REVIEW ARTICLE

GENE THERAPY IN SURGERY

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INTRODUCTION

Gene therapy is a new and emerging technology that employs the process of manipulating genes; the biologic unit of heredity.[1, 2] Essence involves the insertion of a desired gene (termed the transgene) into the recipient's cells. When considering the study of gene therapy, it is essential to make the distinction between therapies on somatic cells versus therapies on germ cells (gamete cells, which contain half the normal number of chromosomes and subsequently are capable of reproduction)(2). The essential difference lies in the fact that somatic gene therapy involves manipulating genes from an individual and is not inherited. Germline therapy involves manipulation of genes before the formation of an individual and is inherited.[3, 4] the first approved human gene therapy trial started on September 14, 1990. Researchers at the US National Institutes of Health removed white blood cells from the body of 4-year-old Ashanti DeSilva, born with severe combined immunodeficiency syndrome (SCID), a rare genetic disease. Her cells were cultivated invitro, the missing gene was inserted, and the genetically modified blood cells were infused back into her bloodstream. The therapy showed promising results as evidenced by Ashanti's reduced incidence of common colds and general improvement in health. [2, 25]

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PRINCIPLES OF GENE THERAPY

1) Gene Insertion: This technique relies on the random insertion of healthy genes into a defective genome to compensate for the damaged version. Insertion of a suicide gene coding for an enzyme responsible for converting a nontoxic pro- drug into a potentially lethal compound, killing both the recipient cell and its surrounding cells.

2) Gene Repair: Using this strategy the defective gene is repaired within the cell to restore the genetic code.

3) Gene Surgery: The defective portion of the gene is excised from the DNA, and the excised portion is replaced by its functionally normal counterpart. This would be the ideal form of therapy, and remains to date the ultimate challenge for investigators.

Methods of gene delivery:

The choice of gene delivery system (vector) is crucial. Constraints include the requirement for targeting specific tissues or organs, the necessity to maintain prolonged expression of the transgene. Potential side effects on the host, such as toxicity and immunization, must be prevented.

Non-viral methods (transfection):

<u>Chemical methods;</u>

Cationic liposomes: These are lipid bilayers that are rendered cationic (positively charged) and associate in a noncovalent fashion with negatively charged DNA to form liposome-DNA complexes. The rationale for coating nucleic acid with lipids is to allow highly negatively charged nucleic acid molecules to traverse the plasma membrane of the target cell. Lipofection is the term used to refer to gene transfer by this

mechanism (4). The advantages of lipofection include repeated application, lack of toxicity, and ability to carry large amounts of DNA. [5, 6]

Physical methods

Direct injection using a hypodermic needle: Injecting naked plasmid DNA solution directly to the target tissue to enable transgene expression. It is a desirable mode of transfection in that it is versatile and can be used for plasmid DNA solution and liposome DNA complexes, and it has a lower incidence of effects exhibited by some viral vectors, such as elicitation of an adverse immune response and insertional mutagenesis. [4, 5, 6, 7]

Microseeding; Microseeding is a technique for in vivo gene transfer whereby the plasmid DNA solution of choice is delivered directly to the target cells of the skin by a set of oscillating solid microneedles driven by a modified tattooing device.

Particle-mediated transfer (gene gun); Particle-mediated gene transfer using a force to accelerate DNA-coated particles initially was developed by Klein and colleagues in 1987 to deliver genes to plant cells. It employs the approach of bombarding cells and tissues with particles or microparticles coated with DNA. The microparticles are composed of gold or tungsten and measure 1 to 5 lm in diameter. When coated with the DNA of interest, the particles are loaded into a device, known as the gene gun, before being accelerated by a force (either an electrical discharge or high-pressure helium) that drives the particles into the target cell, where the DNA dissociates from the particles and is expressed.

