IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 3 Ver. II (May - Jun. 2015), PP 06-12
www.iosrjournals.org

Gene Therapy: Nipping genetic defect at its root

Mohona Gupta, Undergraduate student,
Department of Zoology, Asutosh College, Kolkata.

Abstract:*The long awaited solution to treat the genetic defects successfully has finally taken shape. Advanced research is on full swing and there has already been several successful trials on several systems. It is not the only solution to genetic defects but by far, the most promising of all available methods. Several ways of implementing this therapy has been devised, the major being in-vivo and ex-vivo while others being the local and systemic gene delivery based on the method of modification. The retrovirus, adenovirus and the adenoassociated virus are among the best suited vectors (all of which are viral vectors) because of their permanent expression of the therapeutic gene. Non-viral vectors have their own advantage such as low immunogenicity, greater capacity for therapeutic DNA, etc. Gene therapy has paved a way in the treatment of inherited diseases, cancers and acquired disorders. Human gene therapy has proven to be a significant addition to the armory of research going on to eradicate the perceived incurable diseases.*
Keywords: *genetic defects, in vivo, ex vivo, gene delivery, therapeutic gene*

I. Introduction

James Watson was heard saying "we used to think that our fate was in our stars, but now we know, in large measures, our fate is in our genes". At the present juncture of scientific development, we can feel the essence of this statement in its true sense. Genes are the functional and physical basis of heredity. It is a characteristic of each and every living individual. Four different nucleotides namely adenine, cytosine, guanine , thymine (for DNA) and uracil (for RNA) are the basic constituents of gene and they reside on the chromosomes. Genes are basically instructions that help to synthesize proteins which determine the form and functioning of an organism. When these genes are refashioned , irrespective of the cause of alteration they produce an abnormal gene resulting in the production of impaired proteins. This eventually leads to the development of a genetic defect which is often heritable. The sole purpose of gene therapy is to correct this defect from its very root by regulating the functioning of the defected gene by any of the numerous tested approaches. For example, a brain tumor is developing by rapidly dividing cancer cells. The reason behind such a development is certainly a defective or mutated gene. In this case, gene therapy would involve the use of herpes virus with its virulence removed, making it harmless but still the virus can insert its genetic material into the host cell. The virus is then injected into a mouse where they make additional copies of themselves.After this, a mouse cell carrying the virus is extracted and inserted into the brain containing the tumor cells. On entering the brain, the virus draws out the target cells and invades them completely. The brain cells will then produce herpes enzymes as the virus has successfully inserted its genome into the brain tumor cells. A doctor can now treat such a patient with a herpes curing drug that would not only perish the tumour cells but also the mouse cells responsible for production of the herpes enzyme.In this way , a seemingly incurable disease can be treated with a lot of ease and high success rate.

What is gene therapy?

Gene therapy refers to a kind of experimental treatment aimed at preventing or fighting a disease. This method involves manipulation at the genetic level. Safe,accurate and effective gene manipulation (including gene insertion or removal) is the prerequisite of successful gene therapy.Genes contain DNA segments and are responsible for synthesizing proteins that largely regulate the form and functioning of the body. Alterations at the genetic level could thus prove to be successful in treating several diseases like HIV, hemophilia, Parkinson's disease and many more. By adding a corrected copy of the defective gene, this treatment ensures fixing a disease at its source and not merely treating its symptoms

Overview of the process of gene therapy

The basic objective of gene therapy is to correct the abnormal gene (s) at a desired or target cell. There are different ways of accomplishing gene therapy.The most common approach is that of replacing a nonfunctional gene by a normal gene into a non-specific location within the genome.

- Swapping of an abnormal gene for a normal gene homologous recombination.
- Repairing of an abnormal gene through selective reverse mutation
- Alteration in the regulation of a particular gene, in short, degree to which a gene is turned on or off.

