REVIEW ARTICLE

Potential Therapeutic Modalities in Cancer Gene Therapy

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Abstract:

In spite of huge concerted efforts, the treatment of cancer, a disease frequently associated with genetic alterations caused due to hereditary or environmental factors, remains a challenge. The last few years have witnessed emergence of several innovative and effective modalities for the treatment of solid tumours and hematological malignancies. Gene therapy has shown enormous potential for cancer treatment, especially for metastatic cancers which unlike localized solid tumours, may not be amenable to surgery or other treatment options. Gene therapy aims to introduce a correct copy of the malfunctioning gene in the tumour environment by using viral or non-viral methods to impede or inhibit its growth. This review provides an overview of three main approaches for cancer gene therapy namely immunotherapy, oncolytic therapy and gene transfer therapy. Immunotherapy augments the host immune system in order to destroy cancer cells while oncolytic therapy uses genetically engineered viruses such as to effectively kill cancer cells. Clinical studies so far have shown that cells can be engineered to express gene products that can specifically target cancer cells and prevents their growth and metastasis. Though gene therapy for cancer is yet to see extensive clinical use, it is likely that in combination with other treatment modalities, it will help in controlling and possibly curing cancer in the near future.

Keywords: Gene therapy, Cancer, Immunotherapy, Oncolytic Therapy, Gene Transfer

Introduction:

Cancer, a leading cause of morbidity and mortality worldwide is a group of diseases involving abnormal growth of cells, with a potential to invade distant sites in the patient due to the ability of cancer cells to leave their original site by local and lymphatic spread to regional lymph nodes or by blood. According to reports, by 2030 the incidence of cancer will see occurrence of 25 million new cases and 17 million deaths annually [1,2]. Tobacco remains the leading cause of cancers, primarily lung and oral cancers, resulting in nearly 22% of the cancer deaths all around the world while infections caused by Hepatitis B, Hepatitis C, *Human papillomavirus* (HPV) and Helicobacter pylori is responsible for approximately 20% of the cancers in developing countries [2]. Considering the global burden and impact of cancer, newer preventive and treatment methods are absolutely necessary. There are several approaches for gene therapy that have reached different phases of research and clinical trials for their efficacy and safety, the three major approaches are namely immunotherapy, oncolytic therapy and gene transfer therapy.

The ability to manipulate cells at the genetic level has opened new avenues in biomedical sciences. Gene therapy, a primarily experimental technique as of now, has emerged as a new treatment modality that works at gene level to treat or prevent disease [3]. Gene therapy conceptually means to insert a functional and correct copy of the defective or non-functional gene in the targeted cells. The material which is transferred into the patient's cell may be gene, gene segments or oligonucleotides, while the target cells are usually cancer cells, pluripotent stem cells etc. The potential of gene therapy for cancer follows logically from the fact that cancer arises as a result of single or multiple genetic alterations. Normal cells in the body follow a systematic sequence of events of growth, division and death. Unlike regular cells, cancer cells, generated due to several environmental causes and genetic aberrations, do not undergo apoptosis, or programmed cell death and instead continue to undergo growth and division. It is not surprising that more than 70% of the clinical trials involving gene therapy involved cancer of one type or the other [4].

The major approaches to gene therapy currently include replacing a malfunctioning disease causing mutated gene with a healthy and functional copy of the gene, inactivating or "Knocking out" a mutated gene that is functioning improperly and introducing a new gene, otherwise non-existent into the patient, to fight and control a disease [5].

For cancer, specifically, this would mean restoring normal functioning of the gene product in every cancer cell or to kill all cancer cells which are unamenable to restoration by activating cell death. In addition, the entry of transgene may aim at protecting the normal cells from induced toxicities or activating the patient's immune cells. Currently, several phase I through phase III clinical trials are under progress for testing the efficacy of these approaches for different types of cancer [6]. Safe delivery and achieving expression effects in only targeted cells remain the most critical considerations for the success of gene therapy.

In this review we will focus on the various gene and cell therapy methods used for cancer treatment with a brief summarization on the existing gene delivery systems. The emphasis has mainly been on three broad categories in the field of cancer gene therapy treatments: immunotherapy, oncolytic virotherapy and gene transfer. A small section of this review also focuses on cancer vaccines and their role in cancer prevention.