Electroporation; The principle of electroporation rests on the ability of electrical field pulses to create pores in the cell membrane. Cells suspended in a medium containing plasmid DNA and treated with electrical field pulses take up and express DNA from the medium.[6, 7, 8, 9]

Viral methods (transduction): Viral vectors are generally more efficient. Once in the cell, they utilize the cellular metabolism of the host to complete their replicative cycle. Regions of the virus genome that are dispensable are deleted and replaced with the foreign gene(s) to be introduced into the cell. Potential hazards associated are tumorogenic state and clinical infection.[18]

Adenoviral vectors: The adenovirus attaches to a specific glycoprotein receptor (the Coxsackie adenovirus receptor) present on most mammalian cell membranes and enters the target cell through the internalization of membrane-bound vesicles (endocytosis). The virus persists in the nucleus without integrating into the genome. Certain key genes are deleted to ensure that the recombinant adenoviral vector is replication-incompetent and lysis-defective. Adenoviruses do not require host cell replication for them to enter the nucleus, and can carry a large amount of DNA. However, expression of the transgene is frequently transient (< 4 weeks), and can be associated with inflammatory reactions and/or an immune response, which compromises the long-term expression of the introduced gene.[18, 19, 22]

Adeno-associated viral (AAV) vectors: The Adeno-associated virus (AAV) requires co-infection with a helper virus, such as the herpes simplex virus or an adenovirus, to produce cell infection. AAV virus vectors have a wide host range; can carry several different transgenes into the host genome, even when the host cell is quiescent.[22]

Hybrid viral vectors: Hybrid viral vectors have been designed to exploit elements from different viruses. One virus may facilitate viral entry into the cell and the other may increase its ability to integrate transgenes into non-dividing cells.

Herpes simplex virus type-1: HSV-1 was described as the first genetically engineered virus for virotherapy for the treatment. It has the large genome that can be replaced with multiple therapeutic transgenes without eliminating its oncolytic ability. In addition, many anti-herpetic drugs are available as a safeguard against unfavorable replication of the virus. NV1066 is attenuated HSV mutant derived from strain F that has a deletion of the internal repeats.[21, 22, 23]

GENE THERAPY TECHNIQUES

The techniques by which all of these methods are applied to gene therapy can be categorized into two main disciplines. [23, 24]

In vivo gene therapy: In vivo gene therapy refers to the transfer of genetic material directly into non-manipulated target cells of the host.

Ex vivo gene therapy: Ex vivo, gene therapy involves prior harvesting and cultivation of cells, their transfection or transduction in vitro, and subsequent transplantation to the ultimate host.

MODES OF GENE THERAPY

Addiction gene therapy: The aim of this approach is to regulate tumour growth by introducing tumour suppressor genes that inactivate carcinogenic cells.

Gene therapy using oncolytic viruses: One of the most promising gene therapy approaches is the use of viruses that replicate only tumour cells, designated oncolytic viruses. This procedure emerged from the discovery of adenoviruses lacking E1B, which did not grow in normal cells but grew in cells without p53, one of the most common characteristics of tumour cells.[31, 32]

Suicide gene therapy: In suicide gene therapy, genes are introduced that stimulate the generation of products that are toxic for the cells. When suicide genes are used in retroviral vectors, protection measures must be taken against the appearance of oncogene-activating mutations. Suicide genes permit the expression of enzymes that can transform non-toxic drugs into cytotoxic substances. Thus, the thymidine kinase gene of Herpes Simplex Virus (HSV) transforms ganciclovir into ganciclovir phosphate.[33, 34, 35]

Suicide gene therapy can also use adenoviruses, although, as mentioned above, some characteristics of these vectors limit their efficacy and safety. Nevertheless, gene transfer of HSVtk gene (Herpes simplex virus thymidine kinase gene) via adenovirus vector in combination with ganciclovir administration may be a good therapeutic option.[33, 34]

