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Gene Therapy in India- Current Status

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ABSTRACT

Gene therapy is being considered as a promising modality for more than two decades now. It has been used for a number of difficult-to-treat conditions and has shown good results in some of the conditions, but not that effective in some others. Overcoming the initially faced hindrances, the research in the field of gene therapy resurged. India is one the major Asian countries where gene therapy-related research and centers have shown remarkable growth, despite certain constraints faced by the researchers. Current article discusses the different types of gene therapy along with its clinical implications and its current status in Indian context.

Keywords: Gene therapy, India, children.

Introduction

Gene therapy is an attempt to treat diseases by replacing defective gene with healthy genes or repairing the defective genes in order to improve the function of genes. World was first introduced to gene therapy in 1995 almost two decades ago when Blaese *et al* published his initial trial results of T lymphocyte directed gene therapy in Adenosine Deaminase (ADA) deficiency associated Severe Combined Immunodeficiency (SCID) (1).

Hindrances to gene therapy started following three deaths which occurred just after clinical initiation of gene therapy. One was due to multiple organ failure as a consequence of severe immune response to the viral vector and other two children died of leukemia (2, 3). These deaths raised issues of ethical concern following gene therapy and lead to complete halt of the trials all over the world. After the initial set back, interest later renewed almost a decade later in the year 2008, when gene therapy restored vision in three young children suffering from Leber's congenital amaurosis (4). After these successful results of gene therapy, United States of America and Europe became the pioneers in gene therapy-related studies and clinical trials. In Asian continent, China and Japan have emerged as the forerunners in the stem cell research, with China launching Gendicine, an injectable gene therapy product (a replication-incompetent recombinant human p53 wild type protein particles combined with adenovirus serotype 5) approved by the China Food and Drug Administration (CFDA) in 2003 for head and neck cancer. Japan has introduced Retronectin reagent, which is a recombinant human Fibronectin Fragment and on injection, enhances retroviral (as well as lentivirus, a form of retrovirus)-mediated gene transfection and transduction by helping the co-localization of target cell and virions. The reagent can enhance

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retroviral-mediated gene transfer to target cells that express integrin receptors VLA-4 and/or VLA-5, thus making the gene delivery more specific as the gene will not be delivered to body cells which lack VLA-4 and/or VLA-5 integrin receptors.

Types of Gene Therapy

It becomes important to understand some of the terminologies and the types that are often used in relation to gene therapy. Alternative terms like genetic engineering, Deoxyribonucleic Acid (DNA)-based therapy and molecular therapy are often used by layman and biologists. Gene therapy is one of the tool of genetic engineering used with purpose to alleviate suffering from hereditary diseases. However, genetic engineering in wider term not only aims to alter genes to correct genetic defects but may also be involved in modifying the genes to enhance the capabilities of the organism beyond what is normal. The latter is a dangerous proposition of genetic engineering.

A. Gene therapy can be classified into somatic cell and germ cell types, depending upon the type of cells that are modified by the therapeutic genes (6, 7). All the gene therapies till date are directed towards somatic cells only.

Somatic Cell Gene Therapy

In this type, genetic changes are directed towards somatic cells. As these cells are non-reproductive, effect is not passed into future generations, making it safer. The disadvantage is short duration of effects of somatic cell therapy as most tissues will be replaced by new tissues.

Germ Cell Gene Therapy

This is the type of gene therapy, where germ cells, i.e. either sperm or ova are introduced with therapeutic gene, leading to the changes that are inheritable, i.e. changes in gene may affect future generations.

B. Based upon the technique of delivery of vectors to the target cell, gene therapy can be further classified into ex-vivo and in-vivo therapy.

Ex-vivo Gene Therapy

Ex-vivo gene therapy is where the defected cells are extracted out of the body and targeted with therapeutic gene. Once successfully modified, they are cultured ex-vivo and transferred back to the host, where now the corrected gene replicates.

In-vivo Gene Therapy

In this modality, a vector that is capable of carrying the therapeutic gene, is used to inject host cells with normal gene.

C. The type of change brought out in the faulty gene classifies gene therapy as either gene replacement or gene addition.

