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Gene Therapy in Urology

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1. Introduction

The application of gene therapy in the field of Urology is not limited to cancer therapy but is also being evaluated for non-cancer related bladder dysfunctions as well as erectile dysfunction (ED). This article will review the use of gene therapy for these conditions; the vectors used and limitations associated with different gene delivery systems and the attempts to overcome these shortcomings.

2. Bladder cancer

Bladder cancer is the 7th most common cancer worldwide. It has a natural history of superficial recurrences and local progression. It is estimated that within 18 months of first diagnosis approximately 50% of patients will have a recurrence (Anderson & Naish 2008). Thus there is a need for frequent monitoring of these patients. In the US the estimated life-time cost of therapy for bladder cancer patients with non-muscle invasive disease was US $21.03 million based on a Medicare database (Cooksley et al. 2008). The majority of this is spent on surveillance and the treatment of recurrences. Tumors occur on the luminal surface of the bladder and the architecture of the bladder permits topical intravesical therapies. The bladder is isolated from other organs and tissues and intravesical therapy permits contact with the entire internal surface of the bladder with minimal systemic side-effects.

The present gold standard therapy for superficial bladder cancers is immunotherapy with Mycobacterium bovis, Bacillus Calmette Guerin (BCG) following local transurethral resection of the bladder tumor (TURBT). BCG induces a mononuclear and neutrophilic infiltrate in the bladder wall which results in an inflammatory response as measured by cytokine production that causes sloughing of both tumor and normal cells (Herr and Morales 2008). The presence of IL-2, IL-8 and IL-18 in the urine of patients has been reported to correlate with response to therapy (Thalmann et al. 1997; Thalmann et al. 2000; Saint et al. 2003). Unfortunately, BCG has several shortcomings: it is a live vaccine and commonly causes side effects and occasionally septicemia. In addition some patients (20-42%) do not respond to therapy (Kamat and Lamm 2000). In place of BCG, recombinant cytokines such as IFN-γ, TNF-α, and IL-2 have been used in a number of clinical trials with encouraging results (Glazier et al. 1995; Den Otter et al. 1998; Stavropoulos et al. 2002). However, recombinant cytokines are costly, unstable in urine and have poor permeability

across the glycosaminoglycan (GAG) layer of the urothelium. Gene therapy is a natural alternative approach to ensure cytokine production in the bladder environment.

2.1 Viral transfection systems for bladder cancer

Initially replication defective viruses were generated with the sole purpose of gene delivery (Thomas et al. 2003). The viruses evaluated included: Adenovirus (type 2 and 5), Adeno-associated virus (*Parvoviridae* family), Herpes simplex virus, Retrovirus (Dumey et al. 2005), Canary pox virus and Vaccinia virus (Lee et al. 1994). Table 1 lists their characteristics.
However, because of the limited transduction capability of some viruses, replicating and conditionally replicating viruses were developed. These viruses amplify the transfection efficiency, as virus transduced cells produce more viruses that can infect the surrounding cells. Replication of wild type viruses also induces cytolysis of infected cells.

2.1.1 Limitations and Improvements

2.1.1.1 Non-specificity of transfection

Siemens *et al*. compared adeno-, canary pox and vaccinia viruses in terms of their ability to transfect tumor cells after intravesical delivery in a murine model of bladder cancer (Siemens et al. 2003). The vaccinia and avian pox viruses were better at transfecting tumor cells than adenoviruses but all three resulted in transfection of extravesical tissue (e.g. kidney, liver, spleen). In contrast Wood et al reported only sporadic extravesical transfection after intravesical adenovirus instillations (Wood et al. 1999).
To reduce non specific viral transduced gene expression, oncolytic adenoviruses have been engineered to express the E1A and E1B genes under the control of the uroplankin II gene promoter (Zhang et al. 2002; He et al. 2009) which limits expression to urothelial cells. Another strategy to target viral replication to tumor cells is to place the adenovirus E1A gene under the control of the telomerase promoter (Lanson et al. 2003), the midkine gene promoter (Terao et al. 2007) or the Cox-2 promoter (Shirakawa et al. 2004). All these genes are highly expressed in tumor cells.
Bladder cancer cells often over-express the epidermal growth factor receptor (EGFR) and targeting EGFR with bi-specific antibodies improved the delivery of adenovirus to cancer cells (van der Poel et al. 2002). A gammaretrovirus carrying a chimeric envelope protein containing a single chain variable fragment (scFv) antibody to the human epidermal growth factor receptor 2 (HER2) was shown to specifically target cells expressing Her2 (Tsai et al. 2010).

