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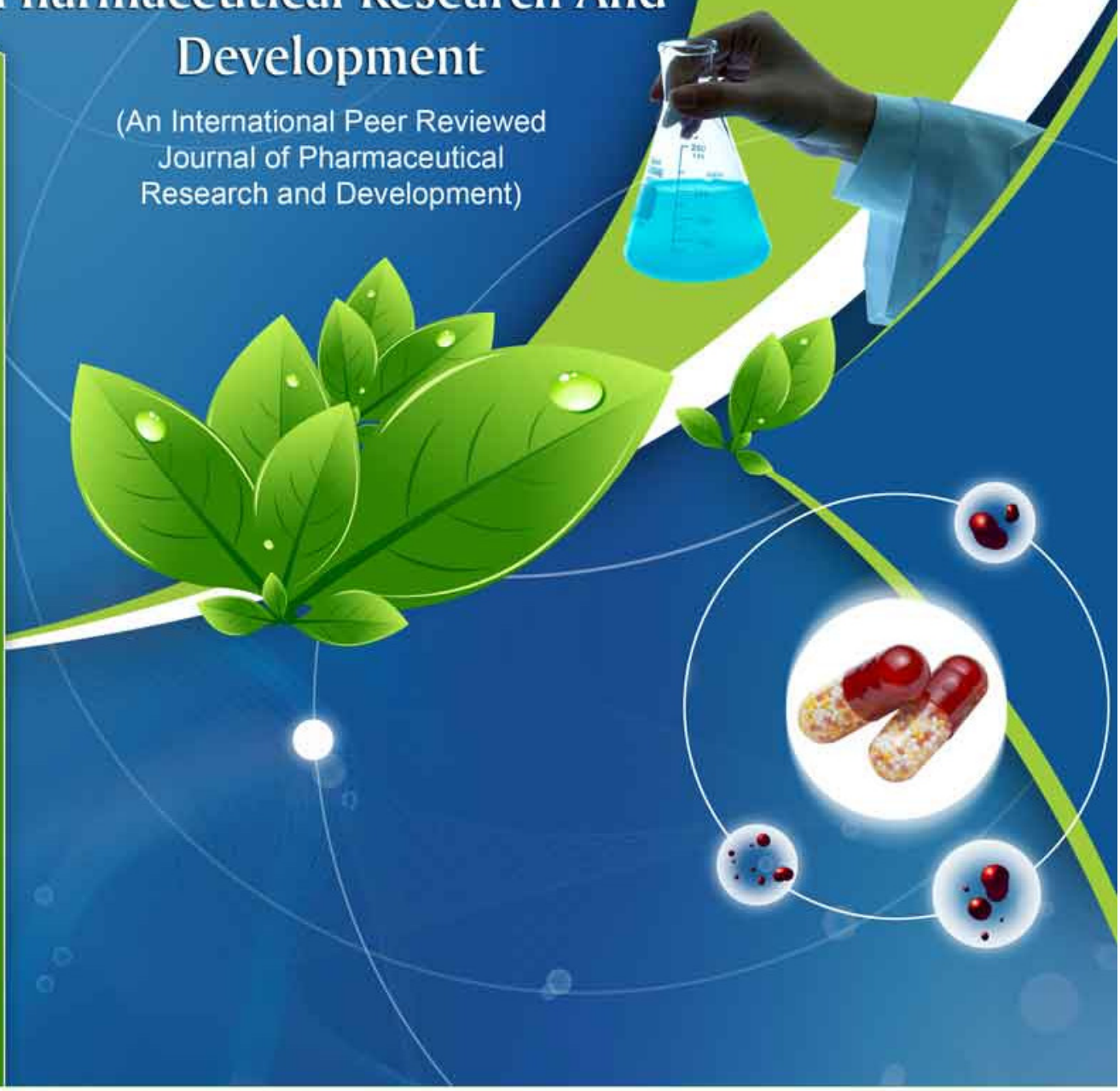

