Gene therapy for cancer

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The structure of DNA was unraveled by Watson and Crick in 1953, and two decades later Arber, Nathans and Smith discovered DNA restriction enzymes which led to the rapid growth in the field of recombinant DNA technology. From expressing cloned genes in bacteria to expressing foreign DNA in transgenic animals, DNA is now slated to be used as a therapeutic agent to replace defective genes in patients suffering from genetic disorders or to kill tumour cells in cancer patients. Thus a new modality of treatment has emerged based on recombinant DNA technology which is termed as gene therapy. Although single gene defects are more amenable to gene therapy, majority of the ongoing clinical trials are for treatment of cancer. Various strategies are now being tested in clinical trials in gene therapy for cancer.

Gene therapy represents a fundamentally new way to treat a disease. The use of genetic material (genes), which can express a protein in the cell or interfere with the synthesis of a protein in the cell, in order to treat a disease, is gene therapy. Replacing a defective gene with a normal gene and thus restoring the lost gene function in the patient’s body is the essence of gene therapy. Originally conceived as an approach to treat autosomal recessive Mendelian disorders, it is now being applied to a broad range of acquired conditions such as cancers, infections and degenerative disorders. Due to safety, legal and ethical issues, gene transfer in humans is presently permitted only in somatic cells and not in germ cells.

It is now widely accepted that cancer has a genetic origin. With the understanding of the genetic basis of cancer, an entirely new approach to the treatment of cancer using gene transfer techniques has evolved. 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 check-point genes may give the cell a growth advantage. These mutated genes or other downstream genes are thought to be good targets for gene therapy. More than half of all ongoing clinical trials for gene therapy aim at cancer.

Gene transfer techniques

Success of gene therapy lies in efficient gene transfer into the cell. The gene (cDNA) is generally cloned into a vector to be able to deposit the foreign gene into the target cell. Selection of the right vector is crucial to gene therapy. An ideal vector should be able to protect and deliver DNA easily across the cell membrane into the nucleus, should have the ability to regulate expression of the gene of interest and minimize toxicity by targeting gene delivery to specific cells. It should be easy and inexpensive to produce in large quantities. Once the therapeutic gene is cloned into a vector with appropriate regulatory sequences (promoter/enhancer), it is introduced into the target cells. The genes can be delivered either ex vivo – where cells from a selected tissue of the patient are removed, exposed to the gene-transfer vector, selected for the transgene using markers, and then the genetically corrected cells are reintroduced into the patient’s body; or in vivo where the vector DNA is injected directly into the body, generally into the tissue to be treated.

Physical and chemical methods of gene transfer

Various methods have evolved in the past few years to transfer genes to the target cells. Physical methods such as (a) microinjection of DNA into the cells or (b) electroporation, although very efficient, have their drawbacks in delivering genes in vivo. Also they are expensive as they involve use of specialized instruments. Chemical methods such as (a) calcium phosphate precipitation, where DNA in trapped in a fine precipitate which is endocytosed by the cell, or (b) DNA bound to the positively charged molecules such as DEAE-dextran or polybrene which then bind to the negatively charged cell membrane, are commonly used in the laboratories. DNA encapsulated in synthetic cationic lipid vesicles which fuse with the cell membrane and release DNA into the cell are being used in a number of gene therapy trials. Cationic liposome-mediated gene transfer is a safe and effective means of delivering genes directly into tumours. This approach prevents undesirable side effects.

Viral vectors for gene transfer

The most efficient method of gene transfer, so far, is by means of viral vectors. Viruses have evolved over the
years to enter the cell and efficiently usurp the cellular machinery to make its own viral proteins. Some of the most commonly used viral vectors are retroviruses and adenoviruses. Viruses, when used as vectors, are generally disabled such that they are unable to replicate on their own. However, recently, replication competent viruses as gene therapy vectors are also being tested.