2.1.1.2 Transfection efficiency

The internal surface of the bladder is covered by uroplakin proteins and the GAG layer which together provide a barrier to transfection of urothelial cells. Agents that disrupt this protective layer such as ethanol, HCl, dodecyl-B-d-maltoside and sodium dodecyl sulphate have been shown to improve viral transduction of the bladder (Engler et al. 1999; Lin et al. 2002; Ramesh et al. 2004).
Though adenoviruses are the most popular viruses for gene therapy they require adhesion with the cellular coxsackie-adenoviral receptor (CAR) for transduction of mammalian cells. Neoplastic tissue unlike normal bladder cells have reduced CAR expression (Buscarini et al. 2007) as a result of epigenetic control mechanisms (Pong et al. 2003).

Virus	Transfection efficiency	Immunogenicity	DNA inserts	Gene expression
Adenovirus	High with CAR receptor	high	8kb	Transient expression, DNA remains episomal
Adeno-associated virus	Good, no receptor	low	4.5kb	Stable, DNA episomes found
Herpes simplex virus	High in neurons	high	>30kb	Stable in neurons and transient in others, episomal
Moloney murine leukemia virus	High in dividing cells	low	8kb	Stable expression, DNA integration into host genome Hematopoietic cells
Lentivirus	Non dividing cells	low	8kb	Stable expression, Integration in host chromosome,
Canary pox virus	Most cells	low	25kb	Transient expression, Viral DNA limited to cytoplasm
Vaccinia virus	Most cells	high	Up to 25kb	Transient expression, Viral DNA limited to cytoplasm

Table 1. Characteristics of viral vectors for gene therapy

To circumvent the need for receptor mediated uptake, polymers have been used to enhance adenovirus transfection of bladder cells (Kasman et al. 2009) or even small molecule excipients such as Syn3 (Connor et al. 2001; Yamashita et al. 2002; Nagabhushan et al. 2007). A recent study has shown that CAR receptor expression and thus adenoviral expression can be increased by treatment with histone deacetylase inhibitors (HDACI) such as trichostatin A and sodium phenylbutyrate (Sachs et al. 2004).

2.1.1.3 Previous Immunity

Vaccinia viruses have long been used in man as vaccines against smallpox. This raised the issue of whether previous immunization would block the effectiveness of these viruses as gene delivery vehicles. Intravesical instillation of vaccinia viruses was successfully demonstrated in pre-immunized mice (Lee et al. 1994). The immunogenicity of adenoviruses is a major limitation in most therapeutic strategies. However it may be advantageous in bladder cancer therapy where non-specific inflammation as a consequence of BCG instillation has been associated with tumor removal.

2.1.1.4 Promoter inactivation

Adenovirus genes expressed from a CMV promoter induced better gene expression than those expressed using a RSV promoter (Freund et al. 2000). But quite often the CMV promoter is inactivated *in vivo*. It has been found that adenoviral transfection together with HDAC inhibitor trichostatin or retinoic acid improved CMV promoter activity. Treatment with these drugs could improve and prolong adenoviral transgene expression (Gaetano et al 2000). The development of tissue specific promoters as described above may resolve this problem.