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Review Article

A THERAPEUTIC APPROACH TO CANCER GENE THERAPY

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ABSTRACT

Cancer can be described as a disease where cellular communication has broken down, allowing transformed cells to escape tight regulatory signals and to replicate autonomously and continuously, ultimately invading and interfering with the functions of normal tissues. Gene therapy of cancer has been one of the most exciting and exclusive areas of therapeutic research in the past decade. Critical developments have occurred in gene therapy targeting cancer cells, cancer vasculature, the immune system, and the bone marrow, itself often the target for severe toxicity from therapeutic agents. The therapeutic approach and efforts have been made to evaluate the target impact in both preclinical and early clinical trials. All therapeutic strategies that are being developed in the treatment of cancer ultimately aim to destroy cancerous cells while leaving normal cells unaffected. This can be achieved by expression of tumour suppressor genes, oncolytic viruses, expression of genes that initiate cell death, gene-directed enzyme pro-drug therapy, and immunotherapy. Cancer Gene Therapy is the essential gene and cellular therapy resource for cancer researchers and clinicians.

Keywords: *Cancer Cells, Oncolytic Virotherapy, Gene Transfer, Immunotherapy, Tumour.*

INTRODUCTION

Gene therapy refers to the introduction functional genetic material into target cells to replace or supplement defective genes, or to modify target cells so as to achieve therapeutic goals. In contrast to all other drugs, this kind of therapy can impart new functions to a cell. Conceived in the 1960s and started in the 1980s gene therapy is still experimental, but holds great promise for curing a number of diseases which at present can, at best, be only palliated or controlled. Gene defects result in failure to synthesize a functional protein or in the synthesis of a dysfunctional Protein.

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Equipping the cell (especially the one which physiologically expresses it) with a normal copy of the defective gene would overcome the deficiency at the site where it is needed on a long term (may be permanent) basis. Apart from inherited genetic disorders with single nucleotide polymorphism (SNP), the major thrust area of gene therapy are a number of acquired diseases such as malignancies, immunological disorders, including AIDS, cardio-vascular, neurological and infective diseases, in many of which even short-term expression of the introduced gene could be therapeutic [1]. In recent years, one of the main focuses of the effort to improve cancer treatment and patient prognosis has been gene therapy. Furthermore, the complex nature of cancer has meant that a variety of therapeutic strategies have been developed along two main avenues: local gene therapies and systemic gene therapies. The strategies for local cancer gene therapy include suppression of an oncogene, activation of a tumour suppressor

gene, and introduction of a suicide gene into cancer cells. Unfortunately, delivering a therapeutic gene to every individual cancer cell in a patient with metastatic cancer has so far proven to be an insurmountable task. It has not been possible to treat all cancerous lesions, which can include undetectable ones such as individual cancer cells and micrometastases. In addition, it is difficult to selectively target cancer cells without affecting normal cells. On the other hand, systemic cancer gene therapy such as immunotherapy appears more promising for both inhibiting tumour growth and preventing metastasis. This chapter will summarize the utility of viral vector-mediated systemic cancer gene therapy in the treatment of human malignancies, focusing on the powerful and promising approach of recombinant adeno-associated virus (AAV)-based systemic anti-angiogenic cancer gene therapy. A number of gene therapy approaches have been taken in clinical trials for cancer therapy, including the delivery of tumour-suppressing genes, notably P53, cytokines and the herpes simplex virus thymidine kinase gene plus gancyclovir treatment.[2-4]

Gene therapy of cancer has been one of the most exciting and elusive areas of therapeutic research in the past decade.[5] Critical developments have occurred in gene therapy targeting cancer cells, cancer vasculature, the immune system, and the bone marrow, itself often the target for severe toxicity from therapeutic agents

Cancer Gene Therapy Approaches

Micro RNA markers are being used to predict response to therapy in head and neck cancer by Mahvash Tavassoli's group at Kings College London (London, UK). Fifty percent of patients do not respond to radio chemotherapy and tumours in general have complex genetic aberrations.[6] Radiation treatment is associated with lifelong disabilities, while promising therapies include radiotherapy plus cetuximab.[7] The future vision for treatment includes targeted therapies to avoid the cost burden and side effects by treating unresponsive patients. Apoptosis is one of the six most important hallmarks of cancer.[8]

Two basic types of gene therapy have been applied to humans, germinal and somatic.[9] Germinal gene therapy, which introduces transgenic cells into the germ line as well as into the somatic cell population, not only achieves a cure for the individual treated, but some gametes could also carry the corrected genotype. Somatic gene therapy focuses only on the body, or soma, attempting to effect a reversal of the disease phenotype by treating some somatic tissues in the affected individual.