One of the main drawbacks of suicide gene therapy is the poor distribution of the vector within the tumour. Although a relatively low anti-tumour response has been observed in clinical trials because of poor transfection efficacy, a high percentage of transfected cells do not appear in vivo due to their ability to induce tumour death.[35]

Introduction of genes to inhibit tumour angiogenesis: Research is in progress on the use of microencapsulated cells in antiogenic cancer therapy. The technique consists of the release of therapeutic proteins to encapsulate recombinant cells. Microcapsules are designed to be permeable to recombinant products and nutrients but not to host immune mediators, due to their large size. These cells are capable of secreting angiostatin, an important angiogenic inhibitor. Angiostatin receptors present ATP synthase on the surface of human endothelial cells, as in the case of αvβ3-integrin and vitronectin. This enables angiostatin to be localized in the tumour instead of organs near the implantation site of the capsule. However, this technique is not adequate for long-term removal of the tumour, especially when it is in an advanced stage. Moreover, it is a prolonged therapy that requires repeated doses and is associated with a high degree of toxicity.[39, 40, 41]

Immunotherapy: The aim of immunotherapy is to increase the patient's immune response to the tumour. The goal of cancer immunotherapy is to induce antibody and/or cytotoxic T lymphocyte (CTL) immune responses against cancer cells. Immunotherapy can be classified into two main categories; active immunotherapies (the stimulation of the immune system) and passive immunotherapies (the creation of immune cells). Active immunotherapies include specific active immunotherapy or vaccine therapy, which elicits hostspecific anti-tumor immune responses. Non-specific active immunotherapy or immunomodulatory therapy, on the other hand, elicits a general immune system response to cancer using cytokines, such as GM-SCF and interleukins. Passive immunotherapies include direct administration of monoclonal antibodies designed to target a specific receptor on the surface of a cancer cell.[33, 39, 40]

Another therapeutic approach may be to use the monoclonal antibody Anti-ICAM-2 alongside the intratumoural gene transfer of interleukin-12.[35]

Excision gene therapy: The aim of this therapy is to remove defective oncogenes, thereby inhibiting the growth of tumour cells. In the medium- and long-term, it may contribute a definitive treatment for oral cancer and precancer that offers greater effectiveness compared with current therapies and markedly reduces the high mortality associated with these lesions. At present, the use of adenoviruses to act at altered gene level and the combination of this technique with chemotherapy or immunotherapy appear to be the most promising approaches to the management.[34, 35, 40]

ETHICAL ISSUES:

The use of gene therapy raises numerous ethical issues, not only those pertaining to the HGP, but also issues concerning the running of clinical trials. Pertinent dilemmas raised by the formation of a genetic database include who should have access to such information.

-Should the data be classified alongside medical records and subject to the same confidentiality laws, or should employers and insurance companies have a right to access?

-More importantly, is it right to subject patients to information about their possible future disease pattern?

-Should this also be the case if no current therapy is available for cure?

-Is it right that the tens of thousands of human gene patents pending are granted to researchers or companies?

In an attempt to address these concerns, many regulatory bodies have been established to overlook the processes involved and enforce some basic principles. The Recombinant DNA Advisory Committee was one such initiative drawn up by the NIH, and the Food and Drug Administration (FDA) focuses on safety and efficacy of genetically altered products (80). Regulations were established whereby research proposals went through a review process consisting of a preliminary approval by the home institution's Institutional Biosafety Committee and Institutional Review Board before a final approval from the Recombinant DNA Advisory Committee.[34, 35, 36, 39, 40]

APPLICATIONS OF GENE THERAPY IN SURGERY

GENE THERAPY IN WOUND HEALING:

The skin is an attractive target for gene therapy for many reasons. The predominant cells of the skin, fibroblasts and keratinocytes, are harvested easily, and protocols exist for their successful culture. This situation not only enables skin cells to be tested in vitro, but also highlights their availability for use as vehicles in ex vivo protocols, because such cells readily can be transplanted back to a host. The superficial location of the skin enables it to be monitored easily for any adverse effects or reactions and renders it accessible to direct DNA transfer by many techniques, including injection, microseeding, and topical application, avoiding unnecessary systemic delivery.[47, 48, 49, 51, 61]

