

Review

Cancer Gene Therapy: Challenges and Opportunities

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Abstract. *Understanding the molecular basis of human disease has been the corner stone of rationale designed molecular therapies. Medicine has a long history of treating patients with cell therapies (i.e., blood transfusions) and protein therapies (i.e., growth factors and cytokines). Gene therapies are the newest therapeutic strategy for treating human diseases. Where will gene therapy be in five years after the euphoria and frustrations of the last 14 years? This is a complex question, but the primary challenge for gene therapy will be to successfully deliver an efficacious dose of a therapeutic gene to the disease tissue. Will the delivery systems return to the early clinical trials by ex vivo gene therapy or will there still be a high demand for systemic therapy? Will systemic therapy continue to depend on viral vectors, or will non-viral and nano-particles become the new mode for gene delivery? The future successes of gene therapy will be built on the improvements in other fields, such as medical devices, cell therapies, protein therapies and nano-particle technologies. This review will characterize the advances being made in the gene therapy field, as well as addressing the challenges for the near future for cancer gene therapy.*

Regenerative medicine

Advances in the medical field have always been pushed forward in fits and starts by the combination of novel observations with emerging technologies. In the past century, medical advances have improved the life expectancy for the Western societies. This milestone has been achieved by advances in public sanitation, vaccinations, improved diets and the wide spread use of antibiotics for infectious diseases. These medical developments have lead to improved treatment of chronic infectious diseases, giving rise to longer life expectancies from ~46 years to ~76 years over the past 100 years. An additional outcome of this improved health care has been a corresponding increase in

the incidence of cardiovascular disease and cancer, due in part to an older population in these industrial societies. With these new challenges, what advances in health care can we expect for the 21st century? Based on the medical advances of the last century, we can expect a medical impact on chronic human diseases with cell therapies, new molecular entities (protein therapies and gene therapies) and small molecules. Our society has come to expect these profound medical advances for this century because of a series of medical breakthroughs in the last century, such as the discovery of insulin, antibiotics, cloning human genes, new biological therapeutics and the culminating achievement of sequencing the human genome. Though some of these breakthroughs have shown great potential, there has been slower progress in exploiting the information of the human genome into medical products (1, 2).

Cell therapies

Cell therapies, in the form of blood transfusions for surgery and bone marrow transplantations for cancer treatment, have become a main stay for medicine. What role these therapies will play in the future is unknown, but recent genomic advances will probably make them obsolete. The role of cell therapies in the form of stem cell therapies offers enormous potential for impacting human disease. There are several challenges to be overcome before we will see any major medical advances in treating human diseases with stem cells. A more fundamental understanding of the biology of the stem cell growth and differentiation will be required. A second challenge would be to better understand the regulation of the signal transduction pathways. This would enable the turning on and off a set of control genes for the correct stem cell growth and differentiation. Finally, educating the public to help them better understand these scientific advances. An educated population would be better prepared to debate the direction and utility of these medical advances. Previously, these medical decisions were left to the governments and medical experts, but this cannot be expected today. The Internet has made current medical information available to the general public, allowing people to become more informed and aware of their medical care choices than ever before. How

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will these new medical advances be accepted in this century, in light of the public nature of medical information is still open for discussion. How these advances will impact the Health Care Industry and the Pharmaceutical Industry also complicates the question of these future medical advances and their impact on the health of our children.

Protein therapies

Protein therapies for patient diseases have been driven by a better understanding of the molecular basis of these diseases. Examples of these new treatments range from insulin for diabetes to cytokines for stimulating stem cells with Epogen for blood cells. The manufacture of biological proteins has been the rate-limiting step for getting these new classes of therapies into the clinic. The Biotechnology and Pharmaceutical Industries appear to have finally overcome some of these manufacturing challenges after twenty years and appear to be poised to move these medical advances forward at a much faster pace. As we gain a better understanding of the molecular basis of human disease, there will be additional protein medical products available for medicine.

Medical devices

Medical Devices combined with biological proteins or drugs have become a new area for medical advances for treating patients with chronic diseases. In recent years several biology advances have lead to successful outcomes with these novel medical devices such as the following:

1. Stents containing proteins or drugs have been used to treat cardiovascular diseases;
2. Collagen containing growth factors have been used to stimulate bone growth;
3. The central nervous system has had cells stimulated to grow with nerve growth factors attached to micro-spheres;
4. Bone and cartilage growth has been stimulated to grow with growth factors in matrix samples for the tissue.

We expect to see many more advances in this field as our knowledge of the biology expands. The advances in these fields will aid the gene therapy field. Conversely, advances in the gene therapy field will advance the treatment of these patients and may have synergistic effects on both fields. This interfacing of two distinct fields will be driven by our knowledge of the human genome and the molecular basis of human disease. These medical products generated by this union can be rapidly disseminated by the digital revolution.