Gene Delivery Methods:

There are essentially two main approaches for gene therapy namely '*in vivo*' and '*ex vivo*' approach (Fig. 1) [7]. Of the two methods the *ex vivo* approach is more commonly used. In this approach, cells are extracted from the patient and a vector carrying the functional gene is then cloned into the cells. Finally the transfected cells are reintroduced into the patient's body so as to produce the protein required to fight the disease. In contrast, the *in vivo* approach involves the delivery of a vector containing the functional gene into the patient's blood stream or to the tumour environment directly which is ultimately directed towards the target cell. The *ex vivo* approach is simpler since it is easier to manipulate target cells externally. Both the methods involve use of appropriate vector which can be viral and non-viral vector for the transfer of the genetic material to host cells.

Non-viral Systems for Gene Delivery:

Non-viral delivery systems include all physical and chemical methods which can be used for

delivery of therapeutic genes to cells without the need of a viral vector. Non-viral delivery system consists of polycationic carriers, polymeric nano/micro particles, gene gun, electroporation, ultrasound transfection and magnetofaction. The non-viral mediated gene transfer method offers advantages in terms of their scale-up production and low host immunogenicity. However, the transfection efficiency of non-viral gene delivery methods is comparatively less than that of viral gene delivery methods [8].

Polycationic Carriers

There are mainly two types of polycationic carrier's namely cationic liposomes and cationic polymers. Polycationic carriers are being used for delivery of genes to several tissues including lung [9], liver, neural tissue [10], heart muscles, spleen, bone marrow [11] and skin cells [12]. Polymers when condensed with DNA are called as polyplexes [13]. Cationic polymers condense negatively charged DNA and protect the same from nuclease degradation in extra cellular fluid as well as in cytoplasm. Polymers such as chitosan [14,15], Polyethylenimine (PEI) [16,17], polyamidoamine [18] and poly-L-lysine [19,20] have been tried for both *in vitro* and *in vivo* gene delivery for various applications including cancer. Among above polymers, PEI is the most versatile system for delivering genes due to its higher transfection efficiency in variety of cell types. The high transfection efficiency of PEI is due to its proton buffering capacity which protects its content from degradation in a wide range of pH, enabling its escape from the endosome of the host cell [21]. Systemic injection of cationic polyplex/DNA complex leads to aggregation due to their interaction with blood components which eventually leads to rapid clearance by Reticuloendothelial System (RES). This problem can be overcome by conjugating polyplex/DNA complex with a hydrophilic polymer such as polyethylene glycol, which increases circulation time of the same upon systemic injection [22]. Modified PEI was used for *in vivo* delivery of p53, a tumour suppressor gene, which showed higher efficiency and stable expression of transgene and eventually led to the induction of spontaneous apoptosis [23]. A study conducted *in vitro* on mice suffering from lung tumours revealed that intravenous site specific delivery of endostatin gene (a potent antiangiogenic factor) complexed with a cationic vector drastically reduced the tumour size thereby highlighting the importance of polycationic carriers as a mode of gene therapy for lung cancer treatment [24]. As a modification of the existing strategies, polycationic carriers are also being utilized in non-invasive aerosol gene therapies due to their ease of handling and biocompatibility. High molecular weight PEI has the capability to attach to airway epithelial cells and subsequently promote transfection. This property has been exploited in suppression of lung metastasis in B16-F10 cell murine melanoma model through aerosol mediated delivery of PEI-p53 complex [25].

Cationic liposomes are being utilized extensively in clinical trials for the treatment of lung cancer, which is the most prevalent cause of cancer associated deaths in the United States [26]. Cationic liposomes share common structural feature of having a positively charged hydrophilic head group and a hydrophilic tail bridged together by a linker such as glycol [27]. The positive charged head group binds with the negatively charged DNA molecule. The hydrophilic tail is made of either aliphatic chain or cholesterol and other steroids. When mixed with DNA, cationic liposome forms a complex structure called lipoplex so that DNA is protected from any

damage and its entry into cell is facilitated. Additional neutral lipid molecules are also used which aid in increasing efficiency of gene transfer by destabilizing the endosomal membrane [28, 29]. The efficiency of gene transfer by cationic liposomes depends on its structure for example size of head group, length of hydrocarbon chain, number of charged groups per molecule and properties of helper lipids [28,30].

