EDITORIAL

Genes and Gene Therapy

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Nobel Laureate Francis Crick was quoted as saying, "We used to think that our fate was in our stars. Now we know that, in large measure, our fate is in our genes."[1] Until recently, genetic and hereditary disorders had counselling as the sole available management. However, with the advent of modern technology, man is achieving yet another milestone. The structure of deoxyribonucleic acid (DNA) was discovered over 50 years ago.[1] From this, a new technique called gene therapy was discovered in the 1980's.[2] Using genes as "medicine", it is an approach to treat genetic disorders where the faulty gene is fixed, replaced or supplemented with a healthy one.[2] While originally aimed at treating life-threatening diseases (inborn errors, cancers and hematological diseases like anaemias and thalassaemias),[3-5] it is now considered for many non-life-threatening conditions, like acquired tissue damage, immunological disorders and systemic protein deficiency.[2]

Introduction of a correct gene will re-establish the normal functioning by inserting it into a location where it replaces a non-functional gene.[6] Different methods employed are gene restoration, gene augmentation, gene correction and gene inhibition.[6] Gene restoration selectively inhibits the abnormal gene, returning to its function. Gene inhibition uses anti-sense oligo-nucleotides that bind to complementary RNA and inhibit it. Gene augmentation adds a functional copy of the lost gene to replace it. For instance, in erythropoietin-responsive anaemia, gene augmentation is required. Gene augmentation and inhibition are used for vascular proliferative disorders[7] while gene restoration is used for the prevention of lung cancer.[8]

Different techniques being employed are the *in-vivo* and the *ex-vivo* technique. In the *in-vivo* technique, the genes are delivered directly to the skin by injection, electroporation, gene gun and topical application employed in cystic fibrosis (adenoviral vector), carcinomas of the lung and breast and Gaucher's disease (retroviral vector).[4] In contrast, in *ex-vivo* technique, the gene is transferred into the skin outside the body, precisely attacking the target cells (keratinocytes or fibroblasts).[9] Examples of the *ex-vivo* technique are renal cell carcinoma, HIV and malignant melanoma.

Another approach for the treatment of cancer involves suicide gene therapy in which tumour cells are modified by insertion of a suicide gene, such as Herpes simplex virus thymidine kinase gene (HSV-TK).[10] By phosphorylation, the HSV-TK gene converts a pro-drug, such as ganciclovir (GCV) into its active form that disrupts DNA replication, resulting in cell death.[10]

Molecules carrying the therapeutic gene are called a vector. These are viral and non-viral. Viral vectors are of two kinds: Adenoviruses and Retroviruses. Retroviruses having RNA are converted by a 'reverse transcriptase' to DNA which attaches to host DNA by an 'integrase'.[11] They in turn break a growth regulator gene leading to uncontrolled growth and carcinogenesis. Adeno-associated viruses (AAV) are non-pathogenic parvo-viruses having a single stranded DNA. They can infect quiescent cells like neuron, useful for treatment of brain, muscle and eye disease.[12]

Non-viral vectors are safer but have poor transduction capacity.[13] This method has produced stable type VII collagen gene expression in Dystrophic Epidermolysis Bullosa (DEB).[14]

Keratinocytes are the cells of choice for gene therapy in skin and systemic diseases because of their easy accessibility and rich vascularization.[15] The genetically modified regions can be easily monitored and aberrant tissue surgically removed. Apart from keratinocytes, fibroblasts, melanocytes, macrophages and endothelial cells can all be used as target cells.[4] The chief cutaneous disorders where this technique is of the foremost importance are: Epidermolysis Bullosa Simplex (EBS), Junctional EB (JEB) and Dystrophic EB (DEB).[16] Other areas of interest are xeroderma pigmentosum, X-linked lamellar ichthyosis,[17] porphyrias, wounds and squamous cell carcinoma. Newer researches include malignancies like lung cancers, osteosarcoma and lymphomas, Alzheimer's disease, sickle cell anaemia, Thalassemia[6] and melanoma.[18]

The above mentioned uses are a few examples. This technology can enhance the ability of children in learning and performing cognitive tasks, increasing the collective human capital embodied in nations that would multiply the national investments in education, scientific and engineering works.

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Limitations of Gene Therapy:

There are certain limitations to the gene therapy. It is short-lived in nature. The therapeutic DNA must remain functional in the cells containing it. Problems with DNA integration and the rapidly dividing cells prevent long-term benefits, leading to multiple rounds.

The new gene may fail to express itself. Immune stimulation may render the therapy ineffective, making repeat therapy difficult. Conditions arising from mutations in a single gene are the best suited for treatment with gene therapy.

Disorders like heart disease, hypertension, Alzheimer's, arthritis and diabetes are multigene disorders, which are difficult to treat.

Likewise there are certain problems with viral vectors as well.[22] Viruses can cause problems like toxicity, immune and inflammatory responses. The vector may recover its ability to cause disease.

