GENE THERAPY – APPROACHES TO TREAT CANCER

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ABSTRACT

Gene therapy is a medical/surgical intervention in which functional gene is introduced or defective gene is replaced in a cell of an organism suffering from an acquired or a genetic disease so as to treat or cure a wide variety of diseases. It is a pharmaceutical therapy with DNA as a drug molecule to be targeted to a specific site. In other words, gene therapy is the correction of a disease by genetic manipulation. For successful delivery of DNA to host cells, either virus is used as a vector or DNA material is inserted directly into the cells. There are various ways to treat cancer like induction of apoptosis, blocking angiogenesis, suicidal genes, DNA vaccination etc. The medical potential is promising, however, it is the task of society to ensure that this promising technology is used profitably and not misused.

KEY WORDS: Gene therapy, cancer, apoptosis

INTRODUCTION

It is now widely accepted that cancer has a genetic origin. Cancer may be the result of DNA damage due to carcinogens or spontaneously during DNA replication. Inability to correct the DNA damage due to mutated DNA repair genes or absence of functional cell cycle checkpoint genes may give the cell a growth advantage. It has been seen that normal human development and physiologic homeostasis depends on the co-ordinate interactions of the products of many genes working together in the body. Mutations of genes result in the failure to synthesize a particular protein or to the synthesis of a defective protein. These result in abnormalities in genes resulting various genetic disorders.

Recent scientific advances have provided a map of the human genome along with a better understanding of the processes that transform healthy cells into diseased cells. This has led to the emergence of a new class of drugs called targeted therapies.[1] The goal of targeted therapy for cancer treatment is to selectively treat cancer cells without harming healthy tissue by acting on pathways that are unique to cancer cells.[2] They achieve their specificity by targeting differences in cancer cells on a molecular level. It also referred to as molecular-targeted drugs or molecularly-targeted therapies [3].cancer is a genetic disease involving multiple and sequential genetic changes that affect oncogenes, tumoursuppressor genes and modifier genes. In addition, there is interplay of various cells in the body which are important in immune surveillance, responsible for removing abnormal cells from the body. The three conventional modalities of treatment of

cancer – surgery, radiotherapy and chemotherapy are often unsuccessful in treating cancer.[4, 5, 6] Gene therapy is the emerging fourth modality for treatment of cancer. It can be used either alone or as an adjuvant to other treatment modalities. The aim of gene therapy is to introduce a defined DNA sequence or functional genetic material into specific cells, to replace or supplement the activity of a defective gene, or to impart a new function to the cell in order to induce it to secrete a protein that has a putative therapeutic function[7, 8]. Thus Gene therapy is a therapeutic approach that is designed to correct specific molecular defects that contribute to the cause or progression of cancer.[9]

Gene therapy can be targeted to somatic (body) or germ (egg and sperm) cells. In somatic gene therapy, the recipient's genome is changed, but the change is not passed along to the next generation. In germline gene therapy, the parent's egg and sperm cells are changed with the goal of passing on the changes to their offspring. [10]

There are two main approaches to gene therapy [10, 11.] It may be helpful to review some facts about human genes and gene delivery techniques.

1. Ex-vivo approach where the target gene is taken out from the body and transgene is introduced into the cell and the cell is reimplanted into the human body. This approach is only applicable to the cells that are capable of reimplantation inside the human body e.g. lymphocytes, fibroblasts, myoblasts, umbilical cord blood, stem-cells, bone marrow cells, hepatocytes etc.
2. In-vivo approach where the transgene is introduced into the target cell inside the body.

Gene transfer is the process of introducing a foreign gene into a cancer cell or the tissue surrounding it.[12, 13] Vectors are required to deliver the gene to the cancer cells. Viral vectors that are replication incompetent are most often used to introduce the foreign gene. Of these, adenovirus is used most frequently. Methods are still being developed to overcome some of the challenges that have been identified in developing gene transfer therapies. Some of these challenges include:

1. Delivering the gene to cancer cells and not to healthy tissues.
2. Delivering the gene to the correct segment of the DNA
3. Of the gene in amounts sufficient to elicit appropriate responses

An ideal vector should deliver gene to a specific cell type, accommodate foreign genes of sufficient size, and achieve the level and duration of transgenic expression sufficient to correct the defect and be non-immunogenic and safe[14.] Presently there are three techniques for delivery genes.