Ways of implementing gene therapy

A. **Mode of action** :-

- In vivo – the principal area of action in this method is inside the body where vectors used deliver the desired gene into the target cell. No cells are removed from the patient's body during this process. This therapy has been tested on Cystic Fibrosis and various kinds of cancer.This process is less invasive and considerably simple but the problem lies in the fact that first, targeting accurately is tough ; secondly, it provides comparatively lesser control than ex vivo and thirdly it is next to impossible to check the safety of this method as vectors are directly administered into the patient's body.
- Ex vivo – the principal area of action in this method is outside the body. Target cells are extracted from the blood or bone marrow of the patient's body, cultured in the laboratory and then exposed to vectors usually viruses which carry the desired gene . This therapy has been tested for HIV and even Alzheimer's disease amongst others.This is a more reliable method as it allows greater control and allows to check the safety before a cell is re-implanted but this method requires high specializations and protocols.

B. **Treatment site** :-

- Systemic- In this method , the gene products are delivered to more than one site via blood circulation. For example, treatment of lung cancer
- Local – In this method, the gene products are directly injected into the site of treatment, for example, in the treatment of synovial tissue during the treatment of rheumatoid arthritis

History/ Historical perspectives

An Austria monk , Gregor Johann Mendel began the study of genetics in 1850 with a series of experiments on green peas, thereby describing the concept and pattern of inheritance as he observed that the traits were inherited as separate units which are now known as genes.Mendel was the harbinger of further scientific achievements that heralded in the era of modern genetics and thus he is rightly called the father of genetics. An American biochemist James Watson and a British biophysicist Francis Crick developed a revolutionary model of double stranded DNA helix until which the physical nature of genes was unknown. Another transilience came in the early 1970's with the discovery of a series of enzymes that made it possible to nick apart genes at a previously determined site along a DNA molecule and also glue them back in a reproducible fashion.

The basic concept of gene therapy started geminating around the 1960's and early 1970's. This occured simultaneously when the development of genetically marked cell lines and the elucidation of mechanisms of cell transformation by papovaviruses polyoma and SV40 were progressing. On gaining knowledge on recombinant DNA technology, cloned genes became accessible which were used to show that foreign genes could correct genetic defects and disease phenotypes in mammalian cells in vitro. Effective retroviral vectors and other gene transfer methods have allowed satisfactory demonstrations of efficient phenotype correction both in vitro and in vivo.

Types of gene therapy

There are basically two types of gene therapy :-

1. Germ line gene therapy – This therapy works on the principle of introduction of functional genes integrated into the genome of the germ cells (ie, sperms and eggs)and is hence heritable. Theoritically, this therapy should be effective in treating genetic disorders but in the recent times very many practical problem including technical troubles and ethical obligations have hindered its progress and application.
2. Somatic gene therapy-This therapy works on the principle that therapeutic genes are transferred to the somatic cells of a patient and therefore it is not heritable.

Gene delivery

A gene cannot be directly inserted into the genome of the patient. It has to be mediated by some other tool and this has been one of the greatest challenges in the approach of gene therapy. To solve this problem, a carrier molecule which is known as a vector is used.

The characteristics of an ideal vector include :

1. Very specific
2. Capable of efficiently delivering one or more genes of the size needed for clinical application
3. Unrecognized by the immune system
4. Purified in large concentrations
5. The vector should not cause any allergic reaction or inflammation
6. It should be safe not only for the patient but also the environment

7. Lastly, it should possess the capacity to express the gene for as long as required which is usually the life of the patient.

