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REVIEW ARTICLE

Ultrasound-targeted microbubble destruction in gene therapy: A new tool to cure human diseases

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Abstract Human gene therapy has made significant advances in less than two decades. Within this short period of time, gene therapy has proceeded from the conceptual stage to technology development and laboratory research, and finally to clinical trials for the treatment of a variety of deadly diseases. Cardiovascular disease, cancer, and stroke are leading causes of death worldwide. Despite advances in medical, interventional, radiation and surgical treatments, the mortality rate remains high, and the need for novel therapies is great. Gene therapy provides an efficient approach to disease treatment. Notable advances in gene therapy have been made for genetic disorders, including severe combined immune deficiency, chronic granulomatus disorder, hemophilia and blindness, as well as for acquired diseases, including cancer and neurodegenerative and cardiovascular diseases. However, lack of an efficient delivery system to target cells as well as the difficulty of sustained expression of transgenes has hindered advancements in gene therapy. Ultrasound targeted microbubble destruction (UTMD) is a promising approach for target-specific gene delivery, and it has been successfully investigated for the treatment of many diseases in the past decade. In this paper, we review UTMD-mediated gene delivery for the treatment of cardiovascular diseases, cancer and stroke.

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Gene therapy for genetic disorders

Gene therapy has been used successfully for the correction of abnormalities of genes, which result in clinical disorders. These treatments have corrected the disorders and clinical symptoms as well as improved the quality of patient lives. For example, in (1) **severe combined immune deficiency (SCID)**, commonly known as the bubble boy disease, children are born without an effective immune system and will succumb to infections outside of the bubble without a bone marrow transplantation from a matched donor. A milestone study representing the first case of a gene therapy "cure", or at least a long-term correction, for patients with this deadly genetic disorder was conducted by investigators in Italy. The therapeutic gene called adenosine deaminase (ADA) was introduced into the bone marrow cells of SCID patients, followed by transplantation of the genetically-corrected cells back into the same patients. The immune system was reconstituted in all treated patients without noticeable side effects, and these children now live normal lives with their families without the need for further treatment.[1] (2) **Chronic granulomatus disorder (CGD)** is a genetic disease that weakens the immune system resulting in susceptibility to bacterial and fungal infections that can be fatal. Using gene transfer technologies similar to those employed in the SCID clinical trial, investigators in Germany treated two patients with CGD. They detected substantial gene transfer in both individuals' neutrophils which allowed for the development of a large number of functionally-corrected phagocytes and notable clinical improvement.[2] (3) In **hemophilia**, patients are not able to produce blood clots and thus suffer from external and internal bleeding that can be life threatening. In a clinical trial conducted in the United States, the therapeutic gene Factor IX was introduced into the liver of hemophilia patients and restored their ability to form normal blood clots. The therapeutic effect, however, was short-lived because the genetically-corrected liver cells were likely recognized as foreign and rejected by the healthy immune systems of the patients.[3] This immune-mediated rejection is similar to that which occurs after organ transplantation, and a curative outcome by gene therapy might be achievable with immune suppression or alternative gene delivery strategies currently being tested in phase I/II clinical trials. (4) **Leber's congenital amaurosis (LCA)** is a rare inherited retinal disease that causes severe visual impairment in infancy or early childhood with an incidence of approximately 1 in 80,000 people. LCA is characterized by nystagmus, sluggish or absent pupillary responses, and severe vision loss or blindness. Researchers at Moorfields Eye Hospital and University College London carried out the world's first gene therapy clinical trial for patients with RPE65 LCA and demonstrated that the experimental treatment is safe and can improve sight. These findings represent a landmark for gene therapy technology and could have significant impact on future treatments for eye disease.[4]

Gene therapy for acquired diseases

It is known that the onset of clinical disorders may be related to genetic alterations. With the development of new technologies, these genetic abnormalities have been identified and have become new targets of gene therapy. It has been demonstrated that some tumor formation are highly correlated with genetic mutation or alteration. (1) **Cancer** researchers have developed several different strategies for utilizing gene therapy in the treatment of a wide variety of cancers, including suicide gene therapy, oncolytic virotherapy, anti-angiogenesis and therapeutic gene vaccines. In fact, two-thirds of all gene therapy trials are cancer based and many of these trials are entering an advanced investigational stage, including a Phase III trial of Ad.p53 for head and neck cancer[5] and a Phase III gene vaccine trial for prostate cancer.[6] In the latter trial, use of sipuleucel-T immunotherapy prolonged overall survival in men with metastatic castration-resistant prostate cancer. Additionally, numerous Phase I and Phase II clinical trials for cancers in other organs are being conducted in academic medical centers and biotechnology companies using novel technologies and therapeutics developed on-site. (2) **Stroke** is the fifth leading cause of death and disability in developed countries.[7] The morbidity and mortality associated with stroke result in severe social and economic burden on patients and their family members. Gene therapy could be applicable to the treatment of severe stroke, and several experimental studies have revealed the usefulness of gene therapy in the protection of neurons against ischemia, reduction of infarct size, and improvement of function.[8–11] (3) **Cardiovascular diseases** include coronary artery disease, heart failure, and cardiac arrhythmias. Nabel et al were the first to carry out successful gene therapy through the transfer of endothelial cells for expression of recombinant genes in the cardiovascular system in 1989.[12] Following this achievement, gene therapy for cardiovascular diseases has been performed worldwide. However, the effectiveness of gene therapy for cardiovascular diseases has been modest because of the lack of gene delivery techniques to provide an adequate dose of a therapeutic gene to specific targets.[13]

In the early phase of gene therapy, naked DNA such as plasmids was directly injected into tissue. Because of lower transfection efficiency, the effectiveness of these treatments has been questioned. To improve transfection, most gene therapy trials have relied on adenoviral-associated platforms for gene delivery.[14] Transfection efficiency has been improved significantly; however, the safety of viral vectors and the need for repeated catheterization raises significant safety issues for clinical trials. Because of these concerns, clinical gene therapy is currently limited to a few highly specialized Institutes. Plasmid therapy, by comparison, is a universally acceptable platform for safe gene delivery. However, DNA instability and the inability to deliver plasmids to specific sites have hindered the application of this approach. A novel gene delivery system is needed in gene therapy. Ultrasound targeted microbubble destruction (UTMD) permits precise, non-invasive gene delivery to target sites and increases transfection efficiency as well as limits off-target transfection.[15–19] In this article, we review the most notable advancements in gene therapy and the use of the UTMD for gene delivery.