Liposomes being synthetic substances are easy to synthesize and not toxic to the host cells. They are not immunogenic either and can carry large piece of DNA inside the cell. Moreover they offer great protection to DNA from nuclease degradation and can also be rendered tissue specific if designed with tissue specific proteins.

N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium (DOTAP) was the first synthesized liposome. Till now several new cationic liposomes have been reported for gene delivery for various applications including cancer of various tissues. For example 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine based nanoliposomes were used for intrapulmonary delivery of anticancer siRNA for lung cancer therapy [31]. DOTAP: cholesterol efficiently used for delivery of tumour suppressor genes p53 and FHIT to human primary lung tumour and significant suppression in tumour growth was observed [32]. Phase I clinical trial was done in lung cancer patients with tumour suppressor gene (TUSC2) administered using DOTAP: cholesterol-nanoliposome. The TUSC2 gene was successfully taken by primary and metastatic tumours and anti-tumour effect was observed [33]. Similarly transfection efficiency of p53 gene to H1299 cells (a human non-small cell lung carcinoma cell line) was found to be higher when administered using cationic solid lipid nanoparticle formulations than commercially available lipofectin [34].

Electroporation and Ultrasound Transfection

Electroporation is a physical transfection technique where gene delivery is achieved by applying an electric field. Electric pulse creates transient nano-sized pores in the cell membrane of host cell through which entry of gene takes place. Efficiency of electroporation depends on pulse intensity, duration and frequency, which varies from one cell type to another [35]. Utility of electroporation for *in vitro* gene delivery was first explored in 1982 by Neumann *et al*. [36], when plasmid DNA was successfully delivered to mouse fibroblast cells. Later in 1991, Titomirov *et al*. [37], used electroporation for *in vivo* delivery of plasmid DNA in the skin cells of newborn mice. The plasmid DNA was introduced subcutaneously and later electric field was applied onto skin. The use of electroporation for DNA delivery for cancer treatment is under clinical trials. Trials are in different phases for the treatment of various types of cancer including melanoma, prostate cancer and human papillomavirus associated cervical cancer [38].

Electroporation has been used for in vivo gene transfer to lung cells without any inflammation and injury. The expression of transgene was detected till 7 days and not only to periphery but also to the parenchyma [39]. Plasmids encoding murine granulocyte macrophage colony-stimulating factor when introduced into B16F10 melanoma tumour model through controlled electroporation, augmented with CD25-depleting antibodies (PC61) showed significant reduction in benign lung cancers as well as lung metastases [40]. Both, the high and low voltage pulse were used to facilitate movement of the negative charged DNA plasmid across the cells. Electroporation resulted into an efficient method to deliver gene into Mehr-80 which is a newly established adherent human large cell lung cancer cell line [41].

Ultrasound mediated transfection mechanism is similar to electroporation however it involves the exposure of cells to ultrasound waves which creates temporary pores in plasma membrane by acoustic cavitation. It temporarily permeabilizes the plasma membrane for uptake of naked DNA molecules [42]. This method is simple, easy to use and safe since it is focused at a particular anatomical location in the body. Use of ultrasound waves for gene delivery evolved during 1990s when ultrasound was used for both *in vitro* and *in vivo*DNA delivery [43,44]. Tissue absorption to ultrasound waves and its efficiency depends upon its intensity, duration, frequency and tissue type. In most of the cases of its therapeutic use, ultrasound frequency of 1–3 MHz with intensity of 0.5–2.5 W/cm^2 is used [45]. A significant advancement in ultrasound mediated gene delivery is the use of microbubbles which greatly enhance the effect of ultrasound cavitation in plasma membrane [46]. Microbubbles when used with ultrasound, release shock waves in the form of high velocity jet which can facilitates disruption of plasma membrane [47,48]. For example ultrasound-targeted microbubble system was used for targeting a wild-type p53 (wtp53) tumour suppressor gene for the treatment of ovarian cancer. pEGFP-N1-wtp53 plasmid mixed microbubbles were exposed to 1 MHz pulsed ultrasound beam which resulted in transfection of wtp53 gene into the ovarian cancer cells [49]. Polymers and their derivatives have been used in the form of nanoparticles and microbubbles to deliver genes with the help of ultrasound [50]. The efficiency of transfection of naked DNA was increased when lipofection was used along with ultrasound waves as compared to transfection by lipofection alone [51]. Ultrasound with the help of microbubbles increased the transfection efficiency of naked DNA into lungs of mice by 15-fold. This suggests that even though ultrasound gets attenuated in presence of air in lungs, the energy of ultrasound is sufficient to penetrate the lung tissue [52].

