

Short 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 rationally 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 defective tissue. Will the delivery systems return to the early clinical trials of 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 success of gene therapy will be built on achievements in other fields, such as medical devices, cell therapy, protein therapy and nano-particle technology. This review describes the advances being made in the gene therapy field, as well as addressing the challenges of the near future for cancer gene therapy.*

Regenerative medicine

Advances in the medical field have always been pushed forward by the combination of novel observations with emerging technologies. In the past century, medical advances improved the life expectancy for Western societies. This was achieved by advances in public sanitation, vaccinations, improved diets and the widespread use of antibiotics for infectious diseases. These medical developments have led 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

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the incidence of cardiovascular disease and cancer in the older population of industrial societies. With the current challenges, what can we expect in health care for the 21st century? Based on the medical achievements of the last century, we can expect a medical impact on chronic human diseases with cell therapy, new molecular entities (protein therapy and gene therapy) and small molecules. Our society has come to expect these profound medical advances in 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 sequencing of the human genome. Although some of these breakthroughs have shown great potential, there has been slower progress in exploiting the information of the human genome towards new medical products (1, 2).

Cell therapy

Cell therapy, in the form of blood transfusion for surgery and bone marrow transplantation for cancer treatment, has become a mainstay 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 therapy in the form of stem cell therapy offers enormous potential for human disease. There are several challenges to be overcome before we see any major medical advances in treating human diseases with stem cells. A more fundamental understanding of the biology of 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 of a set of control genes for correct stem cell growth and differentiation. Finally, there is the question of 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 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 will these new medical advances be accepted in this century, in light of the public nature of medical information, is still open for discussion.

Protein therapy

Protein therapy for human diseases has been driven by a better understanding of the molecular basis of each disease. Examples of new protein 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 this new class of therapy into the clinic. The biotechnology and pharmaceutical industries appear 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 products available for medicine.

Medical devices

Medical devices combined with biological therapeutic agents have become a new strategy for treating patients with chronic diseases. In recent years, research has led to successful novel medical devices such as the following:

1. Stents containing proteins or drugs for treating cardiovascular diseases.
2. Collagen containing growth factors for the stimulation of bone growth.
3. Micro-spheres with attached nerve growth factors for the stimulation of nervous system cell growth.
4. Tissue matrices with attached growth factors for the stimulation of bone and cartilage growth.

Gene therapy

Gene therapy refers to 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 have been over 3,000 patients treated with gene therapy (3) and the observed minimal toxicity could be treated with a non-steroidal analgesic. Unfortunately, one patient died from a high adenovirus load to his liver and two children 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). Stable expression of the normal (wild-type) gene in the stem cells 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 led to overexpression of a pre-oncogene in T cells leading to lymphoproliferation (leukemia). This gene activation may have triggered a selective growth advantage to the transformed *ex vivo* cells and led to a predisposition to cancer in the treated children (2). There have been several strategies to overcome this adverse event, including the use of 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, although there are not many alternatives at this time. 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 on melanoma, prostate and ovarian cancer 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 gene were transduced into autologous cells, normal cells, or cancer cells. After ten years of clinical trials, delivery of these genes showed limited efficacy due to inability to achieve a pharmacological dose of the gene at the target tissue (4).

The goal of the pharmaceutical industry in the forthcoming years will be to produce a gene therapy product that can be delivered systemically. *In vivo* gene therapies so far have focused on viral vectors for gene delivery and have had marginal clinical success. Furthermore, some viral vectors may actually integrate into human chromosomes of normal tissue.

If cancer gene therapy is to be successful, one of the field's biggest challenges will be to achieve 100% selective tumor cell kill.

Gene therapy, challenges

There are four problems to be solved before cancer gene therapy can be successful:

1. Identification of key target genes critical for disease pathology and progression.
2. Identification of the appropriate 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 therapeutic genes and strategies for cancer have been imaginative and wide ranging (1), including 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). Sometimes the inhibition of a target gene and its pathway is not sufficient to inhibit the disease process because the cells have built redundant or alternative pathways (5).

In cardiovascular disease, trans-gene expression of human growth factors (VEGF) in endothelial cells can stimulate collateral growth of blood vessels (6). The sensitivity of the endothelial cells to transduction by trans-genes is attributed to the inflammation reaction at the 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 cells. 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-to 100-fold if the 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 requirements of the cell. In cancer cells, the duration of expression may need only to be short, 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. An efficient transfer system that will stabilize, transduce and express a transgene in the target tissue has not yet been found. Limitations of the present vector technologies have slowed the progress of clinical gene therapy (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 best method for delivering genes may depend on the type of tissue targeted (1, 4, 8, 9).

There are, however, some promising delivery technologies on the horizon for viral therapies, employing replication competent viruses (10). Adenoviruses, herpes simplex virus and Newcastle disease virus have all been modified for replication competent properties in human tumor cells. This has been one of the most popular areas in gene therapy and offers promises for treating

cancer, especially when combined with chemotherapy. However, there are several problems to overcome before this therapy can be successful in patients:

1. Enhanced lytic properties of these viruses;
2. Improved yields with better manufacturing procedures of production for clinical studies;
3. Systemic delivery.

Currently, this is a local/regional treatment for cancer, but systemic 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 constitute a new technology 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 new agents have the potential to be systemically delivered, but their pharmacodynamics and selectivity for the target tissue needs to be validated (12).

Gene therapy, cell delivery

One of the opportunities for gene therapy is to combine therapeutic genes with a cell to overcome the delivery to target tissues (13, 14). The advantages of cell delivery of therapeutic products over previous gene delivery methods are significant: minimal immune response, tissue directed therapy, selectivity and improved potency of the product. However, there are several problems to be solved:

1. Determining optimal transduction of cells.
2. Gene-transformed cells will require a selective growth advantage over defective cells to repopulate the host.
3. DNA repair genes action (minimized 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. Incorporation of a safety mechanism (*i.e.* a suicide gene) to destroy the gene-transformed cells if a problem arises.

In addition, other cell therapies are currently being developed using bacteria, such as a modified *Salmonella*, for gene delivery in cancer patients. These modified bacterial cells are already in Phase I clinical studies (15).

Gene therapy, safety issues

Viral vectors have now been suspected of integrating with the human genome, as well as altering metabolic pathways and inducing immunological responses to the virus and/or its gene products (16). Although over 3,000 patients have been in clinical trials for gene therapy, there are no long-term studies on the genetic and hereditary effects of this new therapy. To date, it seems that there is no evidence of significant adverse events to 99% of the patients undergoing gene therapy.

Conclusion

Understanding of the molecular basis of cancer will lead to more rationally designed therapeutics. Cell, protein and gene therapies may offer many potential opportunities against cancer. By developing novel gene delivery systems, gene repair systems and gene expression technologies, systemic therapies may become a reality. At present, *ex vivo* cells and stem cells may offer the best path for delivery of therapeutic gene products for treating cancer and cardiovascular diseases.

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