of Health in United States. In Europe, Glybera, an alipogene tiparvovec, was the first commercially available gene therapeutic product in western world for treating familial lipoprotein lipase deficiency, which was approved at the end of 2012 [5].

Ex vivo and In vivo approaches

The process of cell isolation from the patient and genetic modification outside the body in cultivated area and subsequent re-implantation into patient is called ex vivo gene therapy. Lower ectopic expression in off-target organs, decreased immunologic response and more robust

administration of the therapeutic agent are among the benefits of this method. However, this method has some limitations such as protein secreting ability of target cells. Gene delivery by vector into the subject as a direct target cell transducer is called in vivo gene therapy. It is far more complex because many difficult issues must be addressed, including selection of appropriate vehicles, or vectors, to carry the genetic load, efficacy of distribution and targeting, ability to regulate gene function or expression in vivo and safety concerns, especially when viruses are used as vectors. One of the first and well known in vivo gene therapy experiments was utilization of an attenuated adenovirus-derived vector for patients with ornithine transcarbamylase deficiency. One out of 17 cases showed severe immune reaction against capsid of the adenovirus, which resulted in death [6]. Improvement in cell targeting and capsid shielding has made this method a potentially useful strategy for gene therapy.

Non-viral gene transfer

Non-viral gene delivery is conducted under chemical and physical methods. Physical route of delivery is based on making transient penetration in cell membrane by mechanical, electrical, ultrasonic, hydrodynamic, or laser-based energy for DNA entrance. Direct injection of naked DNA (5-20 kb) into skin and skeletal muscles, gold or tungsten spherical particles coated with plasmid DNA (2-5 micrometer diameter) bombard ant by gene gun, insertion of a pair of electrodes for cell membrane disruption called electroporation, direct intracellular transfer of any water-soluble particles by hydrodynamics, ultrasonic waves by making nano-pores in cell membrane and magnetofection as a technique for concentrating nucleic acid coated particles into target cell which has got advantage of both physical and biochemical transfection systems at once are between among physical delivery methods [7-10]. Safety, low immunogenicity and toxicity are main advantages of this system. Insufficient transfer efficacy to the target cells and nuclear translocation of the DNA complex are serious problems for gene therapists. Another more desirable route of genetic materials is via chemical method. In this system, negatively charged nucleic acids are compacted by poly-cationic nanomeric particles. The so called nanomeric particles between a cationic liposome and nucleic acid are called lipoplex. The polyplex is a nanomer between a cationic polymer and nucleic acid. Low toxicity and antigenicity are main advantages of cationic delivery systems. Sub-categories of cationic lipids are monovalent cationic lipids, polyvalent cationic lipids, guanidine containing, cholesterol derivative compounds, cationic polymers including poly ethylenimine, poly-l-lysine and protamine, and also lipid-polymer hybrid [11].

Viral gene transfer

Most gene therapy approaches attempting to treat brain tumors have used viruses for in vivo gene transfer to tumor cells. Viruses have undergone millions of years of evolution. Scientists are now attempting to adjust these vehicles for treatment of human diseases. Vectors adapted from viruses stand out because of the ease with which viruses enter cells and then leak out their contents; the viral genes that induce the host to generate the components of new virions [12]. Retroviruses are RNA viruses. Retroviruses can infect (transduction) and transfer the viral genome only to dividing cells. This capacity allows them to be used to selectively target tumors in the brain [13]. The integrated gene may inactivate important regulatory genes, such as tumor suppressor genes, or cause the activation of oncogenes. Such events may lead to new tumor formation (insertional mutagenesis*).* An additional hazard is the possibility of recombination of the replication-defective retroviral vector into a replication-competent virus with uncontrolled replication in the host. Genetic engineering of the vectors minimizes this risk. A subgroup of mentioned vectors is Lentiviruse, which can only infect dividing cells. They can deliver up to 10 kb DNA sequence and because of their unique tropism to neural stem cells, they are utilized in central nervous system targets without significant immune reaction and un-wanted side effects [14]. Adenoviruses are DNA viruses which can be used as vectors in two types. Helper dependant adenoviral vector, also called gutless or gutted, has got two parts; one vector (the helper) contains all the viral genes required for replication but has a conditional gene defect in the packaging domain, the second vector contains only the ends of the viral genome, therapeutic gene sequences and the normal packaging recognition signal, which allows selectively packaged release from cells [15]. Another type is hybrid vectors which are made of the high transduction efficiency of a gene-deleted adenoviral vector and the long term genome integrating potential of adeno-associated and retroviruses viruses [16]. The genome can be manipulated to produce replication-deficient vectors capable of accommodating foreign genes. Insertion of the transgene is usually done at the EIA early region of the virus, inserted without reducing infectivity. These viruses have low pathogenicity in humans. They are not neurotoxic. High titers of the virus can be achieved to allow better levels of expression in treated tissues than those achieved in other vector systems. The adenovirus DNA