Particle Bombardment

This method of gene delivery is commonly called as 'Gene Gun' or micro projectile method and was first used in plants [53] and later on this method found its application in both *in vitro* and *in vivo* gene transfer to mammalian cells [54]. In this method, DNA is coated onto microscopic metal particles such as tungsten, gold, silver and accelerated by a force sufficient enough to penetrate the cells. The physical force to deliver DNA inside cells is generated by an inert gas such as helium pressure discharge. The penetration efficiency of this method is determined by gas pressure to inject DNA coated particles, number and size of particles [55]. The primary disadvantage of this method is the risk of cell damage occurring at the site of gas discharge.

This approach has been extensively used for genetic vaccination and for 'suicide' gene therapy to treat certain cancers. For example human Growth Hormone-specific (hGH) antibody was generated when hGH expression construct was delivered to the epidermis in the mouse model, coated onto gold particle [56]. In an *ex vivo* approach for cancer treatment, a gene encoding Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), which is a potent immuno-stimulatory cytokine, was transfected into the mouse and human tumour cells using the particle bombardment method to generate vaccine against tumour [57]. Rakhmilevich *et al*. [58] reported tumour regression when murine interleukin 12 (IL-12) gene was delivered using particle bombardment method to metastatic murine tumours.

Viral Vectors for Gene Delivery

Viruses are essentially small micro-organisms that contain RNA or DNA as their genetic material in single stranded or double stranded form. The viral genome is encapsulated by a protein coat known as capsid that allows the virus to attach to the host cell receptors and protect it from destruction from cellular enzymes. By itself a virus cannot replicate, but it makes use of host metabolic machinery to propagate and multiply avoiding immunosurveillance by the infected host. These properties make viruses an excellent gene delivery vehicle [59]. The viral vectors used for gene therapy application are replication deficient and genetically modified (Table 1). To achieve this, nearly all or some of the coding sequences of viral genome are deleted but sequences for packaging and integration into host genome are left intact [60]. The main advantage of viruses in gene therapy is prolonged expression of transgene with minimal side effects and relative ease of purification into high titers [61]. Both DNA and RNA viruses have been widely utilized as vectors for gene transfer to several tissues.

Adenoviral Vectors

Adenoviruses are double stranded DNA viruses that are responsible for mild respiratory and digestive infections in humans; hence, for gene therapy modified adenoviruses are used. The ease of infection and large DNA carrying capacity make adenovirus one of the most popular delivery vehicles. They can also be produced commercially on a large scale [62]. The increasing use of adenoviruses as a vector in lung cancer gene therapy strategies stems from their ability to infect both dividing and non-dividing host cells in vitro as well as *in vivo* with relatively high efficiency [63]. When these viruses infect the host cell, they introduce their DNA molecule into the host and their genetic material is transcribed without being incorporated into host genome. Adenoviral vectors have been proven to be useful for cancer gene therapy. For example, Cytosine Deaminase (CD) gene was transferred by an adenoviral vector into lung cancer cells in a Lewis mouse model along with an antifungal drug Fluorocytosine (5-FC). Cytosine deaminase converted 5-FC to the antimetabolite 5-Fluorouracil (5-FU), which killed malignant lung cancer cells [64]. Modified oncolytic adenoviruses are under clinical trials for the treatment of astrocytoma [65].