One of the most promising approaches to emerge from the improved understanding of cancer at the molecular level is the possibility of using gene therapy to selectively target and destroy tumor cells, for example, the loss of tumor suppressor genes (e.g. the P53 gene) and the over expression of oncogenes (e.g. K-RAS) that have been identified in a number of malignancies. It may be possible to correct an abnormality in a tumor suppressor gene such as P53 by inserting a copy of the wild-type gene; in fact, insertion of the wild-type P53 gene into P53-deficient tumor cells has been shown to result in the death of tumor cells.[10] This has significant implications, since P53 alterations are the most common genetic abnormalities in human cancers. The over expression of an oncogene such as K-RAS can be blocked at the genetic level by integration of an antisense gene whose transcript binds specifically to the oncogene RNA, disabling its capacity to produce protein. Experiments in vitro and in vivo have demonstrated that when an antisense K-RAS vector is integrated into lung cancer cells that over express K-RAS their tumorigenicity is decreased.

There are a number of gene therapy-based clinical trials in the field of oncology currently underway worldwide, encompassing a variety of delivery agents, target indications and means of intervention. All therapeutic strategies that are being developed in the treatment of cancer ultimately aim to destroy cancerous cells whilst leaving normal cells unaffected.

This can be achieved by using one (or more) of the following approaches:

Expression of tumour suppressor genes

In many types of cancer, deactivating mutations arise in specific genes whose function is to prevent the uncontrolled growth of the cell. Genes that regulate cell division in this manner are called tumour suppressor genes (TSG); two examples include P53 and Rb. It has been found that re-introduction this type of gene into cancer cells initiates a series of events that results in the ultimate death of the cancer cell. Moreover, because normal cells of the body have the correct copy of these genes, the expression of a TSG in normal cells has no significant pathological effects. Therefore, it is generally considered that TSG expression in tumours is a safe way to specifically kill the cells that comprise the cancer. The only state-approved gene therapy treatment adopts this type of approach and is used in the treatment of both lung cancer and squamous cancer of the head and neck. This drug, called gendicine (gene medicine) and comprises an adenovirus expressing P53, was registered by the Chinese State Food and Drug Administration (SFDA) in 2003 and has been successfully used in the treatment of these two cancers, specifically when administered in conjunction with traditionally used chemotherapeutics.

Oncolytic viruses

For almost a century it has been known that certain types of virus can kill cancer cells if they are induced to replicate inside them. Research is currently being conducted by institutions around the world using both "non engineered" and "engineered" viruses to evaluate their use in the fight against multiple types of cancer. Non engineered viruses are naturally occurring viruses that innately preferentially target and replicate in certain types of tumor cells. Some non-engineered viruses include the Newcastle Disease Virus, Autonomous Parvovirus, and the Reovirus. Conversely, engineered viruses do not innately selectively target and replicate in cancer cells. Scientists must genetically modify ("engineer") the virus to selectively target and/or replicate within specific types of cancer cells. Today, there are three main approaches that are being explored in the development of engineered tumor-specific oncolytic viruses. Although the three approaches differ from one another, they all share a common goal—the destruction of cancer cells as a result of viral replication. The three approaches are as follows:

- Selective Targeting: Capsid Protein Modification: The capsid protein, the external surface of the virus, is modified so that the virus will specifically target cancer cells, completely avoiding normal cells. The virus would then replicate within the targeted cancer cell, ultimately leading to cell death.
- Selective Replication in the Absence of an Antitumor Gene: The virus is genetically modified so that it will replicate only in the absence of a gene believed to inhibit tumor cell growth, such as P53. While the virus "passes through" normal cells, it is triggered to replicate in cancer cells that do not exhibit an antitumor gene, ultimately leading to cancer cell death.
- Selective Replication in the Presence of Unique Tumor Cell Characteristic: The virus is genetically modified so that it will replicate only in the presence of a characteristic (e.g. an antigen) unique to the specific type of cancer. While the virus passes through normal cells, it is triggered to replicate in cancer cells that exhibit a specific characteristic, ultimately leading to cancer cell death. Cell Genesys' oncolytic virus product platform utilizes this approach.