2.2 Non-viral transfection systems for bladder cancer

The strength and weakness of non-viral vectors is the transient expression of the delivered genes. For non-viral gene delivery the genes are encoded on plasmid DNA of bacterial

origin. Non-viral delivery agents include liposomes (N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride, DOTAP), polyethylenimine (PEI), viral envelopes (with fusogenic properties) conjugated to liposomes (Hemagglutinating Virus of Japan (HVJ) liposomes), chitosans as well as physical means such as the use of an electrogun or ultrasound (Harimoto et al. 1998; Lawrencia et al. 2001; Ogawa et al. 2004; Bonnet et al. 2008; Tsai et al. 2009; Zaharoff et al. 2009). Plasmid DNA delivery systems result in cellular entry via the endosomes (Al-Dosari & Gao 2009). Endosomal escape is often difficult and when successful the plasmid DNA is mainly restricted to the cytoplasm. A small amount may make it to the nucleus and exist as episomal DNA molecules that can be lost during replication (Al-Dosari & Gao 2009). Besides the delivery agents, the plasmid DNA sequence can also modulate the efficiency of transfection as discussed below.

2.2.1 Limitations and Improvements

2.2.1.1 Promoter inactivation

It has been reported that the CMV promoter often used for plasmid gene expression does not always result in good gene expression (Loser et al. 1998) as a consequence of promoter inactivation. This can be overcome by using a HDAC inhibitor, as demonstrated with OSU-HDAC42 (Lai et al. 2010). An alternative strategy is to utilize tissue/cancer cell specific promoters such as the COX-2, H19 and human IGF2-P4 gene promoters (Ohana et al. 2002; Zhang et al. 2008; Amit & Hochberg 2010).

2.2.1.2 Transfection efficiency

The primary focus of improving plasmid DNA transfection is improving escape from the endosomes and this is achieved by developing new additives and lipoplexes or even polyplexes that use either acidification or osmotic pressure changes or membrane fusogenic molecules to allow DNA escape (Al-Dosari and Gao 2009). We developed a formulation comprised of DOTAP and Methyl-β-cyclodextrin solubilized cholesterol (MBC) that transfects urothelial cells *in vivo* within 2 hours of exposure. Transfection was confined to the bladder (Lawrencia et al. 2001) and occurred in the superficial and deeper layers of the urothelial tumours (Wu et al. 2003).

2.2.1.3 Specificity of plasmid expression and activity/duration

Antibodies have been used to target delivery of plasmids to tumor cells. ScFv antibody to Her-2 (Tsai et al. 2009) or transferrin have been demonstrated to successfully target plasmids to tumor cells (Pirollo et al. 2008). The latter strategy targets both primary and metastatic disease when delivered systemically. Targeting of plasmid DNA to the nucleus can also be induced by introducing mammalian transcription factor binding sites in the plasmid and this increased the duration of expression (Gill et al. 2009).

Plasmid DNA expression is transient because of the episomal nature of transfected DNA. In bladder cancer therapy this can be overcome by repeated intravesical instillations. Another strategy to improve gene expression is to add a scaffold matrix attachment region (S/MAR) to the plasmid DNA. S/MAR serve both to ensure prolonged gene expression, by reducing the silencing of plasmid DNA as well as to ensure plasmid DNA replication as episomes (Gill et al. 2009).

Integration of plasmid DNA into chromosomal DNA is achievable now. The techniques used include retroviral integrase (Tanaka et al. 1998), sleeping beauty (SB) transposons

(Hackett et al. 2010) or phage recombinase mediated integration (Olivares et al. 2002). Thus in the future a single intravesical instillation may be sufficient for prolonged therapeutic effects.

2.2.1.4 Liposome free delivery

For small molecules such as CpG oligodeoxynucleotides (ODN), intravesical delivery to urothelial cells can be achieved without a transfection agent (Ninalga et al. 2005). But plasmid DNA requires a delivery agent. Nanoparticles (10-100nm in size) with bound plasmid DNA are recognized by cell surface nucleolin on HeLa cells and this results in DNA transport to the nucleus (Chen et al. 2008) and avoidance of endosomal entrapment.