Ad5.SSTR/TK.RGD an infectivity-enhanced oncolytic adenovirus vector expressing a therapeutic thymidine kinase suicide gene is under phase I clinical trial for the treatment of recurrent gynecologic cancer [66]. In a study by Yao *et al*., a gene therapy system was developed using adenoviral vectors conjugated to Polyethylene Glycol (PEG). Viral vector-PEG molecules were conjugated with a CGKRK tumour vasculature homing peptide for site specific targeting after systemic administration [67]. Current phase I clinical trials for the treatment of non-small-cell lung cancer through the administration of adenovirus vector containing wild-type p53 complementary DNA has shown promising results, marked by a significant regression of the tumour. Positive results of the clinical studies have shifted the focus towards developing an effective treatment strategy incorporating both radiation therapy and adenoviral vectors for improved local tumour control [68]. Another effective strategy for the treatment of lung and prostate cancer involves the utilization of a modified adenovirus encoding a nonphosphorylatable Thr34→Ala mutant of the apoptosis inhibitor survivin (pAd-T34A). Targeted administration of pAd-T34A catalysed

the apoptosis and nuclear fragmentation of cell lines of lung, prostate and colorectal cancer thereby inhibiting tumour growth [69]. With the number of cases of cancer reported yearly on a steep rise, vectors such as adenoviruses hold the key to curbing the spread of cancer and providing a cure to this fatal disease in the near future [70].

Retroviral Vectors

Retroviral vectors are the most commonly used viral vector for the reason that it integrates into the genome of the host in a stable and permanent fashion. Retroviruses are RNA viruses with a diameter of 80–130 nm and a genome size of 8–11 kilobases (kb) [71]. The enzyme reverse transcriptase and integrase in the virus allows replication and integration respectively of the viral genome into the host genome. Retroviral vectors are widely used system for gene therapy and several clinical trials are under investigation. Its long research history allows researchers to manipulate viral genome [72].

Retroviral vectors are made replication incompetent by replacing replication genes with a transgene, however sequences for packaging, reverse transcription and integration (LTR sequences) are left intact. This is why, in most of the retroviral vector systems a helper construct is required that provides all essential viral proteins in trans to limit the ability of the virus to replicate freely [73].

Stable integration into host genome, generation of sufficient viral titers and infectivity for a broad variety of cell types are advantages of retro viral vector.

The primary drawback of using retroviruses based on Moloney Murine Leukemia Virus (MMLV) as a gene delivery vector is that they require cells to be actively dividing during the transduction process [74]. As a result, terminally differentiated cells are resistant to infection and transduction by retroviruses however lentiviruses which are retrovirus that can infect non-dividing cells as well, may allow for the wider application of retroviral vectors to gene therapy [75]. Most of the lentiviral vectors are based on the Human Immunodeficiency Virus (HIV) which can carry large size of transgene and provide stable expression of the same. Lentiviral vectors have emerged as a tool for cancer immunotherapy. They can be used to deliver genes for wide range of tumour specific antigens to dendritic cells for them to induce strong antigen-specific T-cell response. *In vivo* administration of lentiviral vectors containing tumour-associated antigens can be used as an alternative to antigen-specific immunization [76]. Moreover, surface of lentiviral vector was modified to express Interleukin-13 (IL-13) and made to bind tumour cells which expressed higher number of IL-13 receptors compared to normal cells. So the engineered viral vector could effectively attach and transduce tumour cells [77]. The use of lentiviruses as vectors in lung cancer therapy is of significant importance. Lung cancer cells are characterized by a mutation in the transcription factor Nuclear factor erythroid-2 related factor 2 (Nrf2) or its inhibitor Keap 1. As an extension of the suicide gene therapy strategy, a Lentiviral Vector (LV) expressing the Herpes Simplex Virus Thymidine Kinase (HSV-TK/GCV) was constructed, and regulated by a Nrf2 enhancer sequence called Antioxidant Response Element (ARE). Suicide gene therapy using LV-ARE-TK/GCV was found to be more effective as compared to the traditional strategy of using LV-TK/GCV in reducing tumour size in the mouse xenograft model of lung cancer. These findings pave the way for more effective therapeutic

strategies in the future, especially for tumour cells in the lung with high constitutive ARE activity [78].