Transcription factors also have epigenetic applications for wound healing. Hypoxia inducible factor 1 is a protein that activates the transcription of hypoxia-inducible genes by binding to a hypoxic response element in the gene promoter. Genes that are regulated by hypoxia inducible factor 1 include VEGF; provision of a transcription factor gene could activate multiple members of the VEGF family of growth factors, inducing revascularization and enhancing wound healing. Exogenous TGF-b upregulated TGF-b mRNA and a1procollagen in keloid-derived and normal fibroblasts and that blocking TFG-b signaling in keloid cells could down regulate collagen gene expression, serving as a potential therapeutic strategy for keloids.[51, 52, 54, 56, 57, 58]

GENE THERAPY IN BRAIN TUMOURS: Although the brain is an area of interest for only a few surgeons, research in this area has provided us with the foundations for genetic therapy. The methodology developed for the treatment of brain tumors has now been adopted

for malignancies in other solid organs. Although traditional approaches such as surgery, radiotherapy, and chemotherapy have improved the quality of survival for many patients, the median survival after diagnosis and adjuvant treatment still remains only about a year (15, 17). Recently, genetically engineered viruses for targeted cell killing have been used successfully in the experimental treatment of gliomas.[15, 17, 34, 35]

GENE THERAPY IN ORAL CANCERS:

OSCC is a good candidate for gene therapy because primary and recurrent lesions are readily accessible for injection or application of the agent.

Addiction gene therapy: Numerous studies have described p53 alteration as an early event in oral cavity carcinogenesis, and mutated p53 expression is frequently observed in noncancerous epithelium adjacent to OCSS.

The percentage of epithelial cells expressing mutated p53 is usually higher with greater severity of the epithelial disorder. For these reasons, one of the tumour suppressor genes most commonly used in gene therapy is the p53 gene, and numerous viral vectors, especially adenoviral vectors, have been developed for its application.

A phase III study is currently under way on adenovirus vector Ad5CMV-p53. This is applied by intramucosal injection followed two hours later by a mouthwash. From the next day, it is administered as a mouthwash twice a day for 2-5 days. This treatment is repeated every 28 days and has shown a capacity to inhibit disease progression in precancerous lesions with no toxic effects. Other tumour suppressors introduced into tumour cells by gene transfer are Rb (retinoblastoma gene) and mda7 (melanoma differentiation-associated gene-7).

Gene transfer of gene p27 was found to inhibit the cell cycle of tumour cells, inducing apoptosis and triggering the suppression of tumour growth. It has been demonstrated that gene p27 mutations are highly related to the appearance of tongue cancer. According to these results, the therapeutic use of p27 gene may in the future prove useful for the treatment of OSCC.

Gene therapy using oncolytic viruses: :Adenovirus ONYX-015, which presents deletion of the E1B region, has been used to control OSCC lesions.The release of an oncolytic herpes virus in a primary tumour after its surgical excision appears to significantly reduce the tumour and regional metastasis . Patients with precancerous lesions were treated with Advexin® mouthwash (Introgen Therapeutics, Inc (INGN), NY), which also administers p53 by means of an adenovirus.[18, 19]

Suicide gene therapy: The thymidine kinase gene of Herpes Simplex Virus (HSV) transforms ganciclovir into ganciclovir phosphate. Gene transfer of HSVtk gene (Herpes simplex virus thymidine kinase gene) via adenovirus vector in combination with ganciclovir administration may be a good therapeutic option for OSCC

Introduction of genes to inhibit tumour angiogenesis: Investigators are also developing vaccines against receptor 2 of the VEGF factor (Vascular Endothelial Growth Factor), also known as FLK-1, with the resulting inhibition of angiogenesis, tumour growth and metastasis. This vaccine also appears to be useful in the treatment of tongue metastasis of OSCC.