2.2.1.5 Inflammation

The CpG sequences on plasmid DNA induce inflammation that could reduce gene expression by either destruction of transfected cells or promoter inactivation (Yew et al. 2000). Minicircle DNA (mcDNA), are supercoiled DNA with only the therapeutic gene cassette. They are generated *in vivo* by site specific recombination in E coli and exhibit improved gene expression in terms of the level and duration of gene expression (Darquet et al. 1997).

2.3 Preclinical evaluation of genes and evolving gene therapy strategies

The different categories of genes used successfully in animal studies are listed in Table 2. Both Sub-cutaneous (sc) and orthotopic models of bladder cancer have been used to evaluate gene therapy. While the data from sc studies have shown the efficacy of the expressed genes, it is the orthotopic models that best reflect clinical disease and therapeutic gene delivery. In general regardless of the delivery system or gene delivered tumor growth reduction or even eradication has been reported in murine models of bladder cancer. Most therapeutic schedules used in the animal studies require repeated instillations of the gene delivery vehicle whether it is a viral or non-viral vector. However, a recent study of viral gene delivery of IFNα indicated that a high dose could reduce the need for increased intravesical instillations (Tao et al. 2006). Transfection using transposons may also reduce the need for repeated transfection of plasmid DNA.

New therapies aim to combine several strategies at once. These include the use of oncolytic viruses and immune modulation using GMCSF (Cozzi et al. 2001; Ramesh et al. 2006); wild type p53 and ribozyme erb-2 (Irie et al. 2006) and Rb94 or oncolytic viruses and chemotherapeutic drugs (Zhang et al. 2002; Pirollo et al. 2008). *In vitro* studies have shown that combining AdHSVTK, ganciclovir and chemotherapy may have therapeutic benefit (Freund et al. 2003). Further the beneficial effect of 5-FU and adenovirus cytosine deaminase gene therapy could be enhanced by irradiation (Zhang et al. 2003). Such multi-factor therapies may be better at eradicating tumor cells. Another strategy is to modify immune cells *in vitro* for cancer therapy. Dendritic cell manipulation by transfection with adenovirus carrying the survivin gene has been shown to induce cytotoxic T lymphocytes (CTLs) (Kikkawa et al. 2009).

2.4 Gene therapy clinical trials

Table 3 lists several Phase I trials that have been carried out for bladder cancer. However, results from only a few of these trials are published.