Gene therapy

Gene therapy is a gene or gene product that can be selectively delivered to a specific cell/tissue with minimal toxicity. This product can inhibit the expression of a specific

defective gene or express a normal gene. During the past five years, gene therapy has been through a series of successes and failures that have left the field frustrated. There has been over 3,000 patients treated with gene therapy (3), and there has been minimal toxicity associated with gene therapy that could not be treated with a non-steroidal analgesic. Unfortunately, one patient has died from a high adenovirus load to his liver and two children have developed abnormal white blood cell growth from bone marrow stem cells transduced with a retrovirus vector (2, 3).

The first successful treatment of a human disease by *ex vivo* gene replacement therapy was the treatment of X-linked Severe Combined Immunodeficiency (X-SCID). The replacement of the normal (wild type) gene in the stem cells was stably expressed and conferred selective growth advantage over the defective T cells (2). Eight patients have been cured of this disease but recently two patients have developed abnormal White Blood Cell growth. There is now evidence that there was retroviral integration at chromosome 11p13 in the LMO2 region. This may have lead to overexpression of a pre-oncogene in T cells leading to lymphoproliferation (leukemia). This gene activation may have lead selective growth advantage to the transformed *ex vivo* cells and lead to a predisposition to cancer in the treated children (2). There have been several strategies to overcome this adverse event, including using a suicide gene in the retroviral vector construct as a fail-safe system, should a similar situation occur again. All the protocols for this therapy are currently on hold until a better understanding of the disease and the treatment can be achieved.

Some scientists have questioned the role of viral vectors in general for the future of gene therapy. There are not many alternatives at this time and viral vectors are the most widely used vector system for gene therapy. Most of the approved European and United States gene therapy protocols are for cancer (~66%), in contrast to monogenetic diseases (~11%) and cardiovascular diseases (~8%). The focus of cancer gene therapy has been in melanoma, prostate, ovarian and leukemia (3).

Gene therapy: *ex vivo* therapy vs. *in vivo* therapy

Early clinical trials of gene therapy started with *ex vivo* delivery of therapeutic genes to patients with monogenetic diseases. Cytokine genes and viral thymidine kinase genes were transduced into autologous cells, normal cells or cancer cells to deliver therapeutic genes to the diseased tissue. After ten years of clinical trials, delivery of these therapeutic genes had limited efficacy due to their inability to achieve a pharmacological dose of the gene at the target tissue (4).

The goal of the Pharmaceutical Industry is to have a gene therapy medical product that can be delivered systemically. *In vivo* gene therapies have focused on viral vectors for gene

delivery and have had marginal clinical successes. Further complicating these delivery systems is the suggestion that some viral vectors may actually integrate into human chromosomes of normal tissue. The probability of producing a gene therapy medical product by a viral vector delivery system currently is at best, only for a niche market in a small disease population and at worst, there may be no satisfactory product.

If Cancer Gene Therapy is to be successful, one of the fields biggest challenges will be to understand the biology of cancer and achieve 100% tumor cell kill. Selectivity has not been achieved with systemic gene therapy because complete transduction efficacy of the tumor cells has not been achieved. This is a high entry barrier for a systemic gene therapy medical product to be successfully marketed by the pharmaceutical industry.

Gene therapy, challenges

There are four issues to be solved before cancer gene therapy will be successful:

1. Identification of key target genes critical for the disease pathology and progression.
2. Identifying the correct therapeutic gene to inhibit disease progression.
3. Optimal trans-gene expression for suppressing the target gene.
4. Delivery of therapeutic product to the target tissue at an efficacious dose.

The target gene requires a fundamental knowledge of the disease. Where is the gene in the signal transduction pathway for the disease? Is the gene an early event or a down stream event in the disease process? Sometimes the inhibition of the target gene and its pathway is not sufficient to inhibit the disease process because the cells have built redundant or alternative pathways (5).

The therapeutic genes and strategies for cancer have been imaginative and wide ranging (1): tumor suppressor gene (p53), inhibition of oncogenes with antisense oligonucleotides (4), ribozymes and short inhibitory RNA, modulation of the cytokine pools, suicide genes such as viral thymidine kinase and ganciclovir and apoptosis genes (4).

In cardiovascular disease, trans gene expression of human growth factors (VEGF) in endothelium cells can stimulate collateral growth of blood vessels (6). The reason the endothelium cells are sensitive to transduction by trans genes is due to the inflammation reaction from plaque build up on the vessel walls.

The optimal trans gene expression requires two critical components: promoters and enhancers to define the duration of trans gene expression in the cell or tissue. There are two types of promoters: constitutive or inducible. The constitutive promoters can be either of viral origin (cytomegalovirus) or tissue specific promoters (7), such as

melanin for melanoma or the prostate specific antigen (PSA) for prostate cancer. Inducible promoters have transient expression and can be induced to express trans genes with hormones or small molecules. Enhancers are placed upstream of the promoters to increase the trans gene expression 2-100 fold if the amount of gene product is required in very high concentrations in the cell. The duration of the trans gene expression will be dependent on the nature of the product and the cell's requirements.