does not integrate into the host cell genome but assumes an episomal position, thus avoiding the potential risks of insertional mutagenesis, inactivation of tumor suppressor genes, and tumorigenesis [17,18]. Herpes simplex virus (HSV) includes disabled infectious single copy, which comprise a glycoprotein H defective mutant HSV genome and they can carry transgenic DNA of up to 150 kb and because of its neurotrophic features, it has the greatest potential for gene delivery to nervous system [19,20]. Every tissue and specific cells within each tissue or organ have significant differences in their transducibility. In general, rapidly dividing cells like cancer and many epithelial cells are readily transduced whereas non-dividing cells like neurons are more difficult to transduce. Hence a vector used for one type of tissue, may not be suitable for another. For the nervous system, adenoviral vectors, lentiviruses and HSV have specific tropism, with adenoviruses having the best safety profile. The basic dogma is non-viral vectors are less efficient but safer; however, viral vectors are increasingly safe and non-immunogenic, and non-viral vectors start to resemble viruses to make them more efficient.

Nervous system as a target for gene therapy

The brain poses three major problems for gene therapy: 1) Neurons in the brain of aults are unable to divide and are difficult to transduce, 2) The blood brain barrier (BBB) prevents vectors and plasmids to get access from the blood to the brain, and 3) Most neurological disorders including epilepsies affect multiple regions of the brain. Notable improvement has been made in developing gene therapy for sensori-neural disorders, in particular blinding retinal degenerative diseases, and other rare diseases such as retinitis pigmentosa, Leber congenital amaurosis and choroideraemia. Gene therapy for retinal blindness is the most progressed protocol, as three different clinical trials have demonstrated efficacy of RPE65 gene augmentation in patients suffering from Leber congenital amaurosis [21]. Non-replicating HSV vectors are promising vehicles for delivery of therapeutic transgenes to the peripheral nervous system. High rate of trigeminal nerve infectivity and long-term persistence in sensory neurons in non-integrated state are among factors for choosing this agent as a suitable vector for mentioned target. Subcutaneous inoculation with a non-replicating HSV vector expressing the opioid peptide enkephalin substantially reduced pain-related behavior caused by inflammation, nerve damage or cancer in rodents [22]. Also, neuropathic pain caused by spinal nerve trauma or diabetes was prevented using later vector expressing the glutamic acid decarboxylase transgene to release inhibitory neuro-transmitter gamma-aminobutyric acid (GABA) in target cells. In addition, HSV vectors that express IL-4 and IL-10 were able to decrease pain in neuropathic pains from both peripheral and central nervous system origins [23,24].

Gene therapy approached for brain tumors

Numerous approaches are widely available for gene therapy in brain tumors, including: gene transfer-mediated drug targeting, transfer of tumor suppressor gene and cell cycle modulators, genetic immune modulation, anti-angiogenic gene therapy and utilization of cytopathic-oncolytic viruses. Gene transfer mediated drug targeting, also known as suicide gene therapy, is most commonly used in clinical trials to attempt to treat brain tumors. It involves the conferring of drug sensitivity by transducing tumor cells with a gene encoding an enzyme that can metabolize a nontoxic pro-drug to its toxic form [25]. The HSV thymidine kinase gene *(*HSV-tk) converts nontoxic nucleoside analogs, such as ganciclovir, into phosphorylated compounds that are used to build the DNA molecule. These compounds directly inhibit DNA polymerase and render the formed DNA molecule unstable, leading to arrest of DNA synthesis and cell death. Thus, glioma cells genetically modified to express HSV-tk are killed after the administration of ganciclovir. Preclinical experiments demonstrated marked tumor elimination, despite gene transfer into only a small fraction of the tumor cells. This cytotoxic effect of transduced cells on adjacent non transduced cells is termed the bystander effect [26]. The bystander effect is mediated mainly by the transfer of toxic phosphorylated forms of ganciclovir to non-transduced cells, presumably via gap junctions. Retroviral vectors target mitotically active endothelial cells in tumor vessel. An immune-associated anti-tumor effect has also been suggested using this approach in experimental animals [27]. Tumor suppressor gene transfer and utilization of cell cycle modulators became attractive due to distinct role of TP53. TP53 can restore cell cycle regulation in TP53-mutated cells and induce apoptosis even in tumors with intact, functional genes by causing enhanced expression of the gene product [28]. Response of cells to DNA damage, cell death, cell differentiation, neovascularization and suppression of transformation by inducing apoptosis or blocking cell cycle progression are among the most important roles of TP53. Also, the TP53 gene up-regulates the expression of other genes, such as the p21 gene, which blocks the cell cycle in the Gl phase, along with the BAX gene, an apoptosis-promoting member of the BCL-2 family. It is well known that low levels of expression of TP53 induce growth arrest, whereas higher