Expression of genes that initiate cell death

Programmed cell death, known as apoptosis, is an essential cellular homeostasis mechanism that ensures correct development and function of multi-cellular organisms. The pivotal importance of correct execution of apoptosis is apparent from the many human diseases with aberrancies in apoptosis, including cancer. During cancer development, various imbalances can arise in the apoptotic machinery. Consequently, sensitivity towards apoptosis is progressively reduced, which ultimately leads to inappropriate cell survival

and malignant progression. However, it has become clear that cancer cells are often reliant on these aberrancies for continued survival. Perhaps counter intuitively, cancer cells can in fact be more prone to apoptosis than normal cells. The apoptosis-prone phenotype of cancer cells is masked and counterbalanced by upregulation of one or more anti-apoptotic mechanisms. Therefore, it is of enormous therapeutic interest to selectively tip the balance of the cellular fate of cancer cells towards apoptosis. Gene therapy can be employed to exploit this weakness in cancer cells by using recombinant gene transfer vectors to express genes that promote programmed cell death. These can either be the ligands that activate death receptor signalling, or the components of the apoptotic machinery that mediate these signals. At present there are wealth of clinical trials underway that exploit the sensitivity of cancer cells to pro-apoptotic signals and it represents an emerging sub-field within the gene therapy arena.

Gene-directed enzyme pro-drug therapy

In this approach tumour cells are killed by the specific action of a toxic drug that is produced from a non-toxic precursor by the action of an activating enzyme. In the clinical setting, an appropriate vector (typically viral) is first used to achieve expression of a prodrug-activating enzyme inside tumour cells. A nontoxic (or minimally toxic) prodrug is then administered and is converted to the active, toxic metabolite in cells expressing the activating enzyme. These cells are subsequently killed. Moreover, untransduced cells might also be killed following prodrug activation, by mechanisms that include direct transfer of activated drug through gap junctions, ingestion of apoptotic bodies from killed cells, effects on tumour vasculature, or immunological responses.

Immunotherapy

Immunotherapy, or the concept of boosting the immune system to target and destroy cancer cells, has been a goal of cancer treatment for over 100 years. Immunotherapy is used to stimulate the body's immune system against cancer. This can be achieved in many different ways. One example is the use of vaccines composed of antigens derived from tumor cells to boost the body's production of antibodies or immune cells (T lymphocytes) to fight the cancer. Typically in this approach, gene transfer vectors are used to deliver cancer antigens to the immune cells, either by engineering the patients own T lymphocytes out of their body and re-administrating them, or by simple injection of the gene transfer vectors encoding the antigens so that the patient's immune cells become infected in vivo. In a second example, a patients anti-tumour immune response can be boosted by the administration of cytokines (natural chemicals in our body that control our immune system) or antibodies that function to specifically attack cancer cells and stimulate the immune system to destroying the tumour.

CURRENT APPROACHES TO GENE THERAPEUTICS AND GENE THERAPY

Immunotherapy Using Recombinant DNA Constructs Expressing Cytokines and Lymphokines. An immune response against syngeneic tumours can be generated in animal models using a variety of tumours induced by chemical carcinogens and viruses. Tumour regression can result from manipulating the human immune response with interleukin2 (IL-2). The response rates of cancer patients to these immune manipulations are low and primarily confined to patients with melanoma and renal cell cancer. In addition, cytokines secreted by tumour cells into which cytokine gene-expressing recombinant DNA constructs have been inserted have elicited antitumor immune responses in preclinical (animal model) studies[11,12]. This suggests that the results of immunotherapy could be improved by the use of recombinant DNA tumour cell vaccines or by adoptive transfer of genetically engineered lymphocytes. A major advantage of this approach is the potential to generate a systemic immune response against the tumour.