Physical: Parental injections, micro-injections, aerosol, electro oration (high voltage current is passed to the target cell to produce pores on the cell surface through which transgene enters the cell) and gene guns[15.]

Chemical: Calcium phosphate, DEAE-dextran, liposomes and lipoplexes (for oral delivery of gene), surfactants and perflurochemical liquids for aerosol delivery of gene[15.]

Viral vectors: These are more promising system of gene delivery with various advantages over physical and chemical method:-

1. Gene transfer is more efficient and specific than physical and chemical method.
2. Multiple and repeated doses are required in case of physical and chemical method, whereas in case of viral vector even a single dose is sufficient.

Strategies for gene therapy for cancer

Knowledge of molecular mechanisms governing malignant transformation brings new opportunities for therapeutic intervention against cancer using novel approaches.

Induction of Apoptosis

Because apoptosis is a gene-controlled process, it is susceptible to genetic manipulation with therapeutic purposes. Several features make apoptotic genes and proteins attractive targets for cancer treatment. First, the growing knowledge on the apoptotic machinery certainly provides many theoretical opportunities to manipulate pathways leading to an increased tumor cell death.[16] Second, recent technological developments enable approaches that allow the genetic and phenotypic modification of cancer cells, and several genetic alterations have been found to be cancer cell-specific, which may allow them to specifically target a tumor cell[17.]

The tumor suppressor TP53 is one of the most frequently altered genes in human cancer.[18] Gene therapy encodes the p53 protein that exerts suppression of proliferate activity of cell through multi protein regulatory pathways of many different target genes and through protein–protein interactions[19] and thereby inducing apoptosis in a subset of cells[20]. P53 functioning as an inhibitor of cell proliferation, it inhibits cell growth in most normal and malignant cells [20, 21] (Fig.1)

The p53 protein is maintained at low levels in cells because of its active degradation by the proteasome mediated by the E3 ubiquitin ligase. Various forms of stress, in particular genotoxic events, stabilize p53 through post-transcriptional modifications that allow p53 to escape degradation. Once stabilized, p53 regulates the expression of many target genes involved in cell cycle control, apoptosis and DNA repair either by direct or indirect transcriptional activation or by repression. Loss of p53 function in cells leads to uncontrolled proliferation and promotes cancer development.[22] In human cancers, p53 is frequently altered by mutation of the gene, which results in the expression of a mutated protein that differs from the wild type by a single amino-acid change.TP53 gene mutations have been found in almost all types of cancers at various frequencies and are very diverse in their nature.[23]

Activation of pro –apoptotic factors like Bax, Bak and Bad as well as inhibition of Bcl-2 family (B Cell Lymphocyte) cause release of cytochrome C from mitochondria. Release of various caspases (Caspase-9 and caspase-3) will lead to apoptosis of cells. (Fig. 2)

Blocking angiogenesis

One prerequisite for tumor formation is neovascularization (i.e. angiogenesis) of

the tumor.[24] Reduced oxygen levels and certain cellular factors up regulate the production of vascularization stimulating factors and their receptors (such as VEGF/VEGF-R) in cancer cells [25], which makes this molecule attractive target for gene therapy. By means of gene therapy, the expression or function of angiogenetic proteins can be inhibited[26,] or anti-angiogenetic proteins (Such as angiostatin and endostatin) can be introduced to the cancer cell[27.]

Immunomodulation by gene therapy

Cancer patients generally have lowered immune response, which can be augmented by gene therapy. It is now possible to genetically alter immune cells to increase their function. Therapeutic genes can be introduced *ex-vivo* either into the tumor cells or into the effector cells such as T lymphocytes or antigen presenting dendritic cells, or even to proximal or distant organ sites in the patient.[28] such a strategy can be used in combination with other strategies or even with any conventional modality of treatment.

