

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 in number of the interstitial cells of Cajal. The *c-KIT* protein is a specific marker of the interstitial cells of Cajal and the product of *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* genetic material, increases *c-KIT* expression and leads to synthesis of the functional *SCL* protein. Intravesical, lentiviral vector-mediated gene transfer has been shown efficacious and safe. Therefore, proper intravesical transfer of RNA encoding *SCL* to the interstitial cells of Cajal may enhance *c-KIT* transcription and activity in the interstitial cells of Cajal of diabetic bladder.

lymphocytes, in which both genes are normally downregulated [18]. Increased *SCL* expression

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 efferent nerves to smooth muscles, and from the urothelium to afferent nerves [7,8]. The under-activity 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 poor self-excitation (poor self-excitation), reduced connections with detrusor cells and nerve terminals (nerve endings), 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 absence (absence) [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-

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]. Elefanti 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 (Effective) 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.

The Hypotheses

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 (and integrated in a stable manner) 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 in 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 *whitespotting*

(*W*) locus in chromatin, where *c-KIT* has been mapped in embryonic precursor cells, leads to ICCs absent (absence) 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.

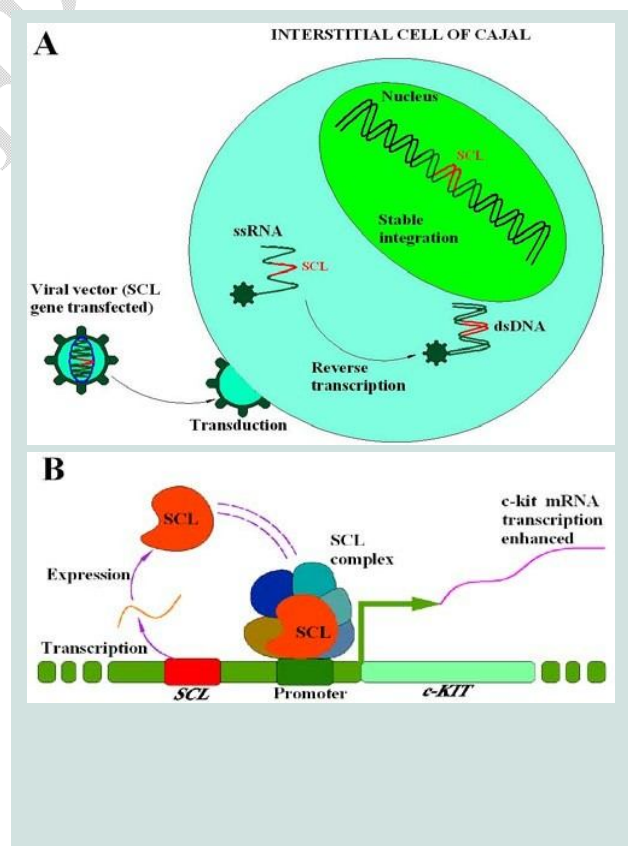
Kurita et al. showed that transduction of exogenous TAL1/*SCL* cDNA into embryonic stem cells using lentiviral vectors is efficient and safe [29]. The viral vectors can deliver significant amounts of genetic information into host cells and integrate the newly synthesized dsDNA into the cellular genome 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 (while) 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 wider 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, taltubulin (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 seem therefore valuable.

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