Current protocols based on this idea use tumour-infiltrating lymphocytes (TILs), tumour cells, or fibroblasts to express cytokine genes. Initially, it was felt that TILs had a propensity to traffic specifically to tumour cells and,

therefore, that expression of cytokines by TILs might avoid the toxicity associated with systemically administered cytokines. Thus, the expression of cytokine genes, such as tumour necrosis factor (TNF), by adoptively transferred gene-transduced TILs could possibly be concentrated at the tumor site. Subsequently, however, this was disproved by a study that showed that tumours did not selectively take up or retain TILs marked with the neo gene.[13]

Another approach is to use autologous tumor cells transduced with a cytokine gene as a vaccine.[14-17] In many instances, however, tumor cells will not be available from patients, and even if available, the transduced cells may not express the cytokines. An alternative approach is to inject fibroblasts that have been engineered to express the cytokine gene. However, this approach also has its disadvantages: In one study, antitumor immunity was not induced by IL-2-expressing mammary stromal fibroblasts.[18]

Yet another approach is to make tumor cells more immunogenic. T cells recognize protein antigens after they are degraded into peptides that bind to histocompatibility complex molecules and are then transported to the tumor cell surface. Tumor cells, however, may be defective in their expression of class I or class II histocompatibility molecules, thus leading to defects in antigen presentation. Additional costimulatory molecules, such as B7-1 and B7-2, may be needed for effective induction of the efferent arm of the immune response to tumor antigens, but tumor cells may be defective in the expression of such molecules.[19] If so, then restoration of costimulatory gene expression might enhance tumor cell immunogenicity.[20] One substantial difficulty with this approach is the heterogeneity and unpredictability of loss of costimulatory molecules in human cancers. For example, one study of prostate cancer showed that cells vary in their loss of histocompatibility and transporter molecules. This approach may therefore require replacement of multiple genes within the tumor cell to elicit an effective immune response. It is also possible that, despite activation of the efferent arm of the immune response to tumor antigens, ineffective transport mechanisms may result in an antigen density too low to be recognized by the cytotoxic effector cells.

Another immunologic approach involves the use of vectors that express tumor-rejection antigens. Recently, immunodominant epitopes on human melanoma cells have been identified that are recognized by TILs and are associated with tumour regression.[21] However, as with the other approaches, there are potential difficulties. The immune response to an immunogen is clonal, but in tumours, antigen expression is heterogeneous.[22] Tumours also produce factors that suppress the immune response.[23]

Developing Gene Therapy for Cancers Using Virus Vectors

Food and Drug Administration (FDA) scientists successfully demonstrated in “test tube” and animal studies that it is possible to target specific cancer cells with viruses that might one day be used for gene therapy. Gene therapy is the use of genes to treat or cure disease by using normal genes to replace a defective one or to supplement the activity of a normal one.

The FDA scientists used viruses modified to carry on their surface a certain protein that binds specifically to another protein on the surface of the cancer cell. The viruses, called lentiviruses, bound to the cancer cells and released their genetic cargo into them, both in the test tube and in tumors growing in mice. For this study, the genes were not therapeutic; rather, they control production of an enzyme called luciferase, which triggers a chemical reaction that produces bioluminescence (light) under the right conditions. The bioluminescence, captured by a special imaging device, shows where the luciferase gene is located. Using this technique, the FDA scientists showed they could deliver a gene directly to tumor cells while avoiding normal cells.

The ability of the lentivirus vector (vehicle for transferring genetic material to a cell) to deliver the luciferase gene is important because it overcomes serious obstacles to using them for

gene therapy. First, it enables scientists to redirect a virus from its normal target cell so it targets the cell of interest, in this case a human glioblastoma (brain cancer) cell. In addition, the use of the modified virus vector avoided unintentional delivery of genes to other, non-target cells.

The FDA scientists, from the Center for Biologics Evaluation and Research (CBER), genetically engineered the lentivirus so it would make the H and F proteins from the measles virus and insert them onto the coating on its surface. The H protein normally helps the measles virus bind to the human cell it infects by latching onto a specific receptor on the cell. It then signals the F protein to trigger fusion of the virus to the cell so the virus can inject its genetic material.