Suicidal genes

Instead of correcting mutated genes or suppressing active oncogenes, a more widely utilized approach has been to introduce exogenous therapeutic gene in to tumor cells. The inserted therapeutic genes can be immunotherapeutic, anti-angiogenetic or chemoprotective, hence called suicidal genes. A particularly interesting strategy with promising efficacy is the prodrug gene therapy or suicide gene therapy. This involves the delivery of a 'suicide' vector to tumor cells. This vector is constructed by coupling a promoter region of a gene expressed in cancer cells with the suicide gene. These suicide genes encode certain enzymes that can convert a relatively nontoxic prodrug into a cytotoxic agent. Cells that are genetically transduced to express such genes essentially commit metabolic suicide in the presence of the appropriate prodrug. Examples of suicidal genes are Herpes Simplex Thymidine Kinase (HSVTK) and cytosine deaminase. Cytosine deaminase is found in fungi and bacteria which convert the antifungal drug flucytosine to an effective anticancer agent, fluorouracil[29.]

DNA vaccination

Immune responses are sufficient to protect animals from a wide variety of live infectious agents, leading to a new class of therapeutic modality called 'DNA vaccines' [30]. It is a new strategy of immunization where genes coding for tumor-specific antigens are injected intramuscularly as naked plasmid DNA where they lodge and commence synthesizing the protein.[31] The tumor protein has been shown to stimulate both antibody-mediated response as well as cytotoxic T lymphocyte response. Genes coding for cytokines have also been used to enhance the immune response against the tumor cells. Encapsulation of the DNA vaccine into biodegradable polymer micro spheres ensures long-term release of the vaccine eliminating the need for subsequent boosters.

Targeting genetic lesions in tumour cells

Antisense molecules are synthetic oligodeoxynucleotides (ODN), which are designed such that they can hybridize specifically to the coding (sense) mRNA inside the cell[31, 32]. The double stranded

RNA cannot be translated and is easily destroyed. *In vivo*, the ODNs can be injected systemically into the patient's body. However, one of the problems is that the ODNs are easily destroyed by the nucleases in the blood. In order to make ODNs stable one of the most common modification of ODNs is replacing the nonbridging oxygen atoms in each of the inter-nucleotide phosphate linkages with sulphur atom[33]. This makes the ODN stable against nucleases, easily soluble in water and simple and inexpensive to synthesize.

CONCLUSION

Gene therapy is at its infancy at present. The methodology is highly specialized, expensive and can only be used in well equipped, specialized medical centers. It is now clear that gene therapy will have a major impact on patient care especially when vectors are developed that can safely and efficiently be injected directly into the patients as drugs. As more and more information from the Human Genome Project becomes available concerning the entire library of genetic information in our cells, the therapy will be utilized not only to cure an array of diseases but also to prevent many disorders. The medical potential is promising, however, it is the task of society to ensure that this promising technology is used profitably and not misused.

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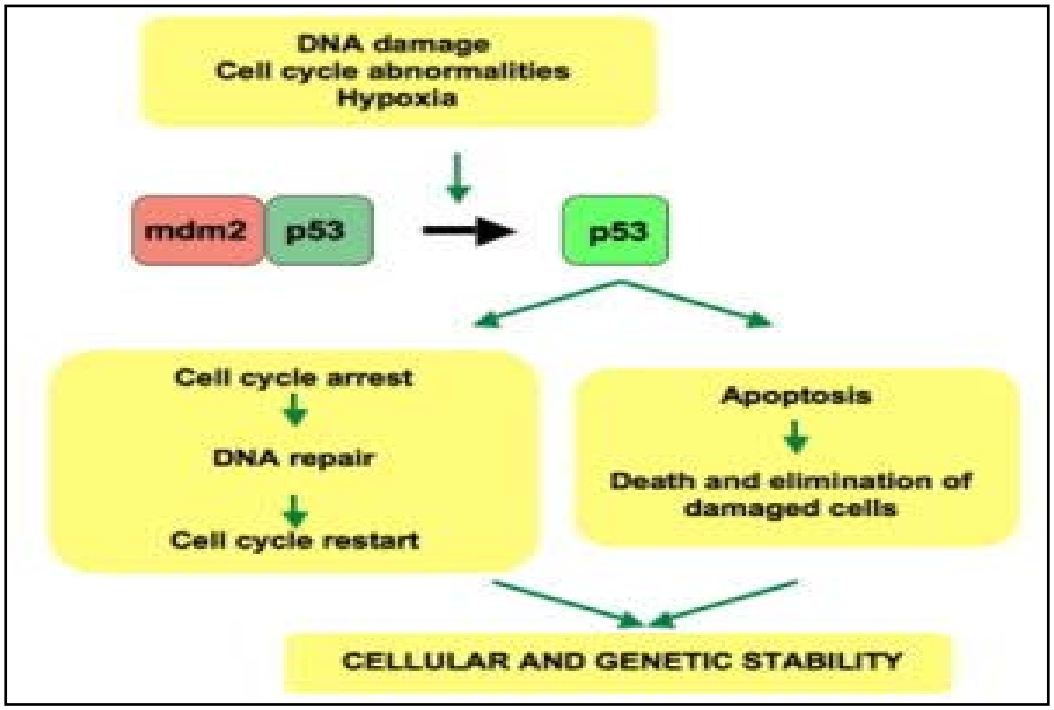