Therapy strategy	Genes	Dosage and cure rate	Reference
Cell cycle modulation	RB94 P53 p53, P21,	Adenovirus delivery and nanocomplex delivery, sc VVp53, increased survival and some mice were cured Adp53 and p21 blocked tumor induction in transformed cells, sc	(Xu et al. 1996; Pirollo et al. 2008) (Fodor et al. 2005) (McGarvey et al. 2001)
Immune modulation	IL2, GMCSF TNF-α IFN-γ IFN-α IL12, IL12 IL12 CD40 Ligand, CpG	Liposome, 1 dose 40%; Liposome, 6 doses, twice a week 50% Liposome, 6 doses, twice a week 50% Co-administered with MBT-2 cells, reduced tumor growth AdIFNα + Syn3, 2 doses, marked tumor reduction Ad.mIL-12 (10^9 PFU) 1 intratumoral injection, cured, sc Chitosan IL12, 4 doses 88-100% cured Liposome IL12, 6 doses 0,3,5 and 10μg DNA, 25-37.5% cured Ad CD40L (10^9PFU) +clorpactin, 3 doses 60% cured CpG ODN, 4 doses, increased survival	(Horiguchi et al. 2000) (Wu et al. 2004) (Zang et al. 2004) (Shiau et al. 2001) (Benedict et al. 2004) (Chen et al. 1997) (Zaharoff et al. 2009) (Horinaga et al. 2005) (Loskog et al. 2005) (Ninalga et al. 2005)
Prodrug activators,	Thymidine Kinase	Plasmid HSV-TK by electrogun + GCV, induced apoptosis in carcinogen induced rat tumor model Ad HSVTK 3 x 10^8 PFU + GCV, >4 fold reduction in tumor growth, sc Chemically induced bladder tumor, AdHSVTK + GCV, growth inhibited	(Shibata et al. 2003) (Sutton et al. 1997) (Akasaka et al. 2001)
Antisense therapy	bFGF, survivin + PLK-1 PCNA + Adp53	Ad-bFGF-AS, Tumor growth significantly inhibited, sc 0.5-1mg/kg 4 doses, suppressed growth of tumors Reduced tumor growth, sc	(Inoue et al. 2000) (Seth et al. 2011) (Zhu et al. 2003)
Oncolytic virus	HSV G207 + Nv1020 Ad E1B defective	$1x10^7$ PFU, 3 doses, 61.5% and 83.3% cured respectively AdE1AdB , tumor growth inhibition	(Cozzi et al. 2001) (Wang et al. 2006)
Toxin delivery	Diphtheria toxin	Calcium phosphate percipitate of plasmid DNA, doses, 40% reduction in tumor eight, sc	(Ohana et al. 2002)
Enhancing Chemotherapy	Cytosine deaminase + etoposide + 5-FU PTEN + doxorubicin P53 + cisplantin	Ad-hTERT-CD $1x10^9$PFU, 5-FC (200 mg/kg), etoposide (2 mg/kg), tumor growth reduced AdMMAC, Reduced tumor growth, sc Combination better than either alone	(Shieh et al. 2006) (Tanaka and Grossman 2003) (Miyake et al. 2000)
Anti-Angiogenesis	AAV Endostatin	$1x10^{11}$ PFU, 6 doses, reduction in tumor growth, sc	(Pan et al. 2011)
Actin regulation	gelsolin	AdGSN 1x10 PFU, 3 doses, tumor suppression	(Sazawa et al. 2002)
Apoptosis	Caspace 3 + 9	PEI/DNA COX-2 driven gene expression with exogenous activation, 5 doses, tumor reduction better than BCG or celecoxcib	(Zhang and Godbey 2010)

sc –stands for sub–cutaneous models.

Table 2. Genes demonstrated to cause tumor reduction in murine models of bladder cancer.

Trial ID	Year	Country	Phase	Status	Vehicle	Gene (ref)
CA-0019	2005	Canada	I	Open	Adenovirus	CG0070 GMCSF
BE-0005	1998	Belgium	I	Closed	Poxvirus	Antigen MAGE-1.A1 and MAGE-3.A1
DE-0013	1997	Germany	I	Closed	Adenovirus	P53 and transduction enhancing agent (Kuball et al. 2002)
UK0057	2000	UK	I	Open	Adenovirus	E1B deleted
SE-0004/ NCT00891748	2006	Sweden	I/II	Closed	Adenovirus	Antigen, CTLA-4 (Malmstrom et al. 2010)
US-0145/ 9601-145	1996	USA	I	Closed	Adenovirus	Rb
US-0219/9710-219/ NCT00003167	1998	USA	I	completed	Adenovirus	P53 (Pagliaro et al. 2003)
US-0500/0110-500	2001	USA	I	Open	Adenovirus	HSV thymidine kinase and ganciclovir
US-0643/ 0404-643	2004	USA	I/II	Open	Adenovirus	CG0070, GMCSF
US-0581/0304-581	2003	USA	I	Active	Vaccinia, Fowl pox virus	GMCSF and CD80, ICAM1, LFA-3
US-0688/ 0501-688	2005	USA	I	Open	Adenovirus	SCH 721015 (IFNalpha 2b)
NCT00109655	2005	USA	I	Active not, recruiting	Adenovirus	Oncolytic (serotype 5) - CG0070
NCT00393809	2006	Israel	I/IIa	Completed	Plasmid	Diphtheria toxin (Sidi et al. 2008)
NCT00536588	2006	USA	I	completed	Adenovirus	SCH 721015
NCT00595088	2008	USA	2b	recruiting	PEI/plasmid	Intravesical DTA-H19
NCT00959868	2009	Canada	I	recruiting	oligo	OGX-427 (antisense hsp27)
NCT01162785	2010	USA	Ib	Not started	Adenovirus	SCH 721015 (rAd-IFN) and Syn-3,

Table 3. Clinical trials for bladder cancer gene therapy. NCT designation indicates data obtained from http://www.who.int/ictrp/en/;
Other alphabet and number designations are for data obtained from http://www.wiley.com/legacy/wileychi/genmed/clinical where the alphabets indicate country of registration and the numbers only designation are for data obtained from http://www.gemcris.od.nih.gov/Contents/GC_HOME.asp . Note some trials were registered on more than one web-site.