Immunotherapy: Patients with OSCC present altered function of immune cells, including NK cells, T lymphocytes and numerous cytokines. Thus, animal studies have shown that IL-2 administration activates T lymphocytes and NK cells and that these in turn activate tumour necrosis factor α (TNF-α), triggered by the strong tumour inhibition effect.

The monoclonal antibody Anti-ICAM-2 alongside the intratumoural gene transfer of interleukin-12. ICAM-2 is a glycosylated protein with surface adhesion that is expressed in endothelial cells and activates lymphocytes. Recent studies found that systemic administration of Anti-ICAM-2 induced the complete regression of OSCC lesions.[18, 19, 33, 38]

GENE THERAPY IN ESOPHAGEAL CANCER:

A number of specific genetic alterations have been identified in esophageal cancer. These genetic events include amplification and/or overexpression of oncogenes, mutations and deletions leading to inactivation of tumor suppressor genes. The identification of these molecular alterations has allowed the and adenocarcinoma developed in Barrett's esophagus and the central role of p53 in regulating growth and apoptosis.[17, 18, 32]

THE p21WAF1-replacement gene therapy antitumor effect of exogenous expression p21WAF1 in esophageal cancer cells (Fujii et al, 2001). Adenovirus mediated expression of exogenous p21WAF-1 effectively reduced cell growth in cell lines with high cell growth ratio (CGR). p21WAF1-mediated growth suppression was associated with the induction of involucrin, a marker of squamous cell differentiation.

Immunogene therapy: Strategies using genetic methodology have been applied including cytokine gene transfection using viral vectors and cytokine plasmid DNA transfer using electroporation or cationic multilamellar liposomes IL-2 ,GM-CSF ,IFN-beta ,IL-21 and -23 and TNF-!, either alone or in combination with other strategies, have shown an antitumor immune response which can be demonstrated by reduced tumor volume,

Suicide gene therapy: The most frequently used suicide gene therapy protocol is the Herpes simplex virus type 1 thymidine kinase (HSV-TK)/ganciclovir (GCV) system for oral cancers.[18 ,19 ,53, 54, 70, 98]

GENE THERAPY IN BREAST CARCINOMA:

Breast cancers expressing oestrogen receptors are responsive to antioestrogen treatment and have a better prognosis than do oestrogen receptor- negative tumors. The loss of oestrogen and proges- terone receptor expression is associated with pro- gression to less well-differentiated tumors. Trans- fection of the human oestrogen receptors into an oestrogen development of new therapeutic approaches targeting specific differences between normal and malignant cells.

The p53-replacement gene therapy Transfection of wt-p53 is an appealing therapeutic strategy for esophageal cancer because of the high frequency of p53 mutations in both squamous cell carcinoma

receptor-negative metastatic breast can- cer cell line resulting in the expression of the oestrogen receptor gene can therefore be considered as a potential therapeutic approach to hormone-independent cancers.[16]

Viral vectors containing a human IL-2 gene were used to transduce a mouse mammary tumor cell line and a nontumorigenic stromal (fibroblast) cell line. The results successfully demonstrated a favourable antitumor response when the helper cytokine was secreted by the tumor cell line vis-~t-vis IL-2 gene-transduced stromal cells.[16, 17, 18, 19, 31]

GENE THERAPY IN LIVERDISEASES:

Because of the central metabolic role played by the liver, many hepatic diseases have become an attractive target for a gene therapy approach. Two strategies have emerged. Ex vivo, gene therapy entails the transplantation of autologous hepatocytes transduced while in culture. Hepatocytes can therefore be readily infected in vitro and subsequently grafted back into the recipient mammal by infusion of cells directly into the portal vein or into the spleen from where they migrate with reasonable efficiency to the liver.[32]

