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Editorial

Gene Therapy and Current Gene Delivery Methods for Erectile Dysfunction

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Editorial

Erectile Dysfunction (ED) has harmful effect on the quality of life of patients and their partners. It has been estimated that more than 152 million men suffered from ED all around the world in 1995 and the number will be over 300 million in 2025 [1]. Although Phosphodiesterase type 5 (PDE5) inhibitors are effective for most patients, they are rarely valid for serious vasogenic and neurogenic ED caused by aging, diabetes mellitus, radical prostatectomy and so on [2]. Emerged in these decades, gene therapy for ED is one of new promising treatment options to solve the problem.

Gene therapy is a therapeutic way to deliver nucleic acid polymers, either expressed as proteins, interfere with protein expression, or correct genetic defects, into cells to treat disease. Penis is an optimal organ to conduct gene therapy for many advantages. The easily accessible organ, slow penile blood flow and intercellular gap junctions make the target genes be injected directly and safely, transfected efficiently, locate and express persistently [3].

Current researchers have indicated that several signaling pathways, such as nitric oxide-cyclic guanosine monophosphate signaling (NO/cGMP) pathway, cyclic adenosine monophosphate pathway, RhoA/ROCK pathway, might mediate penile erection and be involved in the pathogenesis of ED induced by aging, diabetes, cavernous nerve injury, hypertension, hypercholesteremia, etc. In addition to the target genes, the transfection ways are vital to curative effect of gene therapy, which affect their transfection efficiency and safety. For decades, researchers have used multiple approaches in basic studies and attempted to find optimal method to deliver interested genes into penis. Herein, we review current gene delivery methods regarding gene therapy for ED, including naked genes delivery, using virus or stem cells vectors, noncoding RNAs regulation, genome changes, and utilizing special transfection systems.

Naked Genes

Naked genes delivery has the advantage of safety due to its lack of chromosomal integration. In 1997, Garban et al., firstly used plasmid to transfer exogenous inducible Nitric Oxide Synthase (iNOS) cDNA into corpus cavernosum of aged rats, which increased the intracavernous pressure after cavernous nerve electro stimulation for at least 10 days [4]. Another study showed that the transfer of vasoactive intestinal peptide cDNA was capable of altering the erectile response in diabetic rats [5]. However, the defect of naked genes is easy to breakdown by endonucleases that make it effective for a short time.

Virus

Viral vectors, including lentivirus, adenovirus, Herpes Simplex Virus (HSV) and Adeno-Associated Virus (AAV), are widely used in ED gene therapy because of their high efficiency of transfection. Numerous studies have demonstrated their advantages as vectors to transfer target genes into penis. For example, Lin H, et al., used recombinant adenovirus to carry COX2-10aa-PGIS gene to the penis of cavernous nerve injury rats and proved its capacity to improve erectile function [6]. Another research showed that HSV-mediated delivery of NTN was a viable approach for the improvement of ED resulting from cavernous nerve injury [7]. However, they still have the potentials of mutagenesis, carcinogenesis and induction of immune response, which becomes an important reason to restrict their clinical application.

Genetically modified stem cells

Stem cells, including Embryonic Stem Cells (ESCs) and Adult Stem Cells (ASCs), have the capacity of self-renewal and directed differentiation. Genetically modified stem cells, especially the ASCs such as Adipose Derived Stem Cells (ADSCs), Muscle Derived Stem Cells (MDSCs) and Urine derived Stem Cells (USCs), are novel vehicles to transfer target genes in ED gene therapy. Ouyang B et al., reported that USCs modified with Fibroblast Growth Factor 2 (FGF2) could improve the erectile function of type2 diabetic rats [8]. Another study showed that ADSCs expressing Vascular Endothelial Growth Factor (VEGF) gene restored erectile function in diabetic rats [9]. Our research group transferred either iNOS gene or PDE5 siRNA or both of them to autologous ADSCs followed by injecting them to penis and evaluating their effect on erectile function of streptozotocin induced diabetic rats. The data showed that all of the methods mentioned above could significantly improve erectile response of the rat model, but the efficacy of both genes modified way failed to have an advantage over that of treatment with single gene modified ADSCs. Genetically modified autologous stem cells are promising choice as gene vectors, not only due to the erection improvement capacity of target genes, but also the low immunogenicity and potential mechanism of peregrine or differentiation to penile tissue cells from autologous stem cells. However, the safety of application of stem cells, especially the risk of carcinogenesis, remains to be investigated.