Ethical and legal problems also abound.[23] Many believe it to be an invasion of privacy and believe that if prenatal tests are performed, these could lead to an increased number of abortions. Religious groups and creationists may consider the alteration of genes as tampering or corrupting God's work.[23] Since human experimentation is not allowed, the extent of simulated and/or animal research finding's extrapolation to humans remain a question.

Regulation of what should and should not be included in gene therapy is another issue.

Gene Therapy is an ethical and economic quandary about fairness and fate, vanity and virtues. Just like other innovations, it has its drawbacks. It is up to us to act responsibly to limit the use of this technology for the betterment of mankind. While it is permissible for serious diseases of somatic origin, the prospects of using genetic interventions to improve the basic traits of humans is condemnable. Nevertheless, it has the potential to be the future of medicine and its possibilities must be explored.

It is just the beginning!

REFERENCES

1. Noble Prize. Francis Harry Compton Crick [Biography] [Internet]. [updated 2011 Apr 23]. Available from: http://nobelprize.org/nobel_prizes/medicine/laureates/1962/crick-bio.html
2. Culver KW. Clinical applications of gene therapy for cancer. *Clin Chem* 1994; **40**:510-2.
3. Sarma N. Gene therapy in dermatology. *Indian J Dermatol* 2006; **51**:211-6.
4. Roselli EA, Mezzadra R, Frittoli MC, Maruggi G, Biral E, Mavilio F, *et al.* Correction of ß-thalassemia major by gene transfer in haematopoietic progenitors of pediatric patients. *EMBO Mol Med* 2010; **2**:315-28.
5. Cotrim AP, Baum BJ. Gene therapy: some history, applications, problems and prospects. *Toxicol Pathol* 2008; **36**:97-103.
6. Khavari PA, Rollman O, Vahlquist A. Cutaneous gene transfer for skin and systemic diseases. *J Intern Med* 2002; **252**:1-10.
7. von der Leyen HE, Mann MJ, Dzau VJ. Gene inhibition and gene augmentation for the treatment of vascular proliferative disorders. *Semin Interv Cardiol* 1996; **1**:209-14.
8. Fabbri M, Illiopoulos D, Trapasso F, Aqeilan R I, Cimmino A, Zanesi N, *et al.* WWOX gene restoration prevents lung cancer growth *in vitro* and *in vivo*. *Proc Natl Acad Sci* 2005; **102**:15611-16. Epub 2005 Oct 13.
9. Robbins PB, Khavari PA. Strategies for cutaneous gene therapy. *Curr Prob Dermatol* 2000; **12**:198-203.
10. Lin MT, Pulkkinen L, Uitto J. Cutaneous Gene Therapy: Principles and Prospects. *Dermatol Clin* 2000; **18**:177-88.
11. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P, *et al.* LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 2003; **302**:415-9.
12. YangY, Nunes FA, Berencsi K, Furth EE, Gonczol E, Wilson JM. Cellular immunity to viral antigens limits E1-deleted adenoviruses for gene therapy. *Proc Natl Acad Sci USA* 1994; **91**:4407-11.
13. Chen M, Li W, Fan J, Kasahara N, Woodley D. An efficient gene transduction system for studying gene function in primary human dermal fibroblasts and epidermal keratinocytes. *Clin Exp Dermatol* 2003; **28**:193-9.
14. Mecklenbeck S, Compton SH, Mejia JE, Cervini R, Hovnanian A, Bruckner-Tuderman L, *et al.* A microinjected COL7A1-PAC vector restores synthesis of intact procollagen VII in a dystrophic epidermolysis bullosa keratinocytes cell line. *Hum Gene Ther* 2002; **13**:1655-62.
15. Greenhalgh DA, Rothnagel JA, Roop DR. Epidermis: an attractive target tissue for gene therapy. *J Invest Dermatol* 1994; **103**:63S-69S.
16. D'Alessandro M, Morley SM, Ogden PH, Liovic M, Porter RM, Lane EB. Functional improvement of mutant keratin cells on addition of desmin: An alternative approach to gene therapy for dominant diseases. *Gene Ther* 2004; **11**:1290-5.
17. Choate KA, Kinsella TM, Wiliams ML, Nolan GP, Khavari PA. Transglutaminase 1 delivery to lamellar ichthyosis keratinocytes. *Hum Gene Ther* 1996; **7**:2247-53.
18. Ha SP, Klemen ND, Kinnebrew GH, Brandmaier AG, Marsh J, Hangoc G *et al.* Transplantation of mouse HSCs genetically modified to express a CD4-restricted TCR results in long-term immunity that destroys tumors and initiates spontaneous autoimmunity. *J Clin Invest* 2010; **120**:4273-88.
19. Wikipedia. Gene therapy [Internet]. [updated 2011 Apr 22]. Available from: http://en.wikipedia.org/wiki/Gene_therapy#Problems_and_ethics
20. Ananth N. Gene therapy-potential pros, cons and ethics. *Online J Health Allied Sci* 2002; **2**:1.
21. El-Bayoumi, Abdel Aziz, Khalid A. Gene therapy; the state of the art. Riyadh: *Rabat Islamic Educational, Scientific and Cultural Organiztion*; 2002.