Retroviruses, which are RNA viruses, integrate their genome into the host DNA as a provirus and then replicate to make multiple copies of infective particles, which are released outside the cell. The most commonly used retroviral vector is the mouse moloney murine leukemia virus which is made replication-deficient by replacing the structural genes – gag, pol and env, with the therapeutic gene. The gag, pol and env genes are supplied in trans by either a helper virus or a packaging cell line expressing the proteins, in order to package the virus and make infective viral particles. Retroviral vectors infect dividing cells and integrate the therapeutic gene into the target cells.

Adenovirus which is the common DNA virus found during infection in the upper respiratory tract epithelium, infects all cells with a high affinity, but does not integrate its DNA into the host genome. Adenoviruses are made replication incompetent by deletion of all or part of their E1A and E1B regulatory genes. Viruses, although the most efficient gene transfer vectors in use today, have certain disadvantages. Immunogenicity of viral vectors resulting in decreased effectiveness during repeated treatments in vivo is one of the major disadvantages. The future of gene therapy lies in improved and safe viral vectors for gene delivery.

**Strategies for gene therapy for cancer**

Cancer is a genetic disease involving multiple and sequential genetic changes that affect oncogenes, tumour-suppressor genes and modifier genes. Epigenetic changes such as methylation of CpG clusters also play a major role in regulating expression of tumour-suppressor genes or modifier genes contributing to malignant transformation. In addition, there is an 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. 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. Certain genes can sensitize tumour cells to radiation or drugs and hence can be used to enhance the effect of the treatment. Gene therapy can also be used to debulk tumours which can then be removed by surgery. Various approaches are being examined in clinical trials for gene therapy for cancer, some of which are discussed here.

**Targeting genetic lesions in tumour cells**

Approaches targeted specifically to the genetic lesions have been employed in the preclinical studies as well as in the clinical trials. In order to target genetic lesions in the tumour cell, antisense molecules have been widely used. Antisense molecules are synthetic oligodeoxynucleotides (ODN) which are designed such that they can hybridize specifically to the coding (sense) mRNA inside the cell. Targeting mRNA with ODNs is attractive as they form Watson–Crick base pairs with the targeted mRNA. 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 non-bridging oxygen atoms in each of the inter-nucleotide phosphate linkages with sulphur atom. This makes the ODN stable against nucleases, easily soluble in water and simple and inexpensive to synthesize. Such ODNs are termed as phosphorothioate ODNs. There are other derivatives of ODNs which are notable for both, extremely high nuclease resistance and tight binding to single-stranded RNA. The anti-sense nucleic acid can also be expressed from a plasmid transfected into the cell. Synthetic DNA and RNA can also be engineered to contain inherent cleaving activity like ribonucleases H which are ubiquitous enzymes cleaving the RNA part of RNA/DNA hybrids.

Anti-sense ODN can block the expression of specific target genes responsible for human diseases and are being used in therapy. Clinical trials have been initiated using antisense ODNs against a variety of oncogenes, including K-ras, c-myc, bcr-abl and bcl-2 (see Table 1). In a phase I–II clinical study using anti-Bcl-2 therapy combined with chemotherapy in patients with advanced malignant melanomas, the antisense ODN was found to downregulate the target BCL2 protein in metastatic cancer and demonstrated encouraging anti-tumour response in 6 out of 14 patients.

**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 tumour 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. Such a strategy can be used in combination with other strategies or even with any conventional modality of treatment.
Genetically modified tumour vaccines in gene therapy

Cytokine genes: Tumour cells as well as immune-effector cells have been modified by insertion of various genes mainly cytokine and growth factor genes. Cytokines, which are small polypeptides involved in immunity and inflammation, are being extensively used in immunotherapy\(^9\). Genetically modified tumour cells releasing various cytokines have been shown to result in local recruitment of inflammatory cells that in turn can inhibit tumour growth\(^13\). This is accompanied by tumour antigen priming of the host immune system and enhanced tumour immunogenicity resulting in tumour regression. In animal studies, in some instances immunological memory has been generated to resist subsequent challenge with unmodified parental tumour cells\(^14\).

