Gene therapy for urologic disease

Fehmi Narter*
Institute of Experimental Medical Research, Department of Molecular Medicine, Istanbul University, Istanbul, Turkey

Gene therapy for the urologic diseases is focused on urinary tract malignancies, urinary dysfunctions and erectile dysfunction. Urinary system is suitable for minimally invasive molecular medicine therapies. Endoscopic and percutaneous routes are preferable for treatment of urological diseases. With recent advances in minimal invasive treatment methods, technology and molecular medicine, gene therapy, tumor vaccines, diagnostic tests, new therapeutic molecules, stem cell therapies and nanomedicine are becoming elucidated. In this review we will focus on gene therapy for urologic diseases. Gene therapy is safe for urinary tract diseases, because it is not always necessary to use systemic therapeutic routes for gene transfer so systemic toxicities are very rare.

Key words: Urology, gene, gene therapy


Gene Therapy for Urologic Malignancies

Surgery, radiation therapy, chemotherapy are therapeutic options for uro-malignancies. But these can also damage the healthy tissues.

Developments of molecular biology; mechanisms about growth factors, cell receptors, adhesion molecules, oncogenes, tumor suppressor genes, cellular regulation, gene expression, angiogenesis, carcinogenesis are getting clearer. So gene defects can be cured by replacing with the normal (wild type) gene.

Tumorigenesis has a multistep pathway, unknown gene defects and multigene defects have roles its progress. Initiation, proliferation, loss of contact inhibition, invasion, metastasis are steps on this pathway. Multiple gene defects can effect cell cycle regulation, angiogenesis, cell adhesion, immunoreactivity. Because safety, high efficiency, selectivity are important for the ideal gene therapy and because of the difficulties of gene therapy on multigene disorders. Long term follow up, more experimental and clinical trials are necessary. Malignant processes go on with activation of oncogenes and embryonic growth factors and/or deactivation of tumor suppressor genes.

Transfection: To introduce the gene into target cells, different vectors are used to facilitate its entry through the cell membrane.

Gene therapy attempts to correct mutations on DNA. Gene therapy is done by transfection of tumour cells to quenche the oncogenes or activate tumor suppressor genes. Gene therapy concern a technique to translate coding DNA using any transport mechanism into cells for DNA transcription.

Methods for gene therapy are

a) Gene replacement: Many cancers represent over-expression of an oncogene or inactivation of a tumor suppressor gene. This method is not so much succesful in cancer patients yet. Because many gene defects can be implicated in the formation of tumor.

*Correspondence to: Fehmi Narter, MD
Istanbul University, Institute for Experimental Medical Research
Department of Molecular Medicine,
P.O. Box: 7 Çapa 34390 Istanbul, Turkey
Phone&Fax: +90 212 635 1959
e-mail: fehminarter66@yahoo.com
Accepted: May 25, 2006
b) Tumor vaccines: Tumor vaccines are created by the use of cytokine genes transfected into tumor cells. Normally tumor cells escape from immune system through down regulation of cell surface antigens such as major histocompatibility complex (MHC). In this methods tumor cells are harvested from the patient, grown in cell culture, and transfected with cytokine genes (IL-2, IL-6, TNF-alpha, INF-gamma etc.). Cytokines stimulate expression of cell surface antigenic proteins (HLA Class I-II etc) which then enhances immunogenicity. After irradiation, these cells are administered back to the patient. This approach is more complex than the others. For new generation tumor vaccines, viruses or packaged segments of DNA can be used to deliver the cytokine genes into tumor cells. Usually to these in vivo procedures, retroviral vectors are used for transfection. Alternate liposome-cytokine gene complexes can be used for cytokine production within the tumor. IL-2 and GM-CSF (granulocyte-monocyte-colony-stimulating factor) genes are used by intratumoral injection for prostate and renal cancer patients.

c) Suicide genes: The cells treated by suicide gene-sare selective to kill tumorous cells. We use vectors to transport the genes into the cell. The gene itself can be also engineered with a tumor-specific prometer or can be used in combination with the gene product. By this method we use some therapeeutics together and we can switch some benign medications to toxic agents. For example, herpes simplex virus thymidine kinase gene (HSV-Tk) is non toxic, but in the presence of ganclovir, it converts toxic agent to prevent DNA synthesis. Prostate specific antigen (PSA) promoter sequence causes increased androgen sensitivity prostate specific gene expression will be limited only to the target tissue and make our gene therapy only specific to that focused tissue.

Vectors

All vectors must be safe and effective. Naked plasmid DNA vectors when administrated systemically, are rapidly cleared in the bloodstream. Liposome vectors can also be used for efficent gene transfer. This has also no immunogenicity, it is eliminated by liver.

Viral vectors are retroviruses and adenoviruses. Retroviral transfection success is low because of rapid proliferation of tumor cells. Because prostate cancer is slow growing, the retroviral methods may not be effec-
tive in this cases. Retroviral viruses have potential risk for mutagenesis and long term adverse effect. Adenoviral vectors can provide higher efficiency. Genomic integration rates are low, but adenoviruses can kill tumor cells, by oncolysis is independent from transfection. And replication of the transfected adenoviruses may enhance immune response against the tumor antigen. And there are some newly modified adenoviruses (vaccinia viruses etc) that can be more effective for transfection.