Herpes Simplex Virus

Herpes Simplex Virus (HSV) belonging to the alpha herpes virinae subfamily are essentially double stranded DNA viruses possessing an inherent neurotropic nature. Like most viral vectors, the 152 kb linear genome is encapsulated within an inner icosadeltahedral capsid and an outer envelope which facilitates the attachment of the virus to the target cell. Of the two types of herpes simplex virus- HSV I and HSV II, HSV I is being explored and utilized extensively for cancer therapy either in the form of a replication defective vector carrying transgenes or a replication competent vector (oncolytic therapy) [79].The characteristics which contribute to the increasing popularity of HSV I as a vector in cancer therapy include: ability to transfect a broad variety of cell types, capability of accommodating transgenes up to 30 kb in size and potency to cause cell lysis through a cytolytic mode of action. Since the virus is non-integrative in nature it significantly reduces the risk of insertional mutagenesis which commonly occurs with the use of retroviruses [80]. On the contrary, one of the primary concerns regarding HSV is the maintenance of stable transgene expression [81]. HSV I virus is being used as a vector in the treatment of malignant gliomas and other cancers of the central nervous system owing to its neurotropic nature. However this inherent neurotropic nature also limits its application as a vector in the treatment of other types of cancers such as lung cancer [82]. Most gene therapy strategies involving HSV I vector are aimed at delivering a suicide gene called Thymidine Kinase (TK), against the cancerous cells while causing minimal harm to the neurons and glial cells. Thymidine kinase is capable of converting prodrugs such as ganciclovir into toxic metabolites which when integrated into the replicating DNA of actively dividing cells like cancer leads to interruption and halting of DNA synthesis in them [81]. HSV type 1 mutants (ICP34.5 gene) have shown promising results in the treatment of lung cancer. Mutation in the ICP34.5 gene enabled the HSV vector to replicate in rapidly dividing cells. In animal models of lung cancer these vectors have been effective in promoting tumour regression and enhancing survival [83]. Novel strategies which incorporate anti-angiogenic genes into the genome of HSV's are being tested as means of controlling cancer, since angiogenesis plays a crucial role in the maintenance and progression of tumours. In a study conducted by Mullen *et al*. [84] angiogenesis was found to be inhibited in the human HT29 colon carcinoma model through the incorporation of the murine endostatin gene in the genome of the HSV virus.

The purpose of gene therapy is to replace a mutated gene (present within a cancer cell) with a functional exogenous gene. In this regard, a variety of genes such as a cytokines, tumour suppressor genes, chemokines etc. are being utilized by researchers for cancer treatment. Of particular importance is Interleukin 12 (IL-12), which functions as a key regulator of immune response and stimulates components of the cell mediated branch of the immune system such as helper and cytotoxic T lymphocytes. These properties of IL-12, enable recombinant HSV to exert an oncolytic effect on malignant cells of squamous cell carcinoma and hence be utilized as an effective anti-cancer strategy [85, 86].

Approaches to Cancer Gene Therapy:

There are several approaches for gene therapy that have reached different phases of research and clinical trials for their efficacy and safety, the three major approaches are namely immunotherapy, oncolytic therapy and gene transfer therapy.

Immunotherapy

Cancer arises and evolves, in part, by devising mechanisms to escape detection by immuno-surveillance system [87]. Immunotherapy approach is based on the concept of blocking the immunosuppressive mechanisms devised by cancer cells and reactivating the host's own immune system enabling it to target and kill the cancer cells. One of the ways to achieve this aim is by creating cancer vaccines, delivering them to tumour environment by various methods, and producing an immune response (for example - antibodies against specific tumour antigen) which will help in recognition of cancer cells presented with right antigenic and immune stimulatory cellular components [88, 89]. Immuno-stimulatory genes like cytokines when targeted to a tumour produce specific antigens which facilitate the recognition of the tumour by the immune system and promote formation of anti-tumour antibodies. Similarly, infusion of vaccines aimed at stimulating the production of antibodies to recognize the cell surface receptors such as Her2 expressed specifically by tumour cells can promote destruction of cancer cells.

