Future Applications of Intravesical SCL Gene Transfer for the Treatment of Diabetic Neurogenic Bladder

**Keywords:** Intravesical; SCL gene; Treatment; Diabetic cystopathy

**Abstract**

Diabetic cystopathy, a complication of diabetes affecting the bladder, is mainly characterized as reduced contractility of the detrusor and increased post-voiding residual volume, which is induced by the reduction of the interstitial cells of Cajal. The c-KIT protein is a specific marker of the interstitial cells of Cajal and the product of a c-KIT proto-oncogene. The interstitial cells of Cajal in high glucose medium express less c-KIT mRNA and protein. SCL gene, serves to assemble SCL complexes on the c-KIT promoter, sustaining c-KIT transcription. Proper transduction of exogenous SCL gene may upregulate c-KIT gene expression in normal bone marrow cells. This c-KIT regulation involves proper activation of the c-KIT promoter by the SCL protein, which coordinates SCL complex binding on the promoter sequence to activate c-KIT transcription [19]. Elefanty et al. successfully introduced SCL into mutant mice (SCL lacZ/w) and demonstrated that functional SCL protein was synthesized [20]. For the transfer of genetic material to target tissue, the use of intravesical lentiviral vector-mediated gene delivery to bladder cells has been shown to be efficacious and safe for the treatment of bladder cancers [21]. Taken together, increased SCL expression in DCP patients may initiate a cascade of events, resulting in increased c-KIT activity, thus leading to the rescue of bladder function.

**Introduction**

Diabetic cystopathy (DCP) is one of the most common complications in diabetes mellitus, affecting over 25% of diabetic patients [1,2]. The main symptoms of DCP are under-activity of the detrusor muscle and increased post-voiding residual volume, which would induce severe urinary tract infection, vesico-ureter reflux, hydronephrosis, and even uremia and renal failure [3,4]. Previous studies have shown that interstitial cells of Cajal (ICCs) in the detrusor have pace-making ability, which is responsible for contraction of the detrusor [5,6]. ICCs are involved in signal transmission between smooth muscle bundles, from effenter nerves to smooth muscles, and from the urothelium to afferent nerves [7,8]. The underactivity of DCP has the disturbance of spontaneous contractility, caused by reduced suburothelial ICCs in DCP patients [9,10]. ICCs in cultured bladder tissue with high glucose medium were found to exhibit poorly self-excited, reduced connections with detrusor cells and nerve terminals, and expressed less c-KIT mRNA and protein than control cells [11]. The c-KIT protein is a specific marker on the cell membrane of ICCs, which is encoded by the c-KIT proto-oncogene [12,13]. Activation of the c-KIT gene can modulate cell growth, differentiation, and phenotype, while mutation of c-KIT leads to ICCs absent [14,15]. The stem cell leukemia gene (SCL) is a tissue-specific transcription factor of the basic helix-loop-helix family, functions in hematopoietic development, is normally expressed in pluripotent hematopoietic precursors, and is downregulated in maturing cells [16,17]. SCL induces c-KIT expression in chromatin. For example, ectopic SCL expression in transgenic mice sustains c-KIT transcription in developing B-lymphocytes, in which both genes are normally downregulated [18]. Increased SCL expression upregulates c-KIT gene expression in normal bone marrow cells. This c-KIT regulation involves proper activation of the c-KIT promoter by the SCL protein, which coordinates SCL complex binding on the promoter sequence to activate c-KIT transcription [19]. Given the results of previous studies, we hypothesize that intravesical SCL gene transfer by viral vectors, such as the lentiviral vector, has high potential to promote c-KIT gene expression in ICCs and relieve bladder under-activity in DCP patients. During infection of ICCs, the viral vector carrying the SCL RNA is transduced into the ICCs, reverse-transcribed into double-stranded DNA (dsDNA) in the cytoplasm, and transported into the nucleus to stably integrate into the ICC genome. In DCP patients, the exogenous SCL may be expressed, and SCL protein may be synthesized in ICCs. Because DCP causes downregulation of the c-KIT gene, newly produced SCL may activate and sustain c-KIT transcription by binding the promoter. These reactions may enhance/sustain c-KIT gene transcription and protein synthesis in DCP patients; therefore, bladder under-activity may be relieved.

**Discussion**

DCP-related alterations of the detrusor are attributed to several mechanisms: changes in cellular excitability or intercellular communication; changes in receptor density, distribution, and function; alterations to intracellular signal transduction; and molecular or genetic changes [7,8]. All of these mechanisms are induced by abnormalities in ICCs in the bladder [22,23]. At the
molecular level, c-KIT, which is a transmembrane receptor on the ICC cell membrane, is responsible for these abnormalities. Under normal conditions, the stem cell factor (SCF) activates its receptor (c-KIT), and this triggers the activation of MAP kinase and conducts the signal to the cell nucleus to modulate cell growth, differentiation, and phenotype [24]. ICCs and detrusor smooth muscle cells originate from the same cell type, which are embryonic, c-KIT-positive, mesenchymal precursor cells. At the late embryo stage, the mesenchymal precursor cells receiving the c-KIT signal differentiate into ICCs, while precursor cells receiving no c-KIT signal become smooth muscle cells [15]. Mutation of the dominant white spotting (W) locus in chromatin, where c-KIT has been mapped in embryonic precursor cells, leads to ICCs absent in the mouse [25]. In fact, the c-KIT protein is a detection marker, and it plays a crucial role in the control of bladder function. The inhibition of c-KIT receptor induces ATP-K⁺ channel opening and cell membrane hyper-polarization in ICCs; concomitantly, decreased excitability and contractility of the bladder have been detected, which implies that c-KIT protein inhibition in ICCs decreases bladder activity [26,27].