An alternative strategy uses recombinant viruses to deliver the transgene directly to hepatocytes in vivo. The procedure involves a partial hepatectomy followed by portal vein infusion of recombinant retroviral vectors. The partial hepatectomy stimulates regeneration and hence subsequent uptake of retroviruses into dividing cells during this phase. In situ transduction has also been achieved by directly injecting the packaging cells into an estabilished macroscopic metastasis. Packaging cells release retroviral virions that consequently integrate

with the tumor genome. The HSV1-TK gene used in a cognate manner expresses a high concentration of TK, which in turn reduces a nucleoside analogue (ganciclovir) into its active triphosphate form, causing chain termination and subsequent cell death. This study resulted in a near total dissolution of the liver metastasis. Receptor-mediated DNA delivery system utilising the endosomal lysis ability of adenoviruses have shown that DNA can also be delivered into primary hepatocytes, resulting in a high level of gene expression.[31,32,33]

GENE THERAPY IN RENAL TUMOURS:

More recently, a human renal carcinoma line has been transfected with IL-2 and/or interferon a (IFN-(x) gene(s). All the cytokine-producing cells lost their tumorogenicity, and the local production of high concentrations of IL-2 and IFN-a at the tumor site was found to be more effective in preventing tumor growth than was systemic administration of these cytokines. Thus, continuous local delivery of cytokines via transfer of cytokine genes into tumor cells for use as live cancer vaccines is a novel potential strategy for the manipulation of the host-mediated antitumor immune response, and this principle could be extrapolated toward treating patients with advanced renal cancer.[13, 16, 34]

GENE THERAPY IN COLONIC CANCERS

As the first step toward the development of gene therapy treatment for metastatic colorectal carcinoma, the Escherichia coli gene that encodes cytosine deaminase (CD) was cloned and transfected into a human colorectal carcinoma cell line. The expression of CD in eukaryotic cells allows the me- tabolism of the nontoxic prodrug 5-fluorocytosine (5-FC) to the toxic metabolite 5-fluorouracil. This could be utilised as a suicide gene to treat in vivo models of colorectal carcinoma. Monoclonal antibodies can act with both macrophage and natural killer lymphocytes in mediating cellular cytotoxicity against tumor cells. Human colon carcinoma cells have been transduced using monoclonal anti- body genes, thus making them sensitive to immune destruction through coexpression of both monoclonal antibody gene and its reactive antigen. Alternatively, intestinal epithelial cells possess specific low-density lipoprotein (LDL) receptors that can be exploited to accomplish drug delivery and gene transfer via the receptor-mediated endocytosis pathway.[34, 35, 36, 72, 98]

GENE THERAPY IN VASCULAR DISEASES

The third major category addressed by gene transfer studies is cardiovascular disease. Initial experience was in trials designed to increase blood flow to either skeletal (critical limb ischemia) or cardiac muscle (angina/myocardial ischemia). First-line treatment for both of these groups includes mechanical revascularization or medical management, but a subset of patients are not candidates for or fail these approaches. These patients formed the first cohorts for evaluation of gene transfer to achieve therapeutic angiogenesis. The major transgene used has been VEGF, attractive because of its specificity for endothelial cells; other transgenes have included fibroblast growth factor (FGF) and hypoxia-inducible factor 1, α subunit (HIF-1α). The design of most of the trials has included direct IM (or myocardial) injection of either a plasmid or an adenoviral vector expressing the transgene. Both of these vectors are likely to result in only short-term expression of VEGF, which may be adequate because there is no need for continued transgene expression once the new vessels have formed. Direct injection favors local expression, which should help to avoid systemic effects such as retinal neovascularization or new vessel formation in a nascent tumor. Initial trials of adeno-VEGF or plasmid-VEGF injection resulted in improvement over baseline in angiographically detectable vasculature, but no change in amputation frequency or cardiovascular mortality.[27, 28, 29, 30]