Gene Replacement

Gene replacement means replacement of defective gene with a corrected one.

Gene Addition Therapy

Gene addition means restoration of normal function of cell by addition of normal or functional copy of gene into genome. This concept is primarily used in various gene therapy related research on cancer.

Clinical Implications

Cancer

Gene therapy-related research and its clinical application have been mostly utilized in the field of malignancy. By the end of 2009, nearly two third of gene therapy-related research was concentrated on cancers (8). Oncolytic viruses are used to introduce genes into malignant cells, thereby causing death of

malignant cells. Another approach is to deliver p53 gene (tumor suppressor gene) and thereby induce oncolysis. Gendicine that was first approved anticancer drug which was based on this gene therapy principle. Suicide gene therapy is another attempt to treat tumor by delivering of gene coding for enzyme that metabolizes prodrugs into locally active chemotherapeutic drug moiety.

Single Gene Disorder

Gene therapy has a significant role in the treatment of single gene disorders like muscular dystrophies, cystic fibrosis, alpha-1-antitrypsin deficiency, Huntington's disease, lysosomal storage diseases, chronic granulomatous disease, ornithine transcarbamylase deficiency, junctional epidermolysis bullosa, haemophilia, etc. (8).

Immunodeficiency

Over the years with development of gene therapy first major progression has been seen since the first trial in early nineties. After the initial set back where two patients treated for X-linked severe combined immunodeficiency (X-SCID) using retroviral vectors died with leukemia there were clinical trials that had showed clear therapeutic benefits of gene therapy in treatment of both X-SCID and SCID caused by adenosine deaminase (ADA) deficiency. Besides primary immunodeficiency, secondary immunodeficiency states like Human Immunodeficiency Virus (HIV) infection has also evolved as potential candidate for gene therapy. Transgenes can be transferred into haematopoietic stem cells or into T-cells, for specific protection against HIV infection to these cells. They act by disabling HIV-1 protein, or making the milieu unsuitable for HIV-1 replication (8).

Eye Diseases

It was for Leber's congenital amaurosis that there was renewal of faith in gene therapy after the initial set back seen in SCID. Eye being a small organ, hence it is possible that we can transfect a large number of ocular cells. Potential ophthalmologic conditions for gene therapy are glaucoma, Leber's hereditary optic neuropathy, red-green colour blindness and macular degeneration (9). A phase I study is going on to show effects of antiangiogenic cytokine Pigment Epithelium-derived Factor (PEDF) in treating age-related macular degeneration (9). Mancuso *et al* has also shown significant improvement in producing trichromatic colour vision in adult red-green colour blind monkeys by subretinal injection of adeno-associated virus containing a L-opsin gene (10).

Cardiac Diseases

Cardiac diseases are multigenic in origin, hence difficult to treat. There have been trials where scientists have devised techniques to deliver genes for various growth factors like vascular endothelial growth factors (VEGF), Fibroblast Growth Factors (FGF) to promote vascular angiogenesis (11). Though their results did not show significant improvement in stress-induced myocardial perfusion but improved regional wall motion indicated a favorable anti-ischemic effect encouraging further research in the field.

Central Nervous System (CNS) Disorders

Unlike cardiac, in neurological disorders gene therapy has shown promising results to treat Parkinsonism (12) and Alzheimer's disease (13). There have been several trials on gene therapy in Parkinsonism which are still in phase 1 and phase 2 but are showing gene therapy to be safe, tolerable and potential candidate for in-vivo studies (12). Various approaches used are, transmitting the gene for glutamic acid decarboxylase into the subthalamic nucleus (12) or delivery of the gene for neurturin in putamen cell bodies (14). Similarly in Alzheimer's disease gene therapy is being attempted to deliver Nerve Growth Factor gene into the human CNS (13).

Intrauterine Gene Therapy

Prenatal gene therapy or otherwise known as intrauterine gene therapy to resolve the problems of various genetically transmitted diseases is the future of gene therapy. If successful, we can diagnose and treat certain genetic disorders before they manifest in a child. Animal studies have shown some success in the field (15).

