

(Review Article)**Genetic Treatment of Urological Diseases; are We Ready?****Ausama Saadi Abdul Muhsin****ABSTRACT:**

There is an increasing interest in the role of genetics in many urological diseases and malfunctions. The door was opened for early diagnosis, application of genetically based armamentarium to prevent, treat or modify the clinical course as well as prediction of the prognosis of such abnormalities.

Many urologists share the idea that the use of a genetic tool to diagnose or treat their patients still has a long time before coming into a real clinical and surgical practice.

The purpose of this article is to briefly and simply review gene therapy, epigenetic therapy and stem cell therapy principles and prospects in the management of various urological disorders and to encourage standard urology centers' authorities in the Middle East countries to develop, share and improve their national experience which can serve genetic counseling and possible therapeutic management of their own patients to cope with the international revolution in this field.

KEY WORDS: gene therapy, urological diseases
Urologists manage a wide variety of diseases and malfunctions, many of which have underlying genetic bases including:

1) Congenital or developmental abnormality such as vesicoureteral reflux (VUR), autosomal dominant polycystic kidney disease (PKD), disorders of sex development (DSD), hypospadias etc.

2) Functional deficiency such as urinary incontinence and male impotence.

3) Abnormal or cancerous growth in kidney, bladder, prostate, urethra, and testis.⁽¹⁾

4) Urinary stones: Approximately 25% of patients with renal stones have a family history of stone disease and it is possible that urolithiasis is a polygenic defect with partial penetrance. In addition, several disorders that have a clear genetic basis do predispose to stone formation (renal tubular acidosis, cystinuria, xanthinuria).⁽²⁾

5) Male infertility⁽³⁾.

Disease causing genes were studied by the traditional methods like analysis of family pedigrees, and twin studies. For enhanced diagnosis and the development of new therapy it is necessary to pinpoint the underlying gene defects. The range of available techniques includes cytogenetics, loss of heterozygosity, fluorescence in-situ hybridization (FISH), comparative genomic hybridization (CGH), and DNA microarrays. Antisense oligonucleotides and the more recently discovered technique of RNA interference can be used to silence suspect genes and so study their effects in more detail.⁽⁴⁾ Genetic treatment mainly refers to gene therapy, epigenetic therapy and stem cell therapy.

Gene therapy

Genes, which are carried on chromosomes, are the basic physical and functional units of heredity. Genes are specific sequences of bases that encode instructions on how to make proteins. Although genes get a lot of attention, it's the proteins that perform most life functions and even make up the majority of cellular structures. When genes are altered so that the encoded proteins are unable to carry out their normal functions, genetic disorders can result.⁽⁵⁾

Gene therapy is an experimental technique for correcting defective genes responsible for disease development. In the future, this technique may allow doctors to treat a disorder by inserting a gene into a patient's cells instead of using drugs or surgery⁽⁶⁾.

How does gene therapy work?

One of several approaches may be used for correcting faulty genes:

- A normal gene may be inserted into a nonspecific location within the genome to replace a non functional gene. This approach is most common.

- An abnormal gene could be swapped for a normal gene through homologous recombination.

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- The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function.

- The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered.^(5, 6)

The success of gene therapy strongly depends on an efficient delivery

system to allow local transfer and expression of the therapeutic gene (transgene) in the target organ or tissue. There are two different categories of delivery

vehicles: viral and non – viral vectors. Each vector system has a series of advantages and disadvantages that must be taken into consideration in view of the final aim.^(7, 8)

Different types of viruses used as gene therapy vectors such as retroviruses, adenoviruses, adeno – associated viruses, and herpes simplex viruses.