GENE THERAPY FOR PANCREATIC CANCER

A variety of cancer gene therapies have been developed and some of them are already being employed in clinical trials. As most conventional treatment approaches, with the exception of curative surgical resection, are not effective for pancreatic cancer, new therapeutic approaches are expected to offer alternative

strategies. Adenoviruses in which the E1A and E1B regions (collectively known as E1) are replaced by a therapeutic gene expressed by a promoter or enhancer are being widely employed as a new strategy for gene therapy. The widely known tumor suppressor gene, TP53, is used for cancer gene therapy because it induces apoptosis in cancer cells.25 Interleukin 12, a key cytokine not only about the cell-mediated immune response26 but also in regard to exerting strong antiangiogenesis effects on tumor neovascularization, 27 is also being utilized for immunogene therapy. In general, however, the effect of these gene therapy approaches is limited, because replication-defective vectors (E1A deleted adenoviruses) infect only a small fraction of the cells in the tumor. Effective cancer gene therapy requires an efficient and reproducible method of in-vivo gene delivery. Increasing the titer of adenovirus is an alternative method that leads to higher expression of genes in a solid tumor. However, this strategy may cause adverse effects. To avoid the adverse effects in normal cells, gene therapy that specifically targets cancer cells has been developed, using characteristic promoters for cancer cells such as Alpha fetoprotein (AFP) and Prostate-specific antigen (PSA)(31,32,33).Gene therapy that targets TP53 pathway abnormality. As TP53 is functionally inactivated in many human tumors, including pancreatic cancer, one strategy to impede the growth of such tumors, is through transduction of the wild-type TP53 gene to cancer cells, using a viral vector. However, one of the limitations of this strategy, and of other cancer gene therapies, is the low efficiency of gene delivery in vivo, especially into solid tumors such as pancreatic cancer.[31, 32, 33, 34, 36]

GENE THERAPY IN MELANOMA

Over the past decade, immunotherapy and gene therapy have been investigated in the treatment of malignant melanoma. Advances have occurred in several areas, including the use of monoclonal antibodies, alone or in combination with cytokines; tumor vaccines, using whole cell preparations or cloned melanoma antigens; adoptive immunotherapy, with tumor-infiltrating lymphocytes (TILs) and cytotoxic T-lymphocytes; and lately gene therapy. Gene therapies have been designed to increase the immunogenicity of the tumor, increase the effectiveness of the TILs, or alter the basic mechanisms of tumor cell growth and regulation. Insertion of genes can be used to increase the expression of various cytokines, namely interleukin (IL)-4, IL-2, tumor necrosis factor (TNF), ~/-IFN, and granulocyte-macrophage colony-stimulating factor. For the treatment of melanomas, a similar principle has been exploited.[16, 18, 19, 93, 99, 103]

FUTURE PROSPECTS

The concept of a genetic switch is another exciting development. Regulation of transgene expression in target cells is a crucial and challenging aspect of gene therapy. Many switches have been developed that have been modified to maximize their safety and ease of use. The tetR system (agenetics witch controlled by adoserelated response to the presence or absence of tetracycline) invitro and invivo enabling the manipulation of timing and level of expression of a transgene. Combinations of growth factors or their sequential use may be the future answer to accelerating wound healing processes, and with the advent of this therapy, these genetic switches will become increasingly important. As discussed earlier, developments in matrix components and tissue engineering technology offer promise for the future. The development of slow-release matrices, which may prolong transgene expression.[34, 35, 36, 60, 67]

Gene therapy approaches allow for the introduction of enhancers of healing and inhibitors or down regulators. This concept led to the development of epigenetic approaches to wound healing and, specifically, antisense RNA technology. These techniques do not involve the introduction of a DNA sequence that would be used to translate a protein, but rather a sequence that would modify the cell's ability to express its own endogenous genes. The introduction of antisense cDNA to a growth factor would block the cell from translating that protein.[34, 35, 60, 67, 106]

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