Cancer gene therapy is a relatively young science compared to the long ongoing standard approaches to treating cancer, including chemotherapy, radiation and surgery. The first transfer of a gene (neo marker gene, \textit{ex vivo}) to track lymphocyte homing in cancer in a clinical setting was carried out at the National Institute of Health (NIH) in 1989. Besides being relatively new, there has been substantial negative news coverage of gene therapy.

The tragedy at the University of Pennsylvania in 1999 and the more recent news of leukemias that have developed in children undergoing treatment for severe combined immuno-deficiencies have delayed the development of gene therapy. Looking on the bright side, the invention of Advexin and Gendicine was good news for gene therapists. Administration of both Advexin and Gendicine (Adp53 agents) has been reported to be safe and been well tolerated in over 2000 patients. In fact, gene therapy protocols are becoming of equal efficacy as conventional chemotherapy or radiotherapy. With new approaches undergoing clinical testing, it is anticipated that over the next five years we will see registration of a number of efficacious therapeutic gene medicines that will have equal or greater efficacy than conventional approaches.

The \textbf{Status} and \textbf{Promise} of Cancer Gene Therapy

James S. Norris, Ph.D. and Xiang Liu, M.D., Ph.D.

To date, there are 1020 gene therapy clinical trials ongoing worldwide. Sixty-six percent of them are focused on cancer therapeutics. Sixty-six percent of these clinical trials are underway in the United States. Ironically, although the number of clinical trials carried out in China are few, they have nevertheless led to the first approved gene medicine, Gendicine.

Gendicine was developed by Shenzhen SiBono GeneTech, Co. This therapeutic is available for purchase and has been administered to a reported 1500 or more patients, mostly having head and neck cancer. The efficacy of this therapeutic has been demonstrated, in particular when it is combined with conventional types of cancer therapy, including chemotherapy and/or ionizing radiation.

Another similar vector to be marketed as Advexin by Introgen Therapeutics, Houston, Texas, is also in the late stage of approval in the US and Europe. Both Advexin and Gendicine are the first generation adenoviruses that express normal p53. All human cancers may have a malfunction somewhere in the regulatory pathway controlled by this gene. Deregulation of this pathway results in a cell that may gain the ability to grow under abnormal circumstances and become a cancer.
Besides Gendicine and Advexin, a number of biotechnology companies are developing and testing additional viruses for cancer gene therapy (Table 1). In some cases, viral replication has been programmed to occur within the tumor cell to cause the cell to die. It is believed that this approach will lead to additional infection of adjacent tumor cells in a continuous cascade which is hoped to result in tumor eradication. This type of therapy is also being appreciated in combination with conventional chemo- or radiation therapies. Several of these approaches include viruses that are able to both replicate and express cytokines to further enhance tumor killing.

Germany-based Medigene Inc, a subsidiary of Medigene Ag, has developed several cancer gene therapy drugs based on selectively replicating herpes simplex virus as the transport vehicle. The tumors of interest are malignant glioma and metastatic colorectal liver tumors.

Crusade Laboratories based in Great Britain is working with HSV 1716 and has already used it in four trials with 43 patients with glioma as well as one trial with 20 patients presenting with squamous cell carcinoma of the head and neck. Their clinical data reveals that no toxic reactions were observed and that there was good selectively of tumor cells killing. The data demonstrated that long term survival exceeded six years in some cases.

The Chinese firm Shanghai Sunway Biotech company has several products in the pipeline that are based on oncolytic adenoviruses currently undergoing clinical testing. These agents include H101 (originally Onyx 015), developed by Onyx and is now licensed to Sunway. H103, another Sunway product, is a genetically-engineered adenovirus for treating metastatic cancer by inducing a systemic immune response against both local and metastatic disease. Sunway has a third virus, H102, which is being designed to treat hepatocellular carcinoma, a major life-threatening cancer in Asia, particularly in China.

Another company, Cell Genesys, is working on an “Armed Oncolytic Therapy” which is designed to specifically kill tumor cells and to mediate an immune-based killing approach stimulated by GM-CSF. Extensive clinical trials have been carried out with this drug.

Wellstat Biologics has developed PV701, a replication competent Newcastle disease virus that selectively targets viral replication in tumors that have a defective interferon anti-viral response. This virus has been systemically administered and was demonstrated to be safe with only mild to moderate flu-like systems as the most common adverse event. Modest efficacy was observed from the Phase I dose escalation trial.
Oncolytics Biotech Inc is developing a different scientific approach by using a Reovirus to target tumors that have an activated Ras pathway. Their drug, Reolysin, is in Phase I clinical trials. To date, there has been no serious adverse effects related to virus administration.