Vectors used in gene therapy

1. Viral vectors : Presently , one of the most propitious vectors in use are the viral vectors.. Based on the life cycle of the viruses, it is evident that they have a naturally evolved a gene delivery wagon which delivers genes into the patient's genome in a pathogenic manner. The proteins on the surface of the virus can interact with the receptor sites on the target molecule. This in turn initiates a cellular uptake process which is commonly known as endocytosis.It has been observed that most experiments on gene therapy have utilized viral vectors which consisted of elements of a virus that eventually resulted in a replication-incompetent virus. In the initial stage, immediate or immediate early genes were deleted. These viruses had the potential to recombine to produce a wild –type virus which had the capacity to undergo multiple rounds of replication. They worked by replacing the viral gene(s) with a desired promoter and coding sequence .Accomplished replicating viral vectors were produced utilizing packaging cells which provided deleted viral genes in trans. The protein(s) on the surface of the wild type virus were present in the viral vector particle. Therefore, the cell types infected by these viral vectors remained the same as the wild-type virus from which they were derived. In certain cases, the affinity of a particular virus was modified by the expression of a surface protein from another virus, thereby affecting other cell types. This process in which a protein from another virus is used to alter the tropism for a viral vector is commonly known as pseudotyping. The features of such viruses and their virulence is shown in table 1 and 2

Some of the different types of viral vectors used in gene therapy are

- **Retrovirus** : The first viral vector to be used in gene therapy experiments were the retroviruses. They are a special class of virus which has two positive single stranded RNA genome of 7-10 kb as its genetic material and can create double stranded DNA copies with the enzyme reverse transcriptase. Anotherenzyme known as integrase then integrates the copies of this genome into the chromosome of the host cell, thereby enhancing it to contain a new gene. In case this cell divides, the descendants will certainly contain the new genes. An exclusive feature identified in retrovirus which aided in its initial isolation was the speedy induction of tumors in susceptible animals accomplished by the transfer of cellular oncogenes into cells.It has also been observed that retroviruses can cause delayed malignancy as a result of insertional activation of a downstream oncogene or the inactivation of a tumor suppressor gene. Retroviruses are generally classified into seven distinguished genre based on attributes such as nucleotide structure of envelope, morphology of nucleocapsid, assembly mode of virion and the nucleotide sequence , etc. Retroviruses are about 100 nm in diameter,with a membrane envelope which contains a virus-encoded glycoprotein. This glycoprotein specifies the range of cells that can be infected by binding to a cellular receptor, triggering fusion with a certain cellular membrane on either the cell surface or in an endosomal compartment. For example, "the ecotropic Moloney murine leukemia virus (MLV) receptor is a basic amino acid transporter that is present on murine cells but not cells from other species."
  Even though retroviruses are the most extensively used viral vectors, there are a few problems it poses. First, the integrase enzyme can actually insert genetic material at any random position in the host's genome which might result in insertional mutagenesis or uncontrolled cell division leading to cancer. This is marked by either using zinc finger nuclease or by involving some sequences such as beta globin locus control region to indicate the site of integration to specific chromosome. One of the most lucrative application of gene therapy has been the trial using retroviral vector to treat X- linked severe combined immune deficiency.
- **Adenovirus**: In order to aver the problem of inserting genes at random sites, some researchers have started using adenovirus as vectors. Adenoviruses possess a double stranded DNA genome and is famous for causing intestinal and eye infection (especially common cold).As they infect the host cell, adenoviruses transfer their DNA into the host cell but the genetic material of the adenovirus is not incorporated into the host's genome. Instead the genetic material is left free in the host cell's nucleus and the instructions in this extra DNA molecule are transcribed normally. Adenoviruses also affects a broader spectrum of cells than retrovirus. For instance, adenoviruses can affect lung cells in spite of the fact that they divide slowly. Let us now focus on the disadvantages. First, adenoviruses are more susceptible to the attack of the immune system. Secondly, a huge number of these vectors are required for effective therapy which often induces an unwanted inflammatory response in the patient's body. In spite of such drawbacks, scientists recommend the use of adenovirus in the treatment of liver and ovary cancer. The first product of gene therapy to be given the license to treat head and neck cancer is Gendicine, a p53 based adenoviral product .Questions on how safe the usage of adenovirus is were raised only after the death of Jesse Gelsinger in 1999 while he was participating in a gene therapy trial