Difficulties with Somatic Cell Gene Therapy

Multiple rounds of gene therapy are required due to its short lived nature depending upon the turn-over rate of cells replication. The rapidly dividing nature of many cells prevent the gene therapy from achieving long-term benefits. The therapeutic DNA that is introduced into the target cells must remain functional and stable for long duration. Gene therapy is particularly effective in single gene disorders, hence difficult to apply in multigenic disorders. However, Thalassemia and haemoglobinopathies, though amenable to gene therapy present technical challenges in gene regulation.

The other problem faced is with the mode and the type of vectors used for the gene delivery. Initially viral vectors were used to deliver gene but the problems of endogenous virus recombination, oncogenic effects and most importantly unexpected immune response as seen in the very first case of gene therapy remain the concerns (2).

Ongoing Research in the Field of Gene Therapy

Introduction of New Vectors

Various non-viral vectors that are presently being given consideration for gene therapy are naked DNA, oligonucleotides, lipoplexes and polyplexes dendrimers, etc. The advantages these vectors hold over viral vectors is low immunogenicity, rapid turnover and low toxicity. Most of these vectors are still in experimental stage and we are far from development of a perfect vector of gene therapy. Non-viral vectors can further be classified into those limited to in-vitro applications like calcium phosphate transfection which is the system of choice for transmitting plasmid DNA into variety of cell cultures. Another type of non-viral vectors are also there which have both in-vivo and in-vitro applications like cationic liposomes, etc.

Gene Therapy in India

In India though interest in gene therapy took some time but with financial assistance provided by different government agencies, the country has shown rapid improvement in gene therapy-related research placing India third among the major Asian countries having gene therapy laboratories (16). The main aim is to develop new institutions for gene therapy research, strengthening of existing institutions which have good expertise in this area in order to initiate work in molecular genetics for decreasing the burden of genetic disorders in the country. The pioneer of gene therapy-related research in India is Advanced Centre for Treatment, Research and Education for Cancer (ACTREC) where active work on gene therapy for head and neck cancer using synthetic vectors is being carried out (17). It is heartening to note that scientists in over dozen of labs in India are working hard with small steps in contributing towards gene therapy work as depicted in Table 1 (18-24). Hareendran *et al* (18) suggested that targeting specific host cellular proteins is helpful to attenuate the immune barriers which are a key obstacle in clinical application of adeno-associated virus mediated gene therapy. An alternate approach for treating Haemophilia; a using allogenic transplantation in liver where tolerance against donor antigens can be induced by in-vitro allo-antigen primed T-regulatory (Treg) cells has been studied by Kochat and her team. Shetty *et al* (19) have shown that naïve stemness of pluripotent cells can be generated by devising a transgenic method to express a human ortholog of protein Asrij, present on mouse

