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Virus integration and genome influence in approaches to stem cell based therapy for andro-urology ☆

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ABSTRACT

Despite the potential of stem cells in cell-based therapy, major limitations such as cell retention, ingrowth, and trans-differentiation after implantation remain. One technique for genetic modification of cells for tissue repair is the introduction of specific genes using molecular biology techniques, such as virus integration, to provide a gene that adds new functions to enhance cellular function, and to secrete trophic factors for recruiting resident cells to participate in tissue repair. Stem cells can be labeled to track cell survival, migration, and lineage. Increasing evidence demonstrates that cell therapy and gene therapy in combination remarkably improve differentiation of implanted mesenchymal stromal cells (MSCs), revascularization, and innervation in genitourinary tissues, especially to treat urinary incontinence, erectile dysfunction, lower urinary tract reconstruction, and renal failure. This review discusses the benefits, safety, side effects, and alternatives for using genetically modified MSCs in tissue regeneration in andro-urology.

Contents

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1. Introduction

Autologous stem cells or mesenchymal stromal cells (MSCs)-based therapy for urological diseases caused by congenital defects, trauma, cancer, or chronic inflammation has recently offered a promising alternative for tissue regeneration. MSCs possess certain characters of stem cells, i.e. distinct differentiation potential to promote tissue regeneration via their trans-differentiation and trophic factor secretion although they are lack of self-renew capacity of totipotent stem cells [1]. Major benefits of autologous MSCs include avoiding adverse events related to the rejection of implanted xenogenous or allogenous tissues, and reducing risk of tumorigenesis, and bypassing ethical concerns about application of induced pluripotent stem cells (iPSCs) or embryonic stem cells. Skeletal muscle-derived progenitor cells [2–5], bone marrow-derived mesenchymal stromal cells (BMSCs) [6–16], adipose-derived stem cells (ASCs) [17–21], hair follicle stem cells [22], endothelial progenitor cells [23], amniotic fluid-derived cells [24–30] and urine-derived stem cells (USCs) [31–36] are regarded as candidates for cell therapy in genito-urinary tract tissue repair. However, a lower retention rate of implanted cells limits the therapeutic effect of donor stem cells in tissue repair processes. In cell-based therapy, stem cells are often induced to become fully differentiated cells *in vitro* and then implanted into the host to participate in tissue repair. Thus, an extra step of *in vitro* pre-conditioning processing is needed. Furthermore, the lifespan of *in vitro* pre-differentiated cells is shorter than that of non-pre-conditioned cells when they are implanted *in vivo*.

Since blood support is a key for these donor cells to survive in the implanted sites, angiogenic gene manipulation is often used in stem cell therapy to improve donor cell viability, ingrowth, differentiation and functional tissue formation via revascularization *in vivo*. Several studies have reported that MSCs that express vascular epithelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PEDF) or insulin-like growth factor-1 (IGF1) can improve cell survival, ingrowth, innervation, and myogenesis of stem cells via angiogenesis [37–42]. Most experimental studies on genetically-modified stem cells have focused on augmenting the inherent capabilities of these cells to facilitate urological tissue repair *in vivo*. As excellent carriers of transgenes, stem cells can be feasibly manipulated *in vitro* for delivery of therapeutic genes to genitourinary tissues [31,42,43]. With anti-fibrotic and angiogenic properties, MSCs are an optimal gene carrier cell source for urological tissue regeneration compared to other somatic cells.

Stem cell therapy has been used in tissue defect with minimal scarring tissues; gene therapy is suitable in treatment of inherited disorders or neurodegenerative diseases; stem cell and gene therapy offer an alternative for treating a range of diseases, many of which currently have no cure. In this review, we discuss the advantages and limitations of stem cell therapy combined with gene modification, and describe future directions for cellular therapy in improving cell retention, engraftment, differentiation, and host cell recruitment in urinary tract tissue repair.

2. Stem cell therapy

Cell-based therapy provides therapeutic potential for treatment of genitourinary diseases, such as stress urinary incontinence (SUI) due to urethral sphincter muscle dysfunction, erectile dysfunction (ED) due to nerve or endothelial dysfunction, bladder or urethral defects, and renal ischemia injuries. MSCs are commonly used cell sources when the native target cells are unhealthy or unavailable. Multiple types of stem cells have been used in preclinical animal models to repair or regenerate tissue, including pluripotent stem cells i.e. embryonic stem cells (ESCs) [44–47], iPSCs [48] or multi-differentiated potent MSCs. As a cell source for tissue repair, MSCs can secrete paracrine factors, recruit resident stem cells, foster trans-differentiation, and appear to be less prone to malignant tumors. In addition, MSCs can give rise to skeletal, smooth muscle cells, and endothelial cells for creating urethral sphincter, blood vessels, or urinary tract muscle wall [10,11,43,49]. They can be implanted into the host via local administration, intravenously, or by intra-peritoneal injection (Fig. 1).

In cell therapy for ED, SUI, and renal failure, paracrine factors secreted by stem cells appear to play a dominant role in stimulating host cells to participate in tissue repair. Most studies have demonstrated that numbers of implanted stem cells decrease with time during tissue repair [18,24,25,36]. The most likely reasons include: 1) loss of proliferative function after repeated cellular de-attachment processes during culture; 2) over-expansion of the cell population *in vitro* that shortens cell lifespan; and 3) low retention rate of grafted cells due to a poor blood supply, fibrosis, or inflammation at the implantation site. Improving the microenvironment by adding exogenous angiogenic growth factors is a logical approach to increase the rate of stem cell survival *in vivo*.

3. Gene therapy

Gene therapy promises treatment of inherited disorders or defected tissues by drug (DNA) delivered to the nucleus of the patient's cells in order to function. The most common methods of gene therapy in treatment of inherent disease involve 1) replacing the mutated gene by a plasmid DNA); 2) inactivating, or knocking out a mutated gene [42]; or 3) introducing a functional therapeutic gene (such as IGF1) into the host to promote tissue regeneration [42,50–52] or help fight a disease. In gene therapy, plasmid DNA encoding a therapeutic protein is inserted into the host cells. Once inside, the DNA starts expressed by cellular machinery, causing the regeneration of functional proteins, which can in turn be healing the host tissue defects.

DNA is delivered into cells by two main methods: recombinant viruses and non-viral methods (i.e. naked DNA) [53]. In the viral vector approach, viruses insert their genetic material into the cells as part of their replication cycle in the host. Certain viruses can be used for human gene therapy via using the virus after removing DNA as a vehicle to insert the specific function DNA (Table 1).

Table 1
Commonly used vectors in genetically modified MSCs.

	Adenovirus	Retrovirus	Lentivirus	Plasmid
Insert size	~30 kb	8 kb	4 kb	Unlimited
Integration	No	Yes	Yes	Rare
Production	$>10^{11}$	$>10^6$ cfu/ml	10^6	Unlimited
Administration	*ex/in vivo*	*ex vivo*	*ex/in vivo*	*ex/in vivo*
Expression style	Transient	Long-term	Long-term	Transient
Express level	High	Moderate	Moderate	High
Immune	Extensive	Few	Low	None
Safety concerns:	Insertional none	Insertional Inflammatory	Mutagenesis response	Mutagenesis toxic
Cells infected	All cells, including proliferating cells and non-proliferating cells	dividing cells or proliferating cells	all cells	need help