Information about completed, on-going and planned trials were obtained from the following sources: gene therapy clinical trials world wide web-site http://www.wiley.com /legacy/wileychi/genmed/clinical/; the clinical trials.gov, US National Institutes of Health and the Genetic Modification Clinical research Information System database http://www.gemcris.od.nih.gov/Contents/GC_HOME.asp and the World Health Organization International Clinical Trials registry platform portal http://www.who.int /ictrp/en/.

A Phase I trial on vaccinia virus instillation showed increased lymphocyte recruitment and the induction of an inflammatory response in the bladder (Gomella et al. 2001). There were no clinical manifestations of vaccinia toxicity indicating the safety and therapeutic potential of this virus as a gene therapy vector. Adenovirus delivery of p53 was also shown to successfully deliver p53 gene to bladder cells but there was no change in immunohistochemical detection of p53 in bladder tissue (Pagliaro et al. 2003). However, adenovirus therapy was safe and well tolerated (Pagliaro et al. 2003). Delivery of adenovirus carrying p53 with a transduction enhancing agent improved p53 gene delivery and protein expression was found in patient tissue samples (Kuball et al. 2002). Though higher doses of the virus were administered, no dose toxicity was observed (Kuball et al. 2002). Similarly no serious adverse effects were reported by Malmstrom et al. from a recently concluded Phase I/IIa trial using AdCD40L (Malmstrom et al. 2010). They observed gene transfer in biopsies and the infiltration of T lymphocytes (Malmstrom et al. 2010).

A plasmid was used to deliver the diphtheria toxin gene under the control of the H19 gene regulatory sequence in a Phase I/IIa trial for non–muscle invasive bladder cancer (Sidi et al. 2008). They reported mild toxicity and observed complete and partial response in some patients. Thus based on these Phase I trials, both non viral and viral vectors appear to be well tolerated in man.

Several new trials are either in progress or about to commence. These use non-viral and viral delivery vectors as listed in Table 3. A proposal for a Phase I trial for intravesical therapy in bladder cancer patients using plasmid DNA carrying the IFN-α gene and our liposome based delivery system is being evaluated by the Health Sciences Authority, Singapore.

2.5 Other urological malignancies

Though not covered here several clinical trials are on-going, evaluating gene therapy for prostate and renal cancers. In general the vectors and genes used are similar though tissue specific promoters may differ. Information on these trials can be obtained from the web-sites listed above. Unlike bladder cancer however, gene delivery to these tissues is not as simple. Thus tissue specific targeted gene expression has been developed. Another strategy is the use of macrophages transfected *ex vivo* with a plasmid carrying the E1A/B construct under the control of the hypoxia response element (HRE) as well as a replication competent adenovirus with the E1A under the control of the prostate specific antigen promoter. At the hypoxic tumor site the E1A protein is produced and the adenoviruses released infect tumor cells and cause their lysis (Muthana et al. 2011). Such a strategy prevents virus neutralization *in vivo*.

3. Non-cancer related urological problems

The bladder is made up of a reservoir and an outlet (bladder neck, urethra and urethral sphincter) whose activities are controlled by smooth and striated muscles. There are more

patients with bladder dysfunctions related to its primary function of urine storage and voiding than cancer. These include: lower urinary tract symptoms (LUTS), interstitial cystitis (IC), overactive bladder (OAB), spinal cord injuries affecting micturition and urinary incontinence (UI). It is estimated that by 2018, some 2.3 billion people worldwide will be affected by at least one LUTS, OAB, UI and LUTS suggestive of bladder obstruction (Irwin et al. 2011). LUTS is an umbrella term that encompasses urine storage (increased frequency, at least one episode of nocturia per night, urgency and UI), voiding and post-micturition symptoms (Abrams et al. 2003). Another urological problem is erectile dysfunction (ED). Both LUTS and ED are increased with aging and in patients with diabetes (Brown et al. 2005). With the worldwide increase in the incidence of diabetes the incidence of these urological problems will increase.