- **Adenoaassociated virus**: These are one of the most promising vectors discovered in the recent past. They infect a broad spectrum of cells both dividing and non-dividing. They are small in size and belong to Parvovirus family. They have a genome of single stranded DNA. The peculiarity of this virus is that it can insert genetic material at a specific site on chromosome 19 with absolute certainty. Most researchers are of the opinion that AAV's are not disease causing agents and they do not invoke any allergic response.At present, it has paved a way in the field in the treatment of hemophilia (a hereditary blood disease), eye and muscle diseases. Advanced research has also tested it to transfer genes to the brain because this virus can affect non-dividing cells (example, neurons) in which the genome expression lasts long.In spite of the numerous benefits, there are a few drawbacks. First, its payload is very limited as it is small and carries only two genes in its natural state. Secondly, as the virus delivers genes directly into the DNA of the host cell, the risks of causing unintended damage is very high. Thirdly, there have been very many technical problems that have been faced by the researchers while culturing large quantities of the altered virus but this problem has been solved to a great extent in the recent past by the Amsterdam Molecular Therapeutics (AMT). The recombinant DNA which contains only the therapeutic gene and no viral genome does not integrate , rather fuses at its ends to form circular, episomal forms which are considered to be the primary cause of long term gene expression.
- **Herpes simplex virus [HSV]:**It is a human neurotropic virus. The major area of action of HSV is the nervous system. HSV has a large genome in comparison to the other viruses. This enables researchers to insert more than one therapeutic gene into one virus. The plus point of this virus is that it affects a wide spectrum of tissues and can be used for the treatment of diseases caused by more than one gene defect. It can infect liver, pancreas, nerve and lung cells. It has been studied that the wild type of HSV-1 virus infects neurons that are not shunned by the immune system.

B. Non-viral vectors

Direct DNA transfection is the simplest known non-viral method of gene transfer . Clinical trials performed with naked DNA plasmids have been successful but those done with naked PCR products have had greater success. Several other non-viral methods of gene therapy include (i) electroporation-pores created in plasma membrane as a result of electric field (ii) sonoporation- plasma membrane disrupted due to ultrasonic frequencies (iii) manetofaction- utilizing magnetic particles complexed with DNA (iv)gene guns-under high pressure DNA coated with gold particles are shooted into cells (v) receptor mediated gene transfer. Among all the above mentioned methods, the receptor mediated gene transfer is the most promising. It uses DNA conjugated with either specific viral proteins or liposome or both. DNA conjugated with liposomes have been observed to undergo cellular uptake by endocytosis with subsequent exogenous gene expression under experimental ex-vivo conditions. However there are several drawbacks which need to be catered to such as methods for the stable integration of the endocytosed DNA should be settled. There should be a remarkable improvement in target ability, transfection efficiency and DNA carrying capacity. In recent times, several chemical methods have come into the visible spectrum in order to facilitate the delivery of DNA into the cells. One such method is the use of oligonucleotides to inactivate defective genes using an antisense specific to target gene. Another method refers to the use of lipoplexes that are made up of anionic and neutral lipids. The last one involves the use of polyplexes, a complex of polymers with DNA.Researchers have also devised some hybrid techniques such as vibriosomes that integrate liposomes with an inactivated HIV or influenza virus, combining viral vectors with cationic lipids.

Scientists are thouroughly investigating the scenario so as to successfully insert a 47^{th} artificial human chromosome into the target cells. They claim that this chromosome would autonomously exist by the side of the standard 46^{th} chromosome without influencing its normal functioning in any way. They also assure that this would be a large chromosome capable of holding a large amount of genetic code. The scientists also anticipate that this artificial chromosome will not be attacked by the immune system of the body.

Challenges of gene therapy

It is not a new field and has been evolving rapidly over the past decades. However, this approach is constantly under check because of several technical as well as ethical issues.