To create the vector, the CBER scientists further engineered the lentivirus so a protein called interleukin-13 (IL-13) was bound to the end of the H protein. The IL-13 protein allowed the virus to bind only to cells that had large numbers of the so-called receptor protein for IL-13. While certain normal cells carry the IL-13 receptor, certain human cancer cells carry high levels (up to 50-fold higher compared to normal cells). Therefore, the modified virus attaches to the human cancer cell more efficiently than to a normal cell. The scientists showed that the lentivirus vector delivered its genetic cargo to cancer cells that were maintained in a laboratory culture. In addition, the vectors also found and attached to cells of the tumor that had been injected (xenografted) into mice, both under the skin and in the brain. The success of the lentivirus vector as a tool for ferrying genes into the glioblastoma cells suggests that similar vectors could be developed to target additional tumors that have on their cell surface IL-13 receptor or other specific receptors.

Types of viruses are used in gene therapy

Many gene therapy clinical trials rely on retroviruses to deliver the desired gene. Other viruses used as vectors include adenoviruses, adeno-associated viruses, and lentiviruses, poxviruses, and herpes viruses. These viruses differ in how well they transfer genes to the cells they recognize and are able to infect, and whether they alter the cell's DNA permanently or temporarily. Thus, researchers may use different vectors, depending on the specific characteristics and requirements of the study.

Scientists alter the viruses used in gene therapy to make them safe for humans and to increase their ability to deliver specific genes to a patient's cells. Depending on the type of virus and the goals of the research study, scientists may inactivate certain genes in the viruses to prevent them from reproducing or causing disease. Researchers may also alter the virus so that it better recognizes and enters the target cell.

Risks which are associated with current gene therapy trials

Viruses can usually infect more than one type of cell. Thus, when viral vectors are used to carry genes into the body, they might infect healthy cells as well as cancer cells. Another danger is that the new gene might be inserted in the wrong location in the DNA, possibly causing harmful mutations to the DNA or even cancer.

In addition, when viruses or liposomes are used to deliver DNA to cells inside the patient's body, there is a slight chance that this DNA could unintentionally be introduced into the patient's reproductive cells. If this happens, it could produce changes that may be passed on if a patient has children after treatment.

Other concerns include the possibility that transferred genes could be “over expressed,” producing so much of the missing protein as to be harmful; that the viral vector could cause inflammation or an immune reaction; and that the virus could be transmitted from the patient to other individuals or into the environment. Scientists use animal testing and other precautions to identify and avoid these risks before any clinical trials are conducted in humans.

FUTURE DIRECTIONS

Because oncolytic virotherapy is not yet a mature technology, there is plenty of room for improved treatment vectors. In order for virotherapy to be successful, viral particle production rates in the infected cancer cells must outstrip the growth rate of the uninfected cancer cells. This may be difficult to achieve with large established tumors and may mean that virotherapy must be combined with an existing therapy, such as surgery, to decrease the number of cancer cells in the initial treatment. In addition, the most effective treatment delivery method is yet to be determined. In preliminary studies, systemic injection required 1000x the viral load necessary to achieve results than injection intratumorally.

However, once these factors are overcome, there are many benefits to oncolytic therapy. The selective nature of the virotherapy ensures that healthy tissue will be minimally impacted. In addition, when combined with cytotoxic gene expression, this therapy can affect not only rapidly dividing cells, but those in the surrounding tissue making the microenvironment less favorable for cancer growth. The combination of the powerful killing nature of these vectors combined with the selectivity makes them an exciting avenue for lowering the number of cancer deaths.[24]

CONCLUSION

The field of cancer gene therapy is rapidly maturing and will no doubt be part of the future of cancer therapeutics. Several very exciting cancer vaccine treatments are in late stage trials, thanks to the advent of genetic engineering. In addition, gene transfer technology for cancer treatment holds great promise for increasing the effectiveness of current chemotherapeutic treatment regimens. Significant advances have been made in the field of oncolytic virotherapy, and trials are in progresses that incorporate this technique for precancerous, as well as cancerous treatment. Many of the past obstacles to treatment are being actively overcome and current second and third generation therapeutics are being tested. While not all the current trials will lead to a viable therapeutic agent, there is great hope that these advances will help relegate cancer to a manageable chronic disease without severe suffering and death.

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