Prostate cancer

Prostate specific antigens (PSA) and prostate membrane antigens (PSM) are only expressed at normal levels in prostate tissue. PSA gene enhancer or prometer are androgen dependent. PSA promoter driven gene therapy, HSV-Tk, cytosine deaminase, diphteria toxin, tumor necrosis factor genes are investigated. Prostate cancer gene therapies are used like target specific gene therapy (linked to PSA enhancer or prometer sequence) or intratumetal suicide gene therapy. Osteocalcin gene therapy may also have prostate cancer specificity. Alternately tumor supressor gene replacement is also another route. p53 genes block cell proliferation at the G1 point, DNA polymerase is stopped by this gene product. If this gene is absent, DNA injuries can occur, cell proliferation continues, genetic instability and tumorigenic changes come arround. With normal (wild) p53 gene transfection, prostate cancer can be slowed down. Mutant p53 gene is a marker for aggresiveness. c-ras, H-ras, TGF-beta, c-myc, bcl-2 etc are some tumor supressor genes or oncogenes, that can be used as new targets for prostate cancer gene theraphy.

PSA promoters and proteins are immunogenic and stimulates cytotoxic T-cell reactions. Dendritic cells transfected with the PSA or PSM genes can stimulate immune system and cause antitumoral effect.

Alternatively, with retroviral IL-2 or GM-CSF gene transfections (cytokine reaction), immune response can be generated against prostate cancer.

Bladder cancer

Bladder cancer is one of the most sensitive cancers to immune response modifiers such as interferon, BCG. Many oncogenes or tumor supressor genes implicated in the pathogenesis of bladder cancer (Rb, C-CAM1 (adhesion molecule), c-myc, P53, Bdx-1 (apoptosis reg-
All these genes are potential targets for gene therapy. Retinoblastom gene is a tumor suppressor gene and replacement of it is possible for the treatment of bladder cancer. c-myc gene can give an idea of progression and it can be used as a marker of resistance against the chemotherapy. Antisense oligonucleotids are short synthetic stretches of chemically modified DNA or RNA capable of specifically binding to the mRNA of a chosen target gene and reducing its expression. In some gene therapy models with c-myc antisense oligonucleotids (ODN) significant improvements may occur. Adenoviral p53 gene replacement, adenoviral –TK suicide gene systems, down-regulation of oncogenes, tumor vaccines are other gene therapy options. Vaccinia or HSV –IL2, liposome-IL2 gene therapy trials are promising for future developments in this area.

Testis cancer

Testis tumors (especially germ cell tumors) can have some abnormalities in chromosom 12. Deletion 12q has been identified in testes cancer. MGF is a tumor suppressor gene and is located on the chromosom 12p. MDR-1 gene therapy can provide treatment, because this gene regulates resistance against chemotherapeutics.

Renal cancer

Von Hippel Lindau disease (VHL) and its gene is correlate with renal cancer. Deletion of chromosom (3p) is detected for this disease. This gene or p53 tumor suppressor gene replacement therapy are possible for the treatment of renal cell cancer. Tumor antigen of renal cell cancer has been named as G250. G250 antigen is important for vaccine investigation of renal cell cancer. Cytokines stimulate expression of tumor surface antigens like HLA-Cw7 (MHC). IL-4, GM-CSF, HLA-B7, INF-gamma are used in vaccine development of renal cell cancer. Cytokine genes, liposomal HLA-B7 or IL-2 genes,autolog retroviral GN-CSF gene therapies have been investigated still.

Gene Therapy for Bladder Dysfunctions and Incontinence

Incontinence is a very serious medical and social problem. Three main types of urinary incontinence are stress, urge, and overflow incontinence.
galactosidase for treatment of stress incontinence (autolog cell transplantation). Chris et al evaluated the use of K⁺ channel gene therapy for urinary incontinence. Intravesical naked pcDNA /bslocDNA treatment was used to suppress bladder hyperactivity. Over expression of bladder K⁺ channels may also inhibit the bladder hyperactivity. For the overexpression incontinence treatment; HSV-GF gene therapy for the bladder afferent pathways improved bladder function in diabetic rats.

**Gene Therapy for Erectile Dysfunction**

An essential mediator of penile erection is nitric oxide (NO) and it is the product of Nitric oxide synthase (NOS) reaction. NOS hydrolysises L-arginine and produces NO. NO stimulates the activity of the enzyme guanylyl cyclase and increases c-AMP levels and this cGMP decreases intracellular Ca²⁺. NOS has three isoforms (cNOS, iNOS, nNOS). Gene therapy for erectile dysfunction has focused on over-expression of NOS and K⁺ channel gene delivery in corpus cavernosum. Champion et al demonstrated recombinant adenoviral eNOS (AdCMVeNOS) gene transfer to produce endothelial NO for proper erectile functions. Thirney et al assessed iNOS gene transfer into the corpus cavernosum of adult rats. Wessells et al focused on treatment of erectile dysfunction with ex vivo endothelial cell-based gene therapy. Chris et al have demonstrated the efficacy of gene therapy using naked cDNA of Ca²⁺-activated K⁺ channel gene for impotence (hSlocDNA) Lue et al has demonstrated improvement of bladder function in diabetic rats.

Gene therapies for urologic disorders (bladder dysfunctions, malignancies, erectile dysfunctions, male infertility etc.) have been developing to create new therapeutic approaches (Table 1). But some difficulties are still existing. Choice of therapeutic gene products, safety of vectors, selectivity, unknown of gene products, so which methods are the best?, pharmacokinetics of these therapeutics, optimal doses, adverse effects, long term incomes, type of vector and best intake route should be determined, many of the researches in this area are done by rats. As the answers of all these questions will have been discovered day by day, 21st century will start being “the times of gene therapy” and perhaps most of the conventional therapeutic methods will be given up.

**References**