A. Technical Issues: With alarmingly rising expectations so as to give a permanent halt to the genetic disorders, it is also essential for us to know the technical difficulties. A major difficulty scientists face is to keep a control on the virus as it may attack cells other than the target cells. It is also tough to introduce foreign genes into the cell of a host organism and keep it working. Several questions have been raised as to whether it is profitable to invest on gene therapy to treat a rare disorder

B. Ethical Issues: Gene testing is an essential step in the detection of defective genes so as to correct them via gene therapy. This is raising several objections on ethical and legal grounds as a section of people find it an invasion of privacy. Prenatal tests would increase the rate of abortions. People being diagnosed with a particular genetic disorder face a lot of psychological stress which is uncalled for. Insurance

coverage would be practically impossible to find. Another major problem is the regulation of gene therapy. Our society is wrapped up in thoughts of youth and beauty. Gene therapy could certainly be monopolized by the cosmetic industry to enhance beauty and radiance or to "turn back the clock".The point is whether beauty, height, skin colour ,or in short the physical attributes of a human is as important as a genetic defect such as cystic fibrosis.

II. Conclusion

The tremendous accomplishment of gene therapy has made me believe firmly that it will certainly prove to be a boon to mankind if utilized wisely. Gene therapy has successfully overshadowed all the disadvantages it has. It can help a child born with a genetic disease lead a normal life. Curing a fatal disease such as cancer or any other genetic defect and gifting a normal life to a person should be enough reason for all the people opposing it to change their minds. Every coin has two sides, it depends on us which side we chose. It cannot be denied that all the ethical challenges stated above are justified but I don't think it is more important than the life of a fellow human being. Gene therapy has the potential to cure the by far perceived incurable or fatal diseases and researchers need humongous appreciation for such a remarkable contribution. This will change the whole dynamics of our society, whether for good or bad that is something that only time can tell us.

Tables and charts

Table 1: Characteristics of viruses that have been used to generate Viral vectors

Virus	Size & type of genome	Viral Proteins	Physical Properties	Disease in Animals
Retrovirus	7-10 kb of single stranded RNA	Gag, Pro, Pol, Env	100 nm diameter ; enveloped	Rapid or slow induction of tumours
Adenovirus	36 kb double stranded linear DNA	Over 25 proteins	70-100 nm in diameter; non enveloped	Cold; conjunctivitis; gastroenteritis
Adneovirus-associated virus	4.7 kb single stranded linear DNA	Rep and Cap	18-26 nm in diameter; non enveloped	No known diseases
Herpes simplex virus 1(HSV–1)	152 kb double stranded linear DNA	Over 81 proteins	110 nm in diameter	Mouth ulcers and genital warts; encephalitis
Vaccinia virus	190 kb double stranded linear DNA	Over 198 open reading frames	350 by 270 nm rectangles ; enveloped	Attenuated virus that was used to vaccinate against small pox
Baculovirus	130 kb double stranded linear DNA	Over 60 proteins	36 kb double stranded linear DNA	None in mammals; insect pathogens

Table 2 : Summary of relative advantages and disadvantages of vectors used for gene therapy

Vector	Infects non dividing cells?	Maximum size of Insert	Stability of Expression	Titer
Retroviral vectors	No(yes for lentiviral vectors)	<=8 kb	Stable (random DNA insertion)	1 x 10^6 cfu/ml unconcentrated; 1 x 10^8 pfu/ml concentrated
Adenovirus	Yes	8 kb for E1/E3 deleted vectors; 35 kb for "gutless" vectors	Expression lost in 3-4 weeks in normal animals; expression can last weeks to months with immunosupppression. No integration.	1 x 10^12 cfu/ml
Adenoassociated virus	Yes	<4.5 kb	Stable, it is unclear if DNA integrates in vivo	1 x 10^6 infectious particles/ml unconcentrated; 1 x 10^10 infectious particles/ml concentrated
Herpes simplex virus (HSV)-1	Yes	>25 kb	Stable , maintained as episome	1 x 10^10 pfu/ml
Vaccinia virus	Yes	>25 kb	Expression transient due to an immune response; replicates in cytoplasm	1 x 10^8 pfu/ml
Baculovirus	Yes	>20 kb	Unstable	1 x 10^10 pfu/ml