Viruses have evolved a way of encapsulating and delivering their genes to human cells in a pathogenic manner. Scientists have tried to take advantage of this capability and manipulate the virus genome to remove disease-causing genes and insert therapeutic genes^(5, 9). Figure (1)⁽⁵⁾

Methods of non-viral gene delivery have also been explored using physical (carrier – free gene delivery) and chemical approaches (synthetic vector – based gene delivery). Physical approaches, including needle injection, electroporation, gene gun, ultrasound, and hydrodynamic delivery, employ a physical force that permeates the cell membrane and facilitates intracellular gene transfer. The chemical approaches use synthetic or naturally occurring compounds as carriers to deliver the transgene into cells⁽¹⁰⁾.

An ideal vector system which has not yet been constructed⁽¹¹⁾, has to follow three principles; safety, efficacy and stability⁽⁸⁾. The acute immune response, immunogenicity, and insertion mutagenesis uncovered in gene therapy clinical trials have raised serious safety concerns about some commonly used viral vectors. On the other hand, although significant progress has been made in the basic science and applications of various non viral gene delivery systems, the majority of non viral approaches are still much less efficient than viral vectors, especially for in vivo gene delivery^(10, 12).

Among other fields of urology research, gene therapy and stem cell therapy are commonly

mentioned in two urological problems, namely bladder cancer and erectile dysfunction.

Bladder cancer is the fourth most common malignancy among men in the Western world⁽¹³⁾ and the third most common malignancy in Iraq⁽¹⁴⁾. At the initial diagnosis of bladder cancer, 70% of cases are diagnosed as non – muscle invasive bladder cancer (NMIBC) and approximately 30% as muscle-invasive bladder cancer (MIBC)⁽¹⁵⁾.

The prevention of disease recurrence and progression of (NMIBC) is a major clinical challenge. Intravesical instillation of Bacillus Calmette Guérin (BCG) combined with transurethral resection (TUR) of bladder cancer is recognized as the best treatment option for the prevention or delay of recurrence and progression in high-risk NMIBC patients. However, many patients fail to respond to BCG therapy and are at higher risk of disease recurrence and progression.

Genetic management may play a role in segregating primary pT1 bladder cancers that respond poorly from those showing good response to intravesical BCG immunotherapy through gene expression profiles (gene signatures)⁽¹⁶⁾. Thus, individualized therapeutic (urinary bladder sparing versus radical cystectomy) modalities will be applied.

On the hand, gene therapy for MIBC may provide in the future a safe, efficient, and durable urinary bladder sparing treatment option as an alternative to radical cystectomy which is the standard treatment of localized MIBC in most countries of the Western Hemisphere⁽¹³⁾.

The most common strategies in gene therapy of bladder cancer are corrective, inductive and cytotoxic gene therapy.

Corrective strategies seek to restore the physiological function of a mutated gene in a tumor cells (such as P53 tumor suppressor gene), thereby stopping unrestricted cell division, organ infiltration or metastasis⁽¹⁷⁾. Eleven poly – arginine (11R) – fused P53 protein was effectively delivered into bladder cancer cells and significantly inhibited the growth of the cells. Moreover it enhanced the cis-diaminedichloroplatinum (CDDP) dependent induction of apoptosis of the cells. Transurethral application of protein transduction using 11R effectively and selectively delivered exogenous protein into bladder tumors in vivo, suggesting

that P53 protein transduction therapy may become a novel method for bladder cancer therapy⁽¹⁸⁾. Other gene therapy modalities manipulating P53 gene in bladder cancer are under clinical research⁽¹⁹⁾.

Immune inductive strategies trigger a previously non – existing or enhance a weak immune response against bladder tumor. For this bladder tumor cells can be transfected with immune modulating cytokine genes, like IL - 2 and readministered to the patient as a "vaccination".

Cytotoxic strategies allow the selective killing of tumor cells, while sparing benign tissues. Most approaches transfect tumor cells with genes that express either direct or indirect cytotoxic products for the transfected cell. As the cells themselves generate the toxic product leading to their death, these approaches are often called suicide gene therapy. Targeted tumour-specific radiotherapy uses a radionuclide combined with a tumor seeking drug⁽²⁰⁾.