Therion Biologics, a company located in Cambridge, MA, USA, is currently using vaccinia virus for vaccination studies in both prostate and pancreatic cancers. This company has extensive ongoing clinical trials that also include combination of vaccination with vaccine and radiation or chemotherapy. Their concept is to deliver agents, which will train a patient’s immune system to fight cancer with minimal serious side effects. They have wide experience and have had their vaccine components in over 700 patients in 30 Phase I and II clinical trials.

In summary, the field of cancer gene therapy is progressing nicely with one registered drug and many more in the regulatory pipeline. It seems clear that these drugs have great potential to extend life and/or cure patients with localized cancer, particularly when combined with radiation or chemotherapy. Systemic viral therapies are also promising but need additional clinical data. Immune therapies for certain hematological cancers and melanoma also look promising. Time will tell how many of these drugs will pass the necessary regulatory hurdles to enter the general clinical practice.

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Beauty and Beast:  
The Promises and Concerns of 
Gene Therapy Vectors

H. C. Tang and S. Q. Pan

Introduction

Gene therapy can be defined as the delivery and expression of genetic information in cells of an individual to restore health or to ease the consequences of disease. Initially, it was designed to treat inherited diseases that arise due to the genetic defect in a single gene, such as cystic fibrosis and hemophilia. Now gene therapy has been adapted and extended to tackle complex traits that include certain cardiovascular defects, cancer and infectious diseases. It has been used in treating severe combined immunodeficiency (SCID), lysosomal storage disorders (LSDs) and other genetic diseases. Gene therapy has also been applied to organ transplantations in dealing with graft rejection and survival. More recently, novel gene therapy approaches for acquired immunodeficiency syndrome (AIDS) caused by human immunodeficiency virus (HIV) have been shown to be promising new treatments that can be used alone or in conjunction with the conventional small molecule drugs. Despite the controversial ethical issues and the differential regulation of gene therapy in different countries, the British Government has predicted that gene therapy has the potential to become a cornerstone of modern medicine amid the new challenges.

To obtain desirable therapeutic results, it is important to achieve efficient gene delivery and subsequent expression of the transferred gene (transgene). Thus, the use of a suitable gene therapy vector is of paramount importance. Commonly used gene therapy vectors can be divided into two major categories: viral and non-viral vectors. By harnessing the ability of certain viruses to integrate their genome into the host chromosome, viral vectors have been used widely in gene therapy experiments, applications and clinical trials where human gene transfer protocols were performed. Two of the most commonly utilized viral vectors are the retroviral vectors and the adenoviral vectors.

Retroviruses

Retroviruses are a group of RNA-containing viruses that replicate through a unique molecular event: reverse transcription (i.e. the synthesis of DNA from an RNA template). Retroviral vectors are capable of inserting the therapeutic gene of interest into the host genome without eliciting a strong immune response, and thus providing an effective way of maintaining the acquired genetic information in the target cells. However, the random nature of such an insertion has the hidden risk of being able to cause cancer if the insertion occurs at or near a proto-oncogene (potential cancer-causing gene) and results in the disruption of regulation of such a gene. Indeed, this is the case in the recent incidents of leukemia development in X-linked SCID patients, after a successful treatment of the disease with retroviral gene therapy. This latest development and the fact that this approach only works in actively dividing cells directly injected with high viral titers, have raised some serious concerns regarding the use of retroviral vectors. But after re-evaluation of the safety issues, the investigations have shown that there was no sufficient data to warrant the cessation of other retroviral human gene transfer studies, if the use is limited to terminally ill patients. Some possible solutions that include the use of self-inactivating retroviral vectors have been suggested in order to improve the safety of retroviral vectors.
Adenoviruses

Unlike retroviruses, adenoviruses (Ad) are DNA viruses. Although the initial attempts at human gene therapy were dominated by retrovirus vector-mediated gene delivery, adenoviral vectors have become an increasingly powerful tool for gene therapy\(^1\). The ability of Ad to infect dividing and non-dividing cells with high efficiency and the capacity to produce high-titer Ad stocks have aided the use of adenoviral vectors in gene therapy. However, the adenoviral vectors are highly immunogenic in humans and over 50% of adults carry antibodies to Ad. As such, it is difficult to induce long-term expression of the introduced transgene with the use of adenoviral vectors or to achieve good therapeutic effect with repeated administration\(^{11}\). In 1999, the death of the teenager who was suffering from a metabolic disorder after the administration of a very high dose of an adenovirus vector via liver injection has raised concerns about the clinical use of Ad\(^{1,9,11,14}\). Despite the setback, the ensuing investigations have revealed that Ad vector-induced toxicity is complex and the new Ad vectors with reduced toxicity and improved persistence of transgene expression have been developed\(^{14}\). This includes the ‘gutless’ Ad from which all viral coding sequences were removed. Due to such a removal, the use of this vector requires a second ‘helper’ virus to provide the replicative and structural proteins during vector propagation\(^{11,14}\).