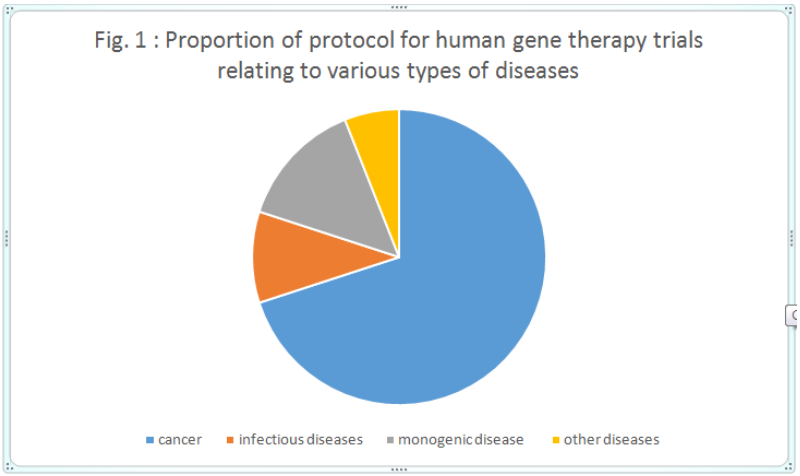

Fig 1: Proportion of Protocol for human gene therapy trials relating to various types of diseases.[1]

References

[1]. Rocholl B. 1996. *Gene Therapy : Revolutionizing Medicine* , available athttp://www.ndsu.edu/pubweb/~mcclean/plsc431/students/brandi.htm [last accessed on 21 April, 2015]

[2]. What is Gene Therapy. *Gene Therapy Net.com* , available at http://www.genetherapynet.com/what-is-gene-therapy.html [last accessed on 21 April , 2014]

[3]. Wang Dan.2014. State-of-the-Art Human Gene Therapy: Part I. Gene Delivery Technologies. *Discovery Medicine*, available at http://www.discoverymedicine.com/Dan-Wang/2014/07/state-of-the-art-human-gene-therapy-part-i-gene-delivery-technologies/[last accessed on 21April, 2015]

[4]. What is Gene Therapy. Learn.Genetics : *Genetic Science Learning Center*, available athttp://learn.genetics.utah.edu/content/genetherapy/gtintro/ [last accessed on 21April, 2015]

[5]. Amer MH. 2014. Gene therapy for cancer: present status and future perspective.Molecular and Cellular Therapies. doi:10.1186/2052-8426-2-27.

[6]. Miller DA. Human gene therapy comes of age. Nature 1992;375:455- 460

[7]. Verma IM, Weitzman MD. *Gene therapy: Twenty-first century medicine*. Annu Rev Biochem 2005;74:711-738

[8]. Misra S. 2013. *Human Gene Therapy : A Brief Overview of the Genetic Revolution*. Journal of the Association of Physicians of India. 61(127) ,available at http://www.japi.org/february_2013/06_ra_human_gene_therapy_a.pdf [last accessed on 21 April, 2015]

[9]. Mountain, A. (2000) Gene therapy: the first decade. Trends in Biotechnology, 18(3), pp. 119-128.

[10]. Evans, C. and Robbins, P. (1995) Current concepts review: possible orthopaedic applications of gene therapy. Journal of Bone and Joint Surgery-A-American Volumes, 77(7), pp. 1103-1115.

[11]. Scherer, L.J. and Rossi, J.J. (2011) Ex vivo gene therapy for HIV-1 treatment. Human Molecular Genetics, 20(R1), pp. R100-R107.