Erectile dysfunction (ED) affects over 50% of men between 50 and 70 years of age, and 40% of men aged about 40 years suffer from some form of erectile dysfunction⁽²¹⁾. Although oral phosphodiesterase type 5 (PDE5) inhibitors (sildenafil, tadalafil, and vardenafil) are effective for the treatment of ED, it is not efficacious in severe ED cases with diabetes, aging or cavernous nerve injury.

Gene-based therapy has been proposed as one of potential new therapies for PDE5-resistant ED. Gene therapy has been investigated in animal models as a mean to restore normal function to the penis; at this time, however, only one human trial (using non-viral gene transfer of Ca²⁺-activated, large-conductance K⁺ channels into the corpus cavernosum of ED patients) showed positive results and published in the peer-reviewed literature⁽²²⁾.

Current status and future prospects of gene therapy.

Current research is evaluating the safety of gene therapy; future studies will test whether it is an effective treatment option. Comprehensive federal laws, regulations, and guidelines help protect people who participate in research studies (called clinical trials). The U.S. Food and Drug Administration (FDA), founded on 1906, regulates all gene therapy products in the United States and oversees research in this area. Researchers who wish to test an approach in a clinical trial must first obtain permission from

the FDA which has the authority to reject or suspend clinical trials that are suspected of being unsafe for participants⁽²³⁾.

FDA has not yet approved any human gene therapy product for sale. However, the amount of gene-related research and development occurring in the United States continues to grow at a fast rate and FDA is actively involved in overseeing this activity⁽²⁴⁾. FDA had declared guidance for industry including (Potency Tests for Cellular and Gene Therapy Products) and (Gene Therapy Clinical Trials – Observing Subjects for Delayed Adverse Events)^(25, 26). FDA oversees the safety and efficacy of other new strategies of gene therapy using nanoparticles as non viral vectors⁽²⁷⁾.

Epigenetic therapy

Epigenetics is defined as heritable changes in gene expression that are, unlike mutations, not attributable to alterations in the sequence of DNA. The predominant epigenetic mechanisms are DNA methylation, modifications to chromatin, loss of imprinting and non-coding RNA. Epigenetic regulation of gene expression appears to have long-term effects and wide-ranging effects on health⁽²⁸⁾.

Disruption of epigenetic processes, which are essential for normal development and maintenance of tissue-specific gene expression patterns in mammals, can lead to altered gene function and malignant cellular transformation. The initiation and progression of cancer, traditionally seen as a genetic disease, is now realized to involve epigenetic abnormalities along with genetic alterations⁽²⁹⁾.

There is now increasing evidence suggesting that several characteristic features of chronic kidney disease such as hyperhomocysteinemia, subclinical inflammation, increased oxidative stress and others may affect the human epigenome. In addition, animal studies have suggested a possible link between nutrition and environmental exposure during the periconceptual period and epigenetic changes in the expression of major genes implicated in kidney organogenesis; these changes result in a diminished number of nephrons in the developing kidney, which predisposes to an increased risk for hypertension and chronic kidney disease in future life.

Epigenetic drugs are already in clinical use for the treatment of cancer as well as under investigation for the use in other diseases^(30, 31),

such as congenital neurogenic bladders in which such drugs may reverse the abnormal collagen production of smooth muscle cells from neuropathic bladders⁽³²⁾.

Stem cell therapy

Stem cells are characterized by their potential immortality and are capable of self-renewal and differentiation⁽³³⁾. In mammals, there are two broad types of stem cells: embryonic stem cells, which are isolated from the inner cell mass of blastocysts Figure (2), and adult stem cells, which are found in various tissues⁽³⁴⁾. The promise of stem cells is to provide a source of non – diseased material for the generation of patient-specific cells or tissue for replacement and reconstruction⁽³⁵⁾.