Adeno-associated viruses and lentiviruses

Besides retroviral vectors and adenoviral vectors, other viral vectors that are likely to assume a greater importance in the near future include the adeno-associated virus (AAV) and lentivirus\(^9\). AAVs are small single-stranded DNA-containing viruses that were initially discovered as a contaminant in adenovirus preparations\(^{15}\). AAV and recombinant AAV (rAAV) vectors are unique, as they are based upon a class of viruses that commonly inhabit a human host without causing any detectable pathology. Although AAV or rAAV vectors require a ‘helper’ virus such as adenovirus for replication, they can establish long-term latency within the host cells in the absence of such a ‘helper’ function. With the discovery of many new AAV serotypes and the progress in rAAV production technology, clinical trials utilizing these vectors are expanding, even though the usual problems of human immunity to these viral vectors are still not fully addressed\(^{16,17}\).

Lentivirus-based vectors (lentivectors) have not been formally approved for use in clinical research, due to much anxiety concerning the specific safety, ethical issues and public health\(^{18}\). But they have been used widely in recent preclinical studies and for genetic manipulation of embryonic and adult stem cells. They appear to be promising gene therapy vectors because of their ability to provide long-term and stable gene expression and to infect non-dividing cells, such as neurons\(^{18,19}\). Although the best characterized lentivector is based on type I HIV, safety concerns have caused investigators to improve the designs of other lentivectors that are not pathogenic in humans\(^{19}\).

Additional viruses

In addition, the use of type I herpes simplex virus-based gene therapy vectors in the treatment of nervous system disorders has also been explored and examined\(^{20}\). Besides that, alphavirus vectors based on Sindbis virus and Semliki Forest virus had also been characterized as potential gene transfer vectors, and the results have indicated that these potential vectors compare favorably with Ad vectors\(^{11}\). All the aforementioned viral vectors have been progressively modified in an attempt to increase their gene transfer efficiency while reducing their toxicity, immunogenicity and inflammatory potentials. More recently, in order to overcome the limitations of each viral species, chimeric viral vectors possessing the combined favorable features of two or more different viruses have also been generated\(^{23}\).
Non-viral vectors

When a viral vector is used for gene therapy, the inherent risks and potential dangers that it presents can never be totally ruled out. This is also the case for the unpredictability of any toxicity effect in individuals with different genetic and immunological backgrounds. Non-viral gene delivery approaches which are potentially safer have been largely overlooked in the past due to their inefficient delivery and the transient nature of transgene expression. But recent advances have rekindled the hope for using non-viral means to achieve efficient and long-term gene expression in humans. This include the employment of: 1) phage site-specific recombinases or AAV Rep integrase to integrate the therapeutic gene to specific genome sites without deleterious consequences; 2) episomally maintained plasmid DNAs (self replicating vectors or chromosomal vectors) that do not integrate into or interfere with the host genome; and 3) human artificial chromosome that resembles natural chromosomes and replicate in low copy number within the host cells\textsuperscript{23, 24}.

Future directions

Even though the common and widespread use of non-viral agents in the clinical setting is still rather limited, the recent advances have nonetheless represented a great step forward from the traditional approaches of naked DNA delivery by physical methods or delivery mediated by a chemical carrier such as cationic polymer and lipid\textsuperscript{25}. Further research aimed to understand how vectors are trafficked within the cells\textsuperscript{26} and how the non-viral plasmid vectors enter the cell nuclei\textsuperscript{27} may serve as the foundation for improving or increasing the efficiency of transgene expression in target cells.

Regardless of whether it is a viral or non-viral vector, the use of tissue-specific promoters\textsuperscript{28}, the alterations to vector structure or the modifications of parameters like routes of administration, dose, or gene promoter type\textsuperscript{29} have all been shown to affect the immune responses of animal models and patients to the chosen gene therapy vector. Today, it is recognized that there is no ideal gene therapy vector system. As such, when the choice of a vector is to be made, it would do well to bear in mind the advantages and disadvantages of the chosen vector. It is therefore crucial to perform a case-by-case review of protocols for appropriate risk/benefit analysis before a final decision is reached.
References