SCL is a crucial regulator of diverse developmental processes, such as hematopoiesis, neurogenesis, and myogenesis [28]. SCL is required for c-KIT expression and function in the hemopoietic cell line and has been shown to induce c-KIT transcription in chromatin. In a functional screen of TF-1 cells expressing antisense SCL 20, c-KIT receptor function and expression were defective. However, co-delivery of SCL in the sense orientation rescued c-KIT gene expression, which suggests that the latter is a potential downstream target of SCL [19]. Ectopic SCL expression in transgenic mice induces sustained c-KIT transcription in developing B cells. SCL levels determine c-KIT gene expression in hematopoietic cells, and c-KIT expression is directly controlled by SCL. In fact, SCL serves to nucleate the assembly of a multi-protein complex (SCL complex) formed on the c-KIT promoter, which contains SCL, Lim-only 2, GATA-1/GATA-2, E2A, LIM domain binding protein 1, and specificity protein 1 (Sp1) zinc finger protein. The SCL complex activates the c-KIT promoter; specifically, SCL, E2A, and Sp1 have been shown to occupy the c-KIT promoter in vivo in TF-1 cells [16-18]. Therefore, c-KIT is a direct target of the transcription factor SCL and its partners, and activation of the c-KIT promoter depends on SCL. Therefore, exogenously introduced SCL genetic material may help cells sustain or enhance c-KIT function.

Kurita et al. showed that transduction of exogenous TAL1/SCL cDNA into embryonic stem cells using lentiviral vectors is efficient and safe [29]. The viral vectors can deliver significant amounts of genetic information into host cells and integrate the newly synthesized dsDNA into the cellular genome successfully and safely [30,31]. Accordingly, genetically engineered viral vectors, such as lentivirus from the retrovirus family, are currently the most efficient tools of gene delivery. Viral vectors contain a viral promoter, which is used to control the expression of the transgene, and virulence genes are removed. Viral vectors are safe to use in the laboratory with security modifications to eliminate pathogenicity [32]. Viral vectors, e.g., the lentiviral vector, can transduce a wide range of dividing and non-dividing mammalian cell types [33,34]. Thus, it is possible that a lentiviral vector harboring SCL RNA can transduce ICCs into the mammalian bladder. Upon infection, the single-stranded RNA is transduced into the ICCs and reverse-transcribed to dsDNA in the cytoplasm; then the resulting dsDNA would integrate into the genome of the ICCs (Figure 1A). In DCP patients, exogenously introduced SCL may be expressed, SCL protein may be synthesized, and the SCL complex would assemble in the ICCs. In DCP patients with downregulated c-KIT transcription, the assembled SCL complex may bind the promoter to enhance or sustain c-KIT transcription. Thus, ICC function may be restored, and bladder under-activity may be relieved (Figure 1B).

In fact, intravesical viral gene transfer has been applied to treat bladder cancer, and the procedure was well tolerated; the bladder urothelium appears to prevent systemic dissemination of the viral particles [21]. Lentivirus is the commonly used vector for these procedures. Lu et al. successfully applied lentivirus-mediated RNA interference to knockdown clusterin in bladder cancer cells at the RNA and protein levels [35].

Some urologists may suspect the intravesical application of lentiviral particles might cause some concern for local/systemic immunogenic responses, and there is a possibility of poor viral vector permeability through the watertight barrier formed by the urothelium. A previous study in Taiwan developed an intravesical instillation of an in situ biodegradable hydrogel system (15% hydrogel) containing a lentiviral vector harboring WWOX, a rat bladder tumor suppressor gene, to treat bladder cancer. The hydrogel system showed promise for in situ delivery of lentiviral vectors to bladders and
demonstrated gene expression for bladder disease therapy without local/systemic immunogenic responses [36]. Another research using lentiviral vector-mediated antisense oligonucleotides targeting heat shock protein-27 with the tubulin inhibitor, taltobulin (HTI-286), discovered that strong preclinical proof-of-principle for intravesical administration of oligonucleotides in combination with HTI-286 for the treatment of high-grade bladder cancer [37].

Taken together, lentivirus-mediated gene delivery to bladder cells is a safe and promising way to treat underactivity of DCP by SCL gene enhancing c-KIT gene expression to rescue the bladder function, so transfection of DNA via an intravesical route using a viral vector is a new strategy of transgene expression for bladder disease therapy. Therefore, we proposed other researchers to explore this treatment strategy in detail, not only for diabetic cystopathy but also for other clinical problems, which can ameliorate the quality of life of our patients, and seems therefore valuable.

References

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