[12]. Tuszynski, M.H., Thal, L., Pay, M., and Salmon, D.P. (2005) A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. Nature Medicine, 11(5), pp. 551-555

[13]. Gopalan B et al .2004a. Ectopic production of MDA-7/IL-24 inhibits invasion and migraton of humnan lung cancer cells. Mol Ther. 9:510-518.

[14]. Friedmann Theodore (1992) A brief history of gene therapy . Nature Genetics 2,93-98. doi:10.1038/ng1092-93

[15]. Mathews QL, Curiel DT.Gene Therapy: Human Gemline Genetics Modifications-Assessing the Scientific,Socioethical, and Religious Issues. Southern Medical Journal 2007;100:98-100

[16]. Bank A. Human Somatic Cell Gene Therapy 1996;18:999-1007

[17]. Gardlik R, Paiffy R, Hodosy J, Lukacs J, Twrna J, Celec P. Vectors and delivery system in gene therapy. Med Sci Monit 2005; 11:110-121

[18]. Giacca M, Zacchigna S. 2012.Virus-mediated gene delivery for human gene therapy. *J Control Release*161(2):377-388

[19]. Kay MA. State-of-the-art gene-based therapies: the road ahead. *Nat Rev Genet* 12(5):316-328, 2011.

[20]. Vannucci L, Lai M, Chiuppesi F, Ceccherini-Nelli L, Pistello M. Viral vectors: a look back and ahead on gene transfer technology. *New Microbiol* 36(1):1-22, 2013.

[21]. Ponder KP. 2001. An Introduction to Molecular Medicine and Gene Therapy http://dasher.wustl.edu/kathy/papers/kresina-4-77-00.pdf

[22]. Rochat T, Morris MA. Viral vector for gene therapy. J Aerosol Med 2002;15:229-235.

[23]. Baun C, Dullman J, Li Z, et al. Side effects of retroviral gene transfer into hematopoietic stem cells. Blood 2003;101:2099-114.

[24]. Durai S, Mani M, Kandavelou K, Wu J, Porteus MH, Chandrasegaran S. Zinc finger nuclease: custom designed molecular scissors for genome engineering of plants and mammalian cells. Nucleic Acid Res 2005;33:5978-90.

[25]. Gasper HB, Thrasher AJ. Gene therapy for severe combined immunodeficiencies. Expert Opin Biol Ther 2005;9:1175-82.

[26]. Vorburger SA, Hunt KK. Adenoviral gene therapy. The Oncologist 2002;7:46-59

[27]. Peng Z. Current status of Gendicine in China:Recombinant Human Ad-p53 Agent for Treatment of Cancers. Human Gene Therapy 2005;16:1016-1027

[28]. Sibbald B. Death but one unintended consequence of gene therapy trial. CMAJ 2001;164:1612

[29]. Varghese S, Robkin DS. Oncolytic herpes simplex virus vector for cancer virotherapy. Cancer Gene Therapy 2002;9:967-78.

[30]. Wolff JA, Malone RW, Williams P, et al. Direct gene transfer into mouse muscle in vivo. Science 1990;247:1465-68

[31]. Wagner E, Curiel D, Cotton M. Delivery of drugs, proteins and genes using transferring as a ligand for receptor-mediated endocytosis. Adv Drug Deliv Rev 1994;14:113-135.

[32]. Curiel DT. Receptor-mediated gene delivery employing adenoviruspolylysine DNA complexes. In Wolff JA (ed). Gene Therapeutics: Methods and Applications of Direct Gene Transfer, Birkhuauser Boston, Cambridge, Mass, 1994:99-117.
[33]. Pierce EA, Liu Q, Igouchera O, Dmarrudin R, Ma H, Diamond SL, et al. Oligonucleotide-directed single base DNA alteration in mouse embryonic stem cells. Gene Therapy 2003;10:24-33.