Noncoding RNAs

Noncoding RNAs, such as small interfering RNA (siRNA), small activating RNA (saRNA), short hairpin RNA (shRNA), micro RNA (miRNA) and long noncoding RNA (lncRNA) are regulators of genes expression and can affect a diverse range of important biological

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processes. The application of noncoding RNAs was widely involved in studies on ED gene therapy. Our group successfully used a saRNA with the capacity to activate VEGF to induce endogenous VEGF expression in primary human corpus cavernous smooth muscle cells *in vitro* [10]. The results of another study from our group showed that saRNA mediated iNOS over expression in the penis could restore erectile function in diabetic rats via the NO/cGMP signaling [11]. siRNA is an approach to inhibit endogenous gene expression. The gene therapy with siRNA-targeting ROCK2 mRNA significantly improved erectile function by inhibiting ROCK2 pathway in the spontaneously hypertensive rats [12]. Although miRNA and lncRNA related studies are still in the initial stage in the field of ED, Pan F et al., [13] and our team have detected the genome-wide profiling of miRNA and lncRNA expression patterns in corpus cavernosum of age or diabetes related ED models, respectively. The aberrantly expressed miRNAs and lncRNAs could provide novel insight into the pathogenesis and gene therapeutic targets of ED. However, noncoding RNAs may be hard to have persistent effect due to their quick metabolism.

Genome Changes

Genome changes, generally including permanent gene integration into or knockout from the genome, are important methods to identify gene function and long-term safety *in vivo*. So far, however, this approach has rarely been used in ED gene therapy. A study demonstrated that inhibition of the Ninjurin 1 pathway by using Ninj1-knockout mice could safely restore erectile function in diabetic mice [14]. Our research group is conducting a study to evaluate the effect of human tissue kallikrein 1 gene on ED with transgenic rats harboring this target gene. The production process of the transgenic rats could be briefly described as microinjecting a 5.6 kb DNA fragment containing the entire hKLK1 gene into the oocytes from Sprague-Dawley rats under the control of the heavy metal responsive mouse metallothionein promoter. The rats were born to contain the target gene and we proved its preventive effect on age-related ED. Our another research was to study the involvement of JAK2-STAT3 pathway in diabetic mice using JAK2-conditional knockout mice, which can block the expression of JAK2 gene in specific time and specific tissues. Conditional gene knockout is a novel technique for researchers to control specific gene expression in animal models at arbitrary time and space, which is a giant forward in the gene therapy field. The efficiency and long-term safety of this method are in the assessment.

Special transfection systems

In order to enhance the efficiency of transfection, some new biomaterials and physical methods are being used in gene therapy. For instance, a guanidinylated bioreducible polymer has been demonstrated as an efficient and safe gene carrier to the corpus cavernosum of mice with vasculogenic ED [15]. Another study using Water-Soluble Lipopolymer (WSLP) as a vector to corpus cavernosum showed that the WSLP/pDNA complex had higher transfection efficiency than naked pDNA [16]. Besides, eight 40-ms pulses at a voltage of 30 V as specific parameter of surface electroporation was found to enhance the efficiency of gene transfer into mouse corpus cavernosum by increasing cell membrane permeability without injury to the penis [17].

In conclusion, gene therapy creates a new era for the treatment of ED. Although all gene regulation methods hold both sides in ED gene therapy, most of these gene-based strategies show preclinical success. Melman A et al., have finished a phase 1 clinical trial using hMaxi-K to treat ED and showed that the improved erectile response could last for more than 6 months without adverse events [18]. A phase 2 clinical trial of this study is in progress. This brings us confidence in the further development of gene therapy. But we should realize that gene therapy is now in its infancy and we need to find more efficient and safer approaches to optimize it.

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