In a selected set of advanced cancer patients it has been demonstrated that high dose of the cytokine, interleukin-2 (IL-2), results in modification of the host immune system leading to tumour regression. It was shown by Rosenberg’s group\(^15\) that lymphokine-activated killer (LAK) cells could be grown in vitro in the presence of IL-2 and adaptively transferred for cancer. So also, they have shown that tumour-reactive T cells termed as tumour infiltrating lymphocytes (TIL), isolated from the patient’s tumour, can be grown in vitro in the presence of IL-2 and returned to the patient for systemic adoptive immunotherapy\(^16\). In order to further enhance their anti-tumour activity, TIL have been genetically modified with cytokine genes such as TNF-\(\alpha\), adding new functions to the effector cells\(^17\).

Cytokine gene transfer into antigen-presenting cells (APC) is also being investigated to augment anti-tumour immune response. Dendritic cells (DC) are efficient APCs which are able to prime naïve T lymphocytes and regulate steadily the delicate balance between tolerance and activation during the immune response. Several reports have shown that genetically engineered DCs can be a powerful tool for inducing an antigen-specific immune response\(^18\). The use of such modified APCs is a working hypothesis in preclinical studies and in clinical vaccination approaches for cancer treatment. Cytokines currently being tested in cancer vaccine trials include IL-2, IL-4, IL-7, tumor necrosis factor, interferon-\(\gamma\) and GM-CSF.

Co-stimulatory molecules: For efficient activation of T cells, in addition to an antigen-specific signal received by the T cell receptor/CD3 complex, non-specific signals are also required. These are provided as co-stimulatory signals by the CD28 receptor on T cells and their ligands belonging to the B7 family – B7-1 (CD80) and B7-2 (CD86)\(^19\). The B7 co-stimulatory molecules are expressed on APCs – the macrophages and dendritic cells, which efficiently present antigen to the T cells. The co-stimulatory signals lead to production of various cytokines which in turn lead to proliferation, activation and maturation of T cells. Although tumour cells express specific antigens recognized by T cells, they do not express co-stimulatory molecules and hence the tumour-specific antigens are not presented to the T cells. If tumour cells are made to express the co-stimulatory molecules they would present tumour-specific antigens efficiently to the T cells directly without requiring helper T cells or antigen-presenting cells. This strategy has been used in gene therapy to generate an effective systemic immune response where B7 genes have been introduced ex vivo into the tumour cells.

<table>
<thead>
<tr>
<th>Strategy for gene therapy</th>
<th>Targeted gene/therapeutic gene</th>
<th>Type of cancer</th>
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<tbody>
<tr>
<td>Antisense molecules</td>
<td>K-ras, bcr-abl, hcl-2</td>
<td>Small cell lung carcinoma (SCLC)</td>
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<td>Augmentation of immune response</td>
<td>Co-stimulatory HLA-B7 CD-80</td>
<td>CML</td>
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<tr>
<td>Immunomodulation</td>
<td>Cytokine genes such as IL-2, IL-4, IL-12, GM-CSF</td>
<td>Non-hodgkins lymphoma</td>
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<td>Prodrug activation</td>
<td>Herpes simplex thymidine kinase gene</td>
<td>Head/neck cancer, childhood acute lymphoblastic leukemia</td>
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<td></td>
<td>Cytosine deaminase</td>
<td>Various solid tumours</td>
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<tr>
<td>Induction of apoptosis</td>
<td>p53</td>
<td>Brain tumour, ovarian cancer, liver cancer, head/neck cancer, breast cancer, head/neck cancer, hepatocellular carcinoma</td>
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<tr>
<td></td>
<td>BAX</td>
<td>SCLC, head/neck cancer, ovarian carcinoma, brain tumours, non-SCLC</td>
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<td></td>
<td>hREC2</td>
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<td></td>
<td>Caspase-8</td>
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<tr>
<td>DNA vaccines</td>
<td>IL-12, IFN-(\gamma)</td>
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<tr>
<td>Genetic radio-isotope targeting</td>
<td>Sodium iodide symporter</td>
<td>Solid tumours</td>
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<tr>
<td>Inhibition of angiogenesis</td>
<td>Angiostatin, Endostatin</td>
<td>Solid tumours</td>
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<tr>
<td>Chemoprotection of bone marrow</td>
<td>MDR-1</td>
<td>Ovarian cancer</td>
</tr>
<tr>
<td>Oncolytic virus</td>
<td>(kills cells carrying p53 mutations specifically)</td>
<td>Head/neck cancer</td>
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In one of the clinical trials carried out by Gleich et al.,\textsuperscript{20} for squamous cell carcinoma of head and neck, HLA-B7 was used as an immuno-modulator. Plasmid carrying the cDNA for HLA-B7, complexed with a cationic lipid was injected intratumourally into 9 patients. The results were encouraging where 4 patients showed partial response and 2 patients showed complete response.