An international forward step of the increasing interest in stem cell therapy in urology had been achieved by the arrangement of 2011 American Urological Association (AUA) Foundation Basic Science Symposium titled “Stem Cells in Urologic Diseases” in Washington DC, USA on May 2011. The meeting was a joint effort of the (AUA) Foundation, Society for Basic Urological Research (SBUR) and the National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK). It was intended to be a rigorous and in-depth discussion of an overarching topic area in basic research that will attract leaders in the field, leading urology researchers, clinical urologists and junior investigators to the AUA Annual Meeting and that will advance progress in urology research. In this symposium several vital issues and future prospects concerning stem cell therapy in urology were discussed.

Tissue engineering and regenerative medicine. The field of regenerative medicine is advancing rapidly, so the use of tissue engineering strategies that include the use of stem cells and artificially created scaffolds represent the most probable solution for treatment of many urological disorders and transplantation therapies in the future. Although there is an increased number of published studies in animals and a few studies in humans, with very promising results, many of these novel techniques are still being investigated. The transplantation of kidneys produced by tissue engineering that avoids rejection and immunosuppressor treatment, or the production of spermatogonia from stem cells of different origins for the treatment of infertility are goals that seem utopic today, but may become a reality in the long term^(36, 37).

Current tissue engineering techniques are hampered by several problems including choice of appropriate cell type, inadequate development of new blood vessels to the regenerated tissue, tissue innervation and primitive bioscaffold design⁽³⁸⁾.

Adult stem cell injection therapy for the regenerative repair of an impaired rhabdosphincter in stress urinary incontinence is currently at the forefront of incontinence research. The implanted cells will fuse with muscle and release trophic factors promoting nerve and muscle integration⁽³⁹⁾.

Stem cell and urological cancers. The appreciation of the importance of stem cells in normal tissue biology has prompted the idea that cancers may also develop from a progenitor pool (the cancer stem cell (CSC) hypothesis), and this idea is gaining increasing acceptance among scientists. CSCs are sub-populations of cancer cells responsible for tumor initiation, differentiation, recurrence, metastasis, and drug resistance. Recently, the tissue-specific stem cells of the normal urothelium have been proposed to reside in the basal layer, and investigators have isolated phenotypically similar populations of cells from urothelial cancer cell lines and primary tumors. This may explain the development of the two different types of bladder cancer: noninvasive and invasive carcinoma as well as therapeutic applications of these findings^(40, 41).

Stem cell therapy in erectile dysfunction (ED). There are currently very few published reports in animal models and none in human men. Although stem cell therapy offers the potential for restoration of functional tissues (by potential protecting and repairing both nervi erigentes and corpus cavernosum vascular endothelial cells), legitimate concerns remain regarding the long-term fate of stem cells^(42, 43).

DISCUSSION:

There is an increasing interest in the role of genetics and genetic related treatment in many urological diseases and malfunctions. This is reflected by the progressive research in this field. For example in European Urology Journal the online search, updated on September 2011, for gene therapy revealed 1388 articles, for epigenetic therapy 146 articles, and for stem cell therapy in urology 316 articles. Although genetic treatment prospect mainly refers to gene therapy, epigenetic therapy and stem cell

therapy, it may possibly be complemented by a wide spectrum of dietary consumption advices such as low fat diet, high intake of fruits, vegetables and lycopene rich foods were found to be protective in prostate cancer⁽⁴⁴⁾ and green tea in bladder cancer⁽⁴⁵⁾, and life style modifications such as smoking habit which is well known to have associated and or induced alterations of molecular genetics in urological cancers mainly urinary bladder⁽⁴⁶⁾ cancers. Other life style variables may be the target of multi modality treatment of certain genetically based urological diseases⁽⁴⁷⁾.

Because gene therapy involves making changes to the body's set of basic instructions, it raises many unique ethical concerns. The ethical questions surrounding gene therapy include:

- What is normal and what is a disability or disorder, and who decides?
- Are disabilities diseases? Do they need to be cured or prevented?
- Does searching for a cure demean the lives of individuals presently affected by disabilities?
- Is somatic gene therapy (which is done in the adult cells of persons known to have the disease) more or less ethical than germline gene therapy (which is done in egg and sperm cells and prevents the trait from being passed on to further generations)? In cases of somatic gene therapy, the procedure may have to be repeated in future generations.