Table I: Indian Scientists work on Gene Therapy

Investigators	Study	Year (ref)	State
Kochat *et al*	Repression of PARP-1, a DNA damage response protein, improves the transduction of single-stranded AAV vectors both in vitro and in vivo in mice. Findings will help Hemophilia B patents.	2016 (18)	Tamil Nadu
Hareendran *et al*	Examined the role of donor major histocompatibility complex (MHC)-stimulated host CD4(+)CD25(+) regulatory T (Treg) cells in suppressing immune responses against allogeneic uncommitted (Lin(-)) bone marrow cells (BMCs) for correction of bleeding disorder in HA mice.	2015 (17)	Delhi
Shetty and Inamdar	Ectopically expressed Asrij in epiblast stage equivalent-human embryonic stem cells (hESCs) to test for induction of naive pluripotency in primed pluripotent cells. The construct pCAG-Asrij was introduced into hESCs by microporation. Ectopic expression of Asrij in BJNhem20 hESC line was performed by selecting for plasmid transfection, followed by stable cell line generation.	2016 (19)	Bangalore
Misra *et al*	Liposomal transfection mediated gene transfer for tumors expressing Sigma receptors.	2016 (20)	Bangalore
Vij *et al*	They reported an amphipathic peptide Mgpe9 that can penetrate the uncompromised skin, enter skin cells and deliver plasmid DNA efficiently as nano-complexes *in vitro* and *in vivo* without any additional physical or chemical interventions prevalent currently leading to efficient gene expression up to the highly proliferating basal layer of the skin without observable adverse reactions or toxic effects.	2016 (21)	Delhi
Hati Boruah *et al*	Knockdown of myostatin gene (MSTN), transforming growth factor-β superfamily, and a negative regulator of the skeletal muscle growth, by RNA interference (RNAi), has been reported to increase muscle mass in mammals.This could provide an alternative strategy of gene knockout and develop stable caprinefetal fibroblast cells. Furthermore, these stable cells can be used as a cell donor for the development of transgenic cloned embryos by somatic cell nuclear transfer (SCNT) technique.	2016 (22)	Madhya Pradesh
Kumar *et al*	siRNA could be used in cancer therapy if naked nucleic acid could be transported using a suitable carrier. The authors developed a nano-carrier system using mesoporouspolycaprolactone (hmPCL) and showed its efficacy in knocking down cancer cells. This approach may open another way of gene therapy.	2016 (23)	Telengana
Sarkar *et al*	A new Cancer Terminator Virus (CTV), Ad.tCCN1-CTV-m7 was developed which displayed dose-dependent killing of Carcinoma Prostate (CaP) without harming normal prostate epithelial cells *in vitro* with significant anti-cancer activity *in vivo* in both nude mouse CaPxenograft and transgenic Hi-Myc mice (using ultrasound-targeted microbubble (MB)-destruction, UTMD, with decorated MBs).	2015 (24)	Orissa

embryonic stem cells (mESCs) which is essential for maintaining pluripotency. Misra *et al* (20) are working on selective gene transfection as a possible strategy of interest for reducing off-target gene expression and toxicity. Vij *et al* (21) have used nucleic acid therapeutics as an effective topical delivery system to overcome the barrier posed by different layers of the skin in cutaneous disorders. Hati Boruah *et al* (22) showed Ribonucleic Acid interference (RNAi) based co-transfection method could provide an alternate route of gene knockout besides providing stable cells which can be used as a cell donor for the development of transgenic cloned embryos by somatic cell nuclear transfer technique. Alternative and efficient nucleic acid transportation has been demonstrated by Kumar *et al* (23). Sarkar *et al* (24) have studied therapeutic efficacy of combining a BH3 mimetic with a novel Cancer Terminator Virus for treating advanced carcinoma prostate.

Obstacles in Growth of Gene Therapy in India

After the initial work in late nineties, gene therapy remained at backseat for a long time as the government was uncertain whether to give priority to this technology. There is absence of the regulatory framework with inadequate exposure level of regulators who are still not updated with the international standards. Prohibitive cost involved in preparing reagents requiring cyclic guanosine monophosphate (cGMP) conditions is another major hurdle. As a result, researchers in India do not have the freedom to take risk in order to develop technology to save several lives. No established Indian guidelines are available on the preparation of clinical grade reagents for clinical trials. Centralized resources for production and distribution of clinical grade gene vectors are lacking. Laboratories or pharmaceutical companies catering to gene services are meager in number, further adding to the difficulties faced by the researchers. Most of gene therapy research is still lab based, preclinical and mostly limited to cancers.

Road Ahead

Despite the above road blocks, India is fast picking up with the rest of the world in developing research related to gene therapy. Adding impetus to further research in India is the release of revised "National Ethical Guidelines for Biomedical and Health Research Involving Human Participants, 2017" and the National Guidelines for Stem Cell Research, 2017 in October, 2017 by the Indian Council of Medical Research (ICMR). These guidelines would encourage research for somatic cell gene therapy for conditions for which it is the only therapeutic option available with due permission from Department of Biotechnology (DBT) for gene constructs (25).

Gene therapy has been theoretically very sound, but its utility will be demonstrated once it comes into clinical practice. Now with the “Make in India” being a popular mantra encouraged by the Govt. of India, we should not shy out from the active involvement in gene therapy research and trials. More funding for academic research, development of dedicated departments with scope for capacity building and training, integration between researchers and clinicians, increasing public awareness and finally and most importantly with the development of Indian guidelines for gene and cell therapy clinical research trials by the ICMR will help in the transition of gene therapy from infancy to adolescence.

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