Fig 1- Role of p53 in cellular function

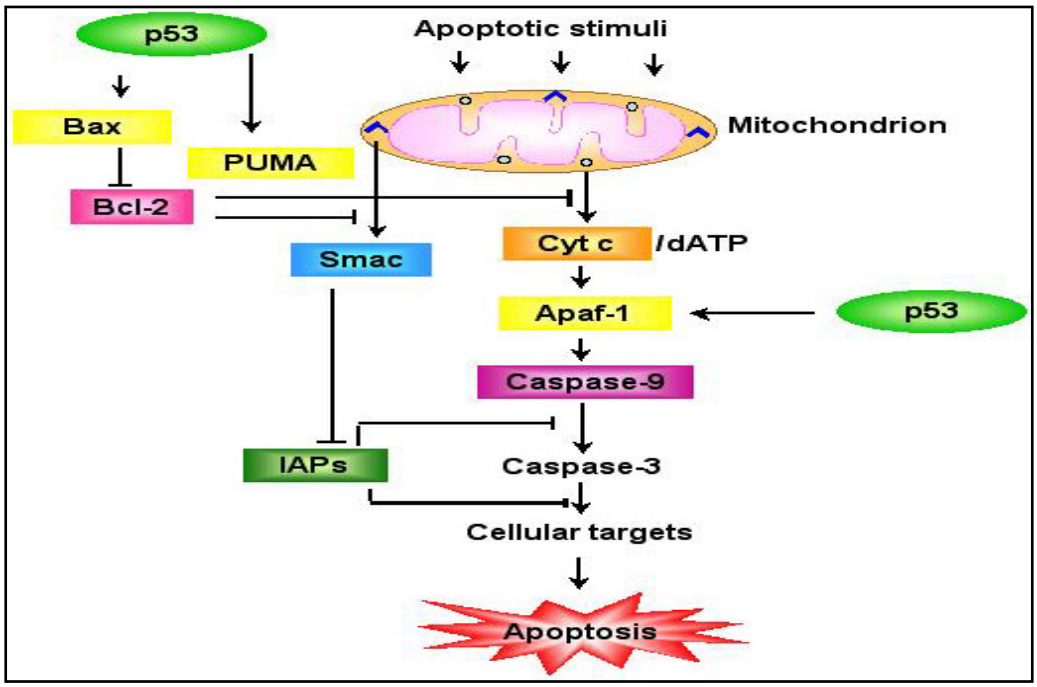

Fig 2- Role of p53 leading to apoptosis

Table 1- Strategies being used in gene therapy for cancer in either preclinical or clinical trials[27]

Strategy for gene therapy	Targeted gene/Therapeutic gene	Type of Cancer
Antisense molecules	K-ras	Small cell lung carcinoma (SCLC)
	bcr-abl	CML
	bcl2	Non-hodgkins lymphoma
Augmentation of immune response	Co-stimulatory HLA-B7 CD-80	Head/neck cancer, childhood acute lymphoblastic leukemia
Immunomodulation	Cytokine genes such as IL-2, Il-4, IL-12, GM-CSF	Various Solid tumors
Prodrug activation	Herpes simplex thymidine kinase gene	Brain tumor, overian cancer, liver Cancer, Head/neck cancer, breast cancer
	Cytosine deaminase	Head/Neck cancer, hepatocellular carcinoma
Induction of apoptosis	p53	head/neck cancer, ovarian carcinoma, brain tumor, non-SCLS
	BAX	
	hREC2	
	Caspase-8	
DNA vaccines	IL-12, IFN-γ	
Inhibition of angiogenesis	Angiostatin, Endostatin	Solid tumors