**DNA vaccine:** DNA vaccination is a new strategy of immunization where genes coding for tumour-specific antigens are injected intramuscularly as naked plasmid DNA where they lodge and commence synthesizing the protein\textsuperscript{21}. The tumour protein has been shown to stimulate both antibody-mediated response as well as cytokotoxic T lymphocyte response. Genes coding for cytokines have also been used to enhance the immune response against the tumour cells. Encapsulation of the DNA vaccine into biodegradable polymer microspheres ensures long-term release of the vaccine eliminating the need for subsequent boosters.

**‘Suicide’ gene therapy**

A commonly used strategy for gene therapy of solid tumours is the ‘suicide’ gene therapy where the therapeutic gene is targeted at the tumour cells, killing the very cell expressing it\textsuperscript{22}. The suicide genes are enzymes that can activate prodrugs which have low inherent toxicity. These enzymes are either viral enzymes such as herpes simplex thymidine kinase (HSVTK), or bacterial or yeast enzymes such as cytosine deaminase (CD). HSVTK converts the non-toxic anti-viral drug – ganciclovir (GCV), into a toxic form by phosphorylation. CD converts the non-toxic drug – 5-fluorocytosine (5-FC), into a toxic form – 5-fluorouracil. HSVTK/GCV strategy as well as CD/5-FC strategy have been used in numerous gene therapy preclinical as well as clinical trials including brain tumours\textsuperscript{23}, head and neck cancers\textsuperscript{24,25}, ovarian cancers\textsuperscript{26} and breast cancers\textsuperscript{27}. The vectors used are either adenoviruses which are injected directly into the tumour or retroviruses which are generally introduced via packaging cells – virus producing cells, injected intratumourally. The intratumoural injections are followed by GCV injections. The genes coding for pro-drug activating enzymes can be placed under tumour-specific promoters, e.g. e-erbB2 promoter which would target the ‘suicide’ gene specifically to breast cancer cells\textsuperscript{28}.

**Apoptosis-inducing genes**

One of the major problems in treating solid tumours by either radiation therapy or chemotherapy is that the tumour cells are often resistant to apoptosis and therefore do not succumb to the conventional treatment. Hence, the therapeutic approaches have been aimed at killing cancer cells by inducing apoptosis. At the molecular level, mutation of the p53 tumour-suppressor gene is found in greater than 50% of human tumours\textsuperscript{29}. p53 plays a major role as a gatekeeper by inducing apoptosis in cells carrying damaged DNA. Wild type p53 has been shown to induce apoptosis in squamous cell carcinoma cell lines\textsuperscript{30} and has also been used in phase-I trials of adenoviral-p53 transfer in patients with advanced squamous cell carcinoma of head and neck in a surgical adjuvant setting\textsuperscript{31}. Wild type p53 has been used either alone or in combination with other apoptosis-inducing genes, or in combination with radiotherapy.

Overexpression of pro-apoptotic molecules such as Bax favour death of cells resistant to ionizing radiation. Expression of Bax could sensitize radio-refractory cells to radiotherapy\textsuperscript{32}. Caspase-8, a member of the family of Caspases is also involved in bringing about apoptosis. Preclinical studies have indicated that caspase-8 effectively induced cell death in gliomas\textsuperscript{33} and could be a useful strategy for gene therapy of gliomas.