Another approach that has been used widely, involves modifying genes in the patient's own T cells (autologous cells) to produce a large number of tumour-specific T cells with an ability to recognize the tumour [90, 91]. Since the immune system of cancer patients is usually low, this approach to directly produce and infuse tumour-recognizing engineered T cells might prove more effective than to generate effector cells by infusing recombinant vaccines.

Regarding cancer, initial efforts to deactivate oncogenes and replace non-functioning tumour suppressor genes were nearly successful. Subsequently new approaches have been developed to transfer genetic mutation directly into target cells aiming to transiently or permanently change their phenotypes. The target cells may be normal cells, cancerous cells, immune mediated cells or pluripotent stem cells etc. such that when the transgene enters the cell, it then assists in its death or restores normal cellular functions.

Oncolytic Virotherapy

The idea of virotherapy for treating cancer started emerging during 1950s, when a vaccine strain of rabies virus was used for the treatment of patients with melanomatosis [92] but the idea actually took a promising shape in 1990s [93]. In this technique, genetically engineered viruses such as vaccinia, adenovirus, herpes simplex virus type I and reovirus were used to effectively kill cancer cells. The main aim of this approach is to achieve a strong cytolytic effect highly restricted to transformed cells alone. The death of infected cancer cells may occur through direct cytotoxic effects of virus or through immune responses generated by the viral infection.

Numerous viruses with inherent anti-cancer activity have been identified and are in different phases of clinical trials [94]. The major advantage of the oncolytic viruses over chemotherapeutic agents is that they can be engineered by in vitro genetic manipulation based on preclinical and clinical findings. Although viral oncotherapy holds tremendous potential for cancer treatment,

yet successful application requires the viruses to fulfill certain criteria of safety and efficacy. To design safe viral oncolytic agents, certain criteria such as cancer specificity, minimizing the chances of regaining pathogenicity, possibility of transmission to healthy individual, undesired side effects and pre-existing immunity require consideration. The important consideration for the use of viruses would be to make the viral infection selective to tumour environment. This has been achieved by engineering the viruses in a way to target the cancer cell specific marker proteins such as Erb2 or PSA as receptors for entry [95]. Moreover, though intra-tumoural delivery ensures tumour targeting of cancer cells to a great extent, yet the systemic delivery of virus without causing host toxicity for treatment of metastatic cancers still remains a challenge.

Gene Transfer Therapy

Gene transfer is the introduction of rationally chosen, selective candidate genes into the host cells using various delivery methods to destroy cancer cells and impede further tumour growth. The recipient cells could be cancer cells or the surrounding healthy tissues and the delivery methods include viral or non-viral methods. The choice of gene to be inserted, the delivery method used for gene insertion, and the duration of expression of the inserted gene determine the success of this approach. Tumour suppression genes, loss of functioning of which inevitably results in tumourigenesis, constitute an important class of candidates considered for gene transfer. Mutations and deletions causing inactivation of one or both alleles of these genes may overhaul control from division of cells leading to aberrant cell growth. For instance, TP53 gene that codes for the tumour suppressor protein p53, an inducible protein not usually present in normal cells, which is activated in response to DNA damaging cellular stress and regulates pathways controlling cellular fate under that stress. In addition p53, as a transcription factor having more than 30 known target genes controls several pathways such as cell cycle, apoptosis, DNA and cell differentiation [96]. It is conceivable that inserting a wild type copy of gene and restoring the function of TP53 (mutated in most human cancers due to genetic alteration (mutation, deletion) may block tumour growth and even reverse it to normal phenotype [97]. Replication-defective adenovirus (Ad-p53) has been used to evaluate this approach and is either under clinical trial [98, 99] or is being used in clinic already [100].

Gene transfer has also shown potential to increase the effectiveness of other treatment modalities like chemotherapy, suggesting that it could be very useful for combination regimens. A highly promising use of this approach is to transfer the sensitivity genes into the cancer cells. For example gene therapy can be used to insert genes to activate a relatively non-toxic pro-drug to form a highly toxic agent. The most widely evaluated system has used thymidine kinase gene of Herpes simplex virus (HSV tk). Ganciclovir, an anti-herpes drug, needs to be metabolized by HSV tk, for exhibiting therapeutic cytotoxic effects. Therefore, insertion of HSV tk into cancer cells followed Ganciclovir therapy may produce selective cytotoxicity in cancer cells. Replication incompetent adenovirus have shown great promise in safe delivery of HSV tk and this approach showed considerable survival benefit in glioblastoma patients [101,102].