levels of expression will induce apoptosis [29]. In addition, MYC family plays a crucial role in cellular proliferation, differentiation, transformation, and apoptosis. In a study, Asai et al failed to prevent the growth of 9L and C6 brain tumors in animals injected with either the c-myc or the p53 gene together with the tumor cells [30]. Genetic immune modulation is based on the use of genetic material to enhance the immune response against tumors by expressing cytokines and lymphokines. Unlike the local effects of suicide and cell cycle modulation strategies, the immune approach has the potential to induce a systemic response against the tumor. Modifying tumor cells in such a way that they may serve as a tumor vaccine and presentation of tumor rejection antigens by antigen-presenting cells are two main strategies in mentioned technique. Tumor vaccines are based on genetic immune modulation theories and can inhibit the local immune-suppressors, block expression of immune-suppressors (TGF-β) and induce expression of various cytokines such as IL-2, IL-4 and granulocyte-macrophage colony stimulating factors [31]. Vascular endothelial growth factor is an important angiogenic factor in gliomas and is over-expressed in many malignant brain tumors, making anti-angiogenic gene therapy an important approach. In vivo transfer of a recombinant adenoviral vector carrying the coding sequence of wild-type vascular endothelial growth factor cDNA in an antisense orientation into subcutaneous human glioma tumors inhibited tumor growth. Tanaka et al constructed retroviral and adenoviral vectors that express a novel, secretable form of the antiangiogenic protein platelet factor 4 (sPF4) which inhibited endothelial cell proliferation in vitro and resulted in hypovascular tumors that grew slowly in vivo. Although genetic vectors administered intravascularly are unlikely to penetrate the BBB and transfer a gene into brain or tumor parenchyma, intravascular delivery of vectors may target endothelial cells in blood vessels of the brain. Studies using intravascular, retroviral-mediated transfer of blockers of angiogenesis are currently in progress [32]. In utilization of cytopathic-oncolytic viruses approach, the idea of using viruses to kill cells directly by their lytic activity is not new. However, recent advances in recombinant DNA technology have paved the way for genetic engineering of wild-type lytic viruses to enable their safe and efficient use for targeted therapeutic purposes. An optimal virus should be able to specifically target and spread to tumor cells, exert a potent lytic effect, cause no toxicity to adjacent normal cells, avoid the immune system, and be controllable pharmacologically. For example, G207 is a double mutant of HSV-1 and is capable of destruction of human glioma cells in monolayer cultures. Another example is an adenovirus mutant that can replicate in and lyse p53-deficient human tumor cells but not cells with functional p53. The application of this virus for brain tumor therapy has not been evaluated yet [33].

CONCLUSION

The strides being made in understanding the molecular basis of human disease and the rapid evolution of recombinant DNA technology enable us to envision new therapeutic modalities, including gene therapy. However, the field of gene therapy is in its infancy, and major technical issues affecting the successful application of this technology are still unsolved. There are some problems in gene therapy techniques in brain tumor treatments, including: 1) screening of new approaches is based on animal models that are far from being representative of the analogous clinical scenarios; 2) the size, consistency, and extent of experimental brain tumors do not reflect the large, necrotic, infiltrative nature of glioblastomas; 3) experimental approaches that aim at immune enhancement use animal models that are practically non-syngeneic to the implanted tumor, and the human response to various viral vectors cannot be predicted from animal experimentation; and 4) many ingenious gene therapy strategies that are effective in preclinical studies will not fulfill their expectations in the clinic. The problem of the distribution of genetic vectors into solid brain tumors and efficient in situ gene transfer remains one of the most significant hurdles in achieving an effective therapeutic gene transfer. Stepwise advances in research will undoubtedly solve many of these issues and allow the translation of basic science into effective clinical applications.

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