Because people who would be affected by germline gene therapy are not yet born, they can't choose whether to have the treatment. The U.S. Government, because of these ethical concerns, does not allow federal funds to be used for research on germline gene therapy in people.

- Preliminary attempts at gene therapy are exorbitantly expensive. Who will have access to these therapies? Who will pay for their use?^(23,5)

The embryonic stem cell research is morally controversial because it involves sometimes the deliberate production, use and ultimate destruction of human embryos. There are many ethical questions raised by in utero therapeutics with stem cells and gene therapy, whether the choice of such treatment belongs to a well informed mother or to the fetus which is not yet born. It is important to separate the services that receive dead fetuses from those that perform the injection of fetal stem cells so that the fetus does not become a mere therapeutic tool.

The Islamic opinion permits such research with full consideration and all possible precautions in the pre – ensoulment stages of early fetus development, if the source is legitimate. Umbilical cord blood banks are now available in the kingdom of Saudi Arabia and United Arab Emirates^(48, 49).

CONCLUSION:

Genetically based management of urological disorders is undergoing an extensive research and it is time for urologists to prepare their mind for such options which are rapidly progressing into clinical trials after being evaluated in animal and laboratory models. This article is also an invitation for our urology research institutions in the Middle East countries to establish the legal, clinical, and economic infrastructure of the genetic related clinical research including that related to urological disorders and to share their experience for such promising research field.

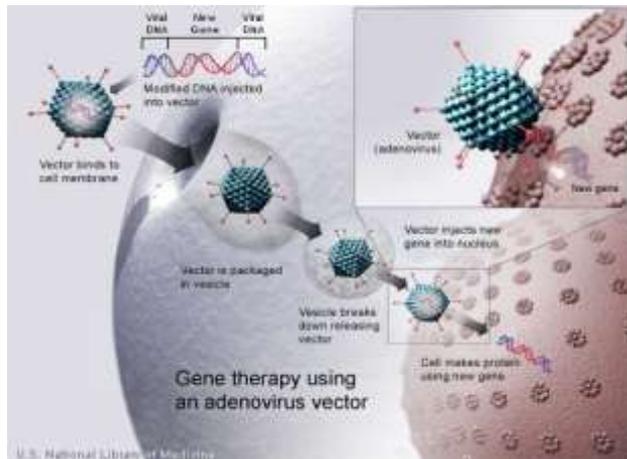


Figure 1: Viral vector in gene therapy

Source: http://www.google/images/viral_vector_in_gene_therapy.
www.acceleratingfuture.com/michael/blog/images/Gene_therapy.jpg
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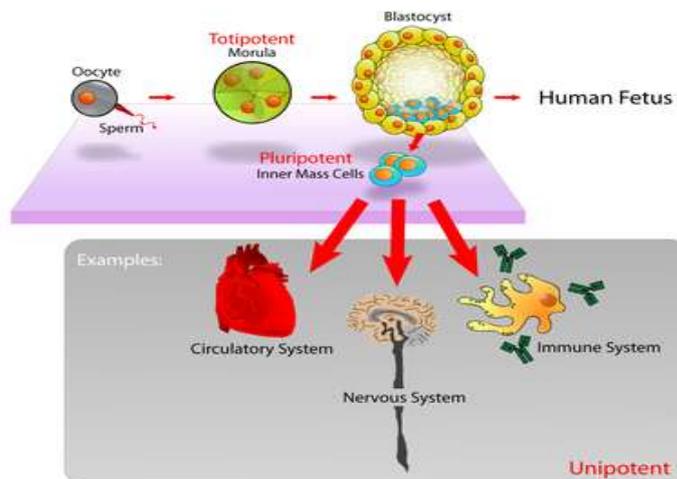


Figure 2: Embryonic stem cell

Source: http://www.Wikipedia,the_free_encyclopedia. File: Stem cells diagram.png.wikipedia.org.

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