Table 1: Viral Vectors for Gene Delivery for Cancer Treatment

Sr. No.	Vector	Description	Application
1	Adenovirus	• Icosahedral geometry with dsDNA. • Genome size of approx. 36 kb. • Non integrative in nature. • Non-enveloped. • Tropism: Dividing and non-dividing cells	Treatment of head and neck cancer [103]
2	Retrovirus	• Genetic material is RNA within the virus. • Integrative in proliferative cells. • DNA insert in a replication-defective viral vector is usually about 8–10 kB. • Enveloped. • Tropism: Dividing cells	Treatment of hepatocellular carcinoma [73]
3	Herpes Simplex Virus	• Consists of a relatively large linear DNA genome of double-stranded DNA 150 kb in length. • Icosahedral capsid. • Enveloped. • Non integrative in nature. • Tropism: Dividing and non-dividing cells	Treatment of high grade glioma [104]

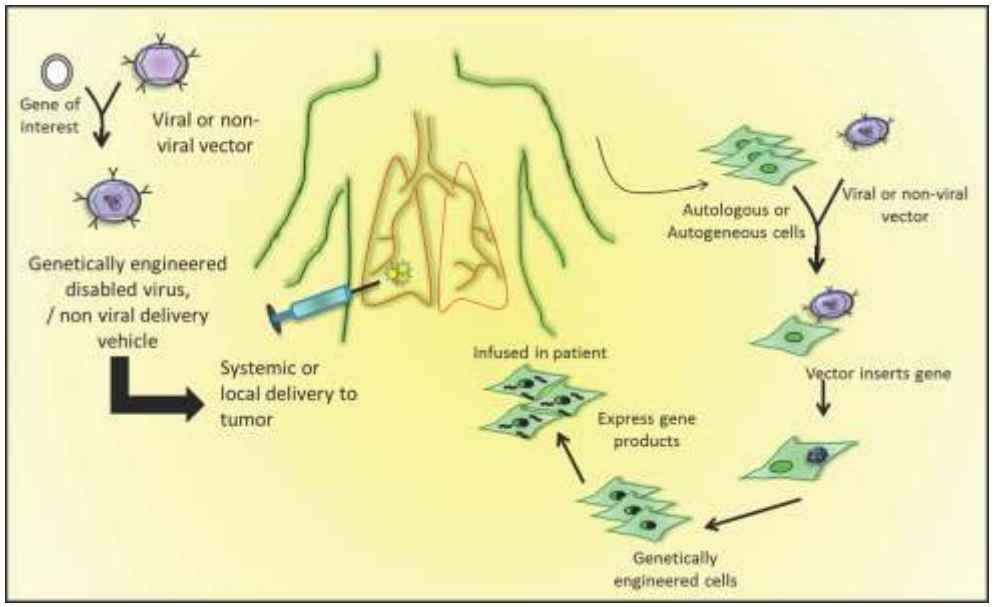

Fig. 1: Ex-vivo and In-vivo Approach to Cancer Gene Therapy

Conclusion:

The advent of genetic engineering holds great promise for increasing the effectiveness of current chemotherapeutic regime for cancer treatment. The field of cancer gene therapy is acquiring limelight and is likely to play a major role in the future of cancer therapeutics. Several vaccine treatments are in late stage of trials. In the future, the wide use of mapping techniques to obtain genomic pattern of patient's tumours as well as assessment of patient's immunity status will pave way for personalized gene therapy as per the need of the patient. Recent progress in developing the repertoire of the tools of gene therapy including the safe and effective gene delivery vehicles will certainly enhance the safety profile and efficacy of gene therapy. The challenge will lie in bringing gene therapy to a large number of cancer patients worldwide in an effective, cheap and relatively less toxic manner.

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