Most current therapies for the above mentioned conditions are palliative rather than therapeutic. Gene therapy however, may provide a way to cure the disease and the recent review by Christ lists some of these strategies (Christ 2011). Most of the bladder related problems seem to be linked to muscle and neuronal defects and because of the latter the most common vectors used for animals studies of bladder dysfunctions are HSV vectors.

3.1 Urinary incontinence

Urinary incontinence is a general term used to cover three types of incontinence namely stress, urge and overflow (Chancellor et al. 2001). Stress incontinence occurs when the urinary sphincter muscle is unable to prevent urine leakage following jumping or coughing. This is more common in women than men. Treatment approaches include exercise, surgery and collagen injections into the sphincter muscle. Often multiple injections are required which adds to the cost of treatment and some patients are allergic to bovine collagen. Tissue engineering and *ex vivo* gene therapy are alternative therapeutic strategies that are being evaluated (Shokeir et al. 2010). Phase I trials of the delivery of autologous myoblasts and fibroblasts from muscle biopsies to female patients with stress incontinence have shown some benefit (Mitterberger et al. 2007). In mice, combining myoblast and gene therapy such as the ex vivo transformation of myoblasts with the VEGF gene resulted in improved volume and function of muscle cells (Delo et al. 2008).

Urge incontinence is characterized by increased urinary urgency and frequency caused by involuntary bladder contractions leading to uncontrollable urine leakage. One study has evaluated intravesical non-viral delivery of a cDNA for the K+ channel and showed that this resulted in increased K+ channels in the smooth muscle of the bladder and amelioration of bladder overactivity (Christ et al. 2001).

Overflow incontinence results from nerve damage such that patients cannot urinate. One common cause is diabetes related neuropathy. Diabetes related cystopathy is often irreversible and restoring bladder functions to diabetic patients is difficult (Sasaki et al. 2002). Animal studies have identified nerve growth factor (NGF) as a good candidate for gene therapy for diabetes induced incontinence (Apfel et al. 1994). HSV delivery of NGF increased NGF in the bladder wall and dorsal root ganglion and improved voiding function in streptozotocin (STZ) induced diabetic rats (Goins et al. 2001; Sasaki et al. 2004).

3.2 Interstitial cystitis/painful bladder syndrome

Interstitial cystitis or painful bladder syndrome occurs predominantly in females (Persu et al. 2010). It is believed to result from underlying inflammation in the bladder. It cannot be adequately treated by drugs and prolonged drug therapy can lead to dependency and

tolerance to drugs that may require dose escalation to remain effective. Thus it is a candidate for gene therapy. Delivery of the preproenkephalin gene by HSV (Yokoyama et al. 2009) has been shown to be beneficial in reducing pain. A HSV vector carrying the ionotropic glycine receptor (GlyR) whose expression was induced by glycine had an analgesic effect (Goss et al. 2011). This vector when inoculated into the bladder wall of an inflammation model of IC/PBS in rats was activated by systemic glycine delivery.

3.3 Overactive bladder

Overactive bladder (OAB) is defined as "urinary urgency, usually accompanied by frequency and nocturia, with or without urgency urinary incontinence" (Haylen et al. 2010) Detrusor over activity is considered a single marker for OAB. It can occur as a result of spinal injury and could result in a lack of control of micturition. This has been demonstrated in animal models of spinal injury. HSV vector delivery of glutamic acid decarboxylase has shown benefit in a rat model of spinal injury (Miyazato et al. 2009; Miyazato et al. 2010).