In cancer cells, the duration of expression may need to last for only a short time up to 30 days. In contrast, genetic diseases may require, long-term expression from months to years.

Gene therapy, viral delivery

Delivery is one of the most difficult challenges facing the gene therapy field. Finding an efficient transfer system that will stabilize, transduce and express a transgene in the target tissue has not been achieved. Limitations of the present vector technologies have slowed the progress of gene therapy to the clinic (1, 4). All the viral gene strategies used to date have significant delivery limitations and at best have very narrow indications for cells and tissues. The therapeutic controversy for gene therapy strategies has always been delivery. The best method for delivering genes will depend on the type of tissue targeted (1, 4, 8, 9).

There are, however, some promising delivery technologies on the horizon for viral therapies; replication competent viruses (10). Adenoviruses, herpes simplex viruses and Newcastle disease viruses have all been modified for replication competent properties in human tumor cells. This has been one of the most researched areas in gene therapy and offers many promising leads for treating cancer especially when combined with chemotherapy. However, there are several challenges to overcome before this therapy will be successful in patients:

1. Enhanced the lytic properties of these viruses in tumor to achieve higher by stander effects;
2. Improved yields with better manufacturing procedures for clinical studies; and
3. Systemic delivery.

Currently, this is a local/regional treatment for cancer, but systematic delivery is required if replication competent viruses are to become therapeutic products.

Gene therapy, non-viral delivery

There are some non-viral technologies that offer several advantages over the previously described viral methodologies. Non-viral delivery systems have reduced adverse immune responses, are easier to manufacture and can be produced for the pharmaceutical industry in large quantities (1, 4, 8, 11). Chemically synthesized nanoparticles are a new technology

for the field of gene therapy and offer several new strategies for successful systemic gene therapy delivery (12).

Some of these new chemical compositions are polymers in nanometer size particles containing either DNA/stearyl polylysine coated lipids or Peptoids (DNA coated with glycine oligomers) or Cationic molecules (DNA/combined with positively charged B-cyclodextrin/adamantane and poly ethylene glycol). These molecules have been shown to be effective in cancer related angiogenesis. These are promising results and if the chemists continue to collaborate with the gene therapy field, some of the issues for delivery may be overcome in the near future. The new technologies have the potential to be systemically delivered but pharmacodynamics and selectivity for the target tissue needs to be validated. This could be a promising technology for systemic cancer gene therapy (12).

Gene therapy, cell delivery

One of the opportunities for gene therapy is to combine its therapeutic genes with a cell to overcome the delivery to target tissues (13, 14). The advantages of cell delivery of therapeutic products are significant: minimal immune response, tissue directed therapy, selectivity and improved potency of the product that has not been achieved with previous gene delivery methods. In contrast, the challenges to make this work successfully in a patient are several:

1. Determining optimal transduction of cells with a trans-gene or product.
2. Gene-transformed cells will require a selective growth advantage over defective cells to repopulate the host.
3. DNA repair genes (minimizes mutations in the gene-transformed cells).
4. Genomic stability (for optimal gene expression)?
5. Determining cell type for therapy, embryonic, stem cells or activated differentiated cells?
6. Incorporating a safety mechanism if a problem arises to destroy the gene-transformed cells (*i.e.* a suicide gene)

At this time, the issues may seem daunting, but cell delivery may be a novel way to look at the gene delivery problems. In addition other cell therapies are currently being developed using bacteria, such as a modified Salmonella bacteria, for gene delivery in cancer patients. These modified bacterial cells are already in Phase 1 clinical studies (15).

Gene therapy, safety issues

Viral vectors have now been suspected of integrating to the human genome, as well as altering metabolic pathways and inducing immunological responses to the virus and/or its gene products (16). Manufacturing of clinical grade gene therapy products has been a challenge for the past 15 years; however, with the experience of working with biological

products in the biotechnology industry, many of the earlier mistakes, hopefully, will be overcome. Although over 3,000 patients have been in clinical trials for gene therapy, there are no long-term studies on the genetic and heredity effects of this new therapy. To date there is no evidence of significant adverse events to 99% of the patients undergoing gene therapy. Better reporting to the FDA may be required for optimal monitoring, if gene therapy is going to be accepted as a new modality of treatment for the 21st century.

Conclusions

A more complete understanding of the molecular basis of human disease will lead to more rationally designed therapeutics. Cell, protein and gene therapies will offer many potential opportunities to impact the pathology of these diseases. By developing novel gene delivery systems, gene repair systems, gene expression technologies, then systemic therapies will become medical products for Pharmaceutical Industry. At the present time, *ex vivo* cells and stem cells may offer the best path for delivery of therapeutic gene products for treating human diseases. These new advances will be first tried in the fields of cancer and cardiovascular diseases. In the near future, we will be worrying about how to treat diseases of the mind, instead of diseases of the body.

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