**Blocking angiogenesis**

Tumours require a constant supply of oxygen, nutrients, hormones and growth factors for their existence and dissemination. This is provided by formation of new blood vessels or angiogenesis. Experimental tumours have been shown to regress by inhibiting angiogenesis and this has made it a suitable target for gene therapy\textsuperscript{34}. Two popular inhibitors of angiogenesis are angiostatin and endostatin. These are naturally generated by proteolysis of larger proteins such as plasminogen (for angiostatin) and collagen XVIII (for endostatin). In Phase I clinical trials with human recombinant endostatin no dose-limiting toxicity was observed\textsuperscript{35}. However, for continuous administration of the protein gene therapy approaches are preferred. Genes coding for the angiogenesis inhibitors can be introduced either directly into the patient’s cells \textit{ex vivo} or through generic cells that have been genetically modified to overexpress the protein of interest. In order to protect the generic cells from immunological destruction, two groups have made anti-angiogenic cell factories embedded in alginate beads and implanted them in animal models for brain tumours\textsuperscript{36,37}. Both the groups have shown considerable reduction in tumour growth.

**Combination of gene therapy with other modalities of treatment**

Cancer gene therapy approaches are often designed as single-agent therapy; however, greater therapeutic effect might be obtained if combined with an established conventional treatment regimen such as chemotherapy or...
radiotherapy. As mentioned earlier, gene therapy using pro-apoptotic molecules sensitizes the tumour to radiation as well as to chemotherapy.

Normal bone marrow cells are highly susceptible to killing by chemotherapeutic drugs. Hence, marrow toxicity is a major complication of high-dose chemotherapy. In order to protect the bone marrow multi drug resistance (MDR-1) gene has been introduced into the bone marrow cells. MDR-1 gene encodes a 170 kDa P-glycoprotein which is an energy-dependent cellular pump which actively effluxes the commonly used and potentially toxic drugs including paclitaxel and anthracycline. This strategy has been used for protecting bone marrow in patients with metastatic breast cancer.

Other strategies for tumour cell kill – oncolytic viruses

Alternative cancer therapy approaches have been based on oncolytic viruses which selectively attack tumour but not normal cells. ONXY-015 is a mutant adenovirus which lacks the E1B-55 kD protein and is thus incapable of replication. However, it was observed that ONXY-015 could replicate in and cause cytopathogenicity in tumour cells which carry p53 mutations. Selective intratumoural replication and tumour-specific tissue destruction has been documented in phase I and II clinical trials in patients with recurrent, refractory squamous cell carcinoma of head and neck (SCCHN). However, less than 15% of these patients showed any clinical benefit. Khuri et al. then undertook a phase II trial of intratumoural ONXY-015 injection in combination with cisplatin and 5-fluorouracil in patients with recurrent SCCHN. This combination of replication-competent viruses along with chemotherapeutic drugs was well tolerated in patients and showed tumour-selective augmentation of chemotherapy efficacy by ONXY-015. This strategy is now being carried out in phase III trials where all clinically detectable tumours in the SCCHN patient will be injected as a method of local control in order to assess survival benefit.

Future of gene therapy

Any conceptually new therapeutic approach takes a long time to establish as a routine treatment. It took a few decades for antibiotics and immunization to make an impact in medicine. Gene therapy is only a decade old and we are learning and improving a great deal from the ongoing human clinical trials. Gene therapy will have a greater impact than antibiotics and immunization in this century. Gene therapy has already shown promising results for treatment of monogenic disorders such as SCID, hemophilia as well as for cancer. At present, in the phase I human clinical trials of gene therapy for cancer, the focus is on patients with advanced or recurrent incurable cancer. Although this patient population is a standard choice for establishing the safety of novel therapies, the greatest chance of eventual success with the currently available gene transfer vehicles and gene therapy strategies will most likely be in those patients with early stages of the disease as well as those with minimal residual disease. The success of this treatment modality will ultimately depend upon the ability to target every cancer cell, express the gene of interest at high levels and minimize toxicity by targeting gene delivery to specific cells. To move gene therapy to mainstream disease therapeutics it will be necessary to devise strategies to administer a gene therapy reagent like any common drug probably in an injectable form. The ongoing revolution in cell and molecular biology, combined with the unfolding of the human genome and advances in bio-informatics, has made the concept of cancer-specific gene therapies more viable and promising.