3.4 Erectile dysfunction

Approximately 150 million men are projected to suffer from ED and the incidence of ED increases with age. Normal erectile function occurs as a result of 3 synergistic events namely: neurological mediated penial arterial inflow increase; cavernosal smooth muscle relaxation and restriction of venous outflow from the penis (Andersson & Wagner 1995). In ED one or more of these events may be impaired. The penis is an excellent candidate for gene therapy because it is easily accessible, has limited blood flow and a slow cellular turnover (Bivalacqua & Hellstrom 2001). Though therapy is available for erectile dysfunction there are a significant number of patients who do not respond to available therapy (Yoshimura et al. 2010). The recent review by Harraz et al. provides an excellent overview of gene therapy strategies used in animal models of ED that have been shown to resolve this problem (Harraz et al. 2010). Rather than reproducing that information only recent publications not included in that review are mentioned here. Over expression of the transient receptor potential (TRP) channels 6 (dominant negative) by transfection with a plasmid caused a decrease in calcium levels in the corporal smooth muscle and improved erectile function in diabetic rats (Jung et al. 2010). Using a STZ induced diabetes model to evaluate erectile dysfunction, it was found that implantation of mesenchymal stem cells transfected with VEGF improved erectile function compared to implantation of mesenchymal stem cells alone (Qiu et al. 2011).

3.5 Clinical trials

Only two clinical trials are listed on the http://www.gemcris.od.nih.gov/Contents /GC_HOME.asp for urological conditions unrelated to cancer. One is the Phase I trial for ED and the other is a trial for overactive bladder syndrome. Both trials used plasmid DNA carrying the calcium activated potassium channel (Melman et al. 2007). The results of the Phase I trial for ED indicate the safety of this delivery system.

4. Major issues and future prospects for gene therapy

One major issue is the safety of gene therapy in terms of its impact on the environment as well as long term safety in patients. Schenk-Braat et al found that only half of all registered

clinical trials included viral shedding data (Schenk-Braat et al. 2007) and what data was available was primarily for the time after virus delivery and not at the time of delivery. These questions should also be raised for plasmid based gene therapy. Long term follow-up data on patients who have received gene therapy may further ameliorate the safety concerns of this therapy. This will result in gene therapy being more readily applied to other non-malignant conditions. The development of better plasmids and ways to integrate plasmids into the chromosome may lead to the greater use of plasmid rather than viral vectors for urological gene therapy.

MicroRNAs (MiRNA) are new targets for cancer therapy. These are small non-coding RNA molecules that bind to complementary sequences in the protein coding regions of mRNA and block their translation. Their expression levels vary in cancer and normal tissues (Catto et al. 2011). MiRNA-203 and MiRNA-221 have been shown to modulate the growth and apoptosis of human bladder cancer cell lines (Lu et al. 2010; Bo et al. 2011) and these could be new targets for therapy. Given the function of miRNA it is possible that these molecules could also be targets for non-cancer related bladder dysfunctions. This has not yet been explored and identifying such molecules may improve our knowledge of the development of these conditions.

5. Conclusion

The application of gene therapy for urological conditions is being evaluated in many preclinical disease models. In general the results obtained are encouraging and soon these therapies may move to Phase I trials.

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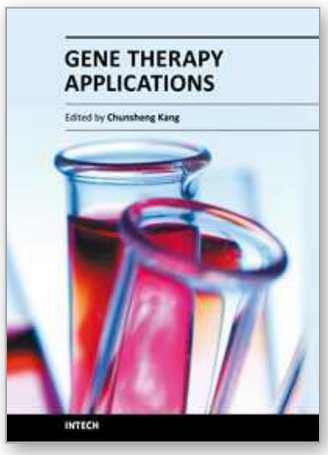

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The aim of our book is to provide a detailed discussion of gene therapy application in human diseases. The book brings together major approaches: (1) Gene therapy in blood and vascular system, (2) Gene therapy in orthopedics, (3) Gene therapy in genitourinary system, (4) Gene therapy in other diseases. This source will make clinicians and researchers comfortable with the potential and problems of gene therapy application.

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