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Review

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Gene Therapy in Surgery

Part I: Methods for Gene Transfer – Application to Cancer*)

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Key-words: Gene therapy – cancer – septic shock – organ transplantation.

Schlüsselwörter: Gentherapie – Karzinome – septischer Schock – Organtransplantation.

Summary: Background: With the increasing body of knowledge in molecular biology, gene transfer respectively gene therapy becomes more and more a valid therapeutic option.
Methods: This is a critical review of gene therapy protocols for treatment of different types of cancer. Furthermore, the pathophysiological mechanism, therapeutically strategies as well as experimental approaches toward gene transfer in septic shock and organ transplantation are critically elucidated.
Results: Gene transfer as a therapeutic option was first successfully applied in children with severe combined immunodeficiency (SCID) in 1990. The majority of gene marking or gene therapy protocols approved for human clinical trials to date are related to the treatment of cancer. Besides viral vectors for brain tumors, non-viral vectors, liposomes particularly, with almost no side effects are increasingly used.
Conclusions: Different approaches of gene transfer in cancer patients are under investigation. Experimental data of septic shock treatment and rejection therapy of the allograft in organ recipients with gene transfer are encouraging for future applications in clinical trials.

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Gentherapeutische Strategien in der Chirurgie

Teil I: Methodik des Gentransfers – Anwendung bei Krebserkrankungen

Zusammenfassung: Grundlagen: Die Fortschritte in der Molekularbiologie zusammen mit der Erforschung des „Humanen Genom"-Projektes lassen den Gentransfer bzw. die Gentherapie zunehmend als therapeutische Option bei verschiedensten Erkrankungen Eingang gewinnen.
Methodik: Die folgende Arbeit stellt einen kritischen Überblick über die klinischen Protokolle der Gentherapiestudien bei Karzinomen dar. Weiters werden der pathophysiologische Mechanismus, die verschiedenen Therapieansätze sowie der erfolgreiche, experimentelle Gentransfer im septischen Schock wie auch bei der Organabstoßung nach Transplantation dargestellt.

*) **Part II:** "Application to septic shock and to organ transplantation" will be published in Acta Chir Austriaca 1997; 29: Issue 1. References are listed at the end of part II.

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Ergebnisse: Der erste erfolgreiche Gentransfer wurde bereits 1990 bei Kindern mit „schwerem kombinierten Immundefekt" (SCID) durchgeführt. Die meisten Gentransferprotokolle behandeln verschiedene Karzinome. Neben viralen Vektoren, die sich besonders gut für nichtproliferierende Gewebe, z. B. zentrales Nervensystem, eignen, werden zunehmend nichtvirale Vektoren, insbesondere Liposomen verwendet.

Schlußfolgerungen: Neben der bereits erfolgreichen Anwendung des Gentransfers bei der Behandlung verschiedener Malignome, lassen experimentelle Daten auch einen erfolgreichen Einsatz des Gentransfers beim septischen Schock und bei der Abstoßungstherapie nach Organtransplantation erwarten.

Introduction

Advancements in molecular biology, which encompasses the study of the genetic basis of disease, have produced new **diagnostic methods** (e.g., DNA tests for the rapid detection of microorganisms like hepatitis-C virus) and **drug therapies** for both congenital and acquired diseases. The growth in the understanding of human genetics also led to the initiation of the first human gene therapy 6 years ago at the National Institutes of Health, USA (1).

Gene therapy is the insertion of a functioning gene into the cells of a patient to correct an inborn error of metabolism (i.e., genetic abnormality or birth defect) or to provide a new function in a cell (e.g., insertion of an immunostimulatory gene into cancer cells to vaccinate patients against their own cancer). This is a very broad definition that includes the potential treatment of all genetic disorders, as well as cancer, infectious diseases, and auto immune disorders through the genetic modification of cells of the human body to prevent or eliminate disease. This article should demonstrate and elucidate potential future applications of gene therapy in surgical patients.

Methods for gene transfer

A number of methods have been developed for introducing genes into living cells. Available gene transfer techniques include chemical, physical, and fusion methods, receptor mediated endocytosis and recombinant virus vectors (Table 1). The **direct injection of DNA** in vivo can be attempted in a number of fashions:

1) Direct injection of "naked" DNA (not coated or bound to antibody, protein, or lipid) directly with a syringe and needle into specific tissue (e.g., muscle), or infused through a vascular bed (2,3).

2) Direct injection of DNA that is contained in artificially generated lipid vesicles, or liposomes (4, 5). The lipid coating allows the DNA to survive in vivo, bind to cells, and be endocytosed into the cell.

3) Direct injection of DNA conjugated to a carrier (e.g., antibodies to a specific cell surface moiety or attachment to asialoglycoprotein to target the DNA to receptors in the liver). These techniques are under development for use as antibody targeting for cancer therapy and for genetic manipulation of the liver (6).

The advantage of these direct injection systems is their simplicity, as compared to the production of recombinant virus vectors, thus diminishing the potential risks and the expense of the procedure. The disadvantages of direct injection methods are not so much a poor gene transfer efficiency any more, but rather a low level of stable integration of the injected DNA, if desired. Long-term expression of non-integrating vectors will be a problem, especially in the proliferating cell types, and repeated injections may be required. However, in cell types that do not regularly proliferate (e.g., muscle), the injected DNA may continue to express its genes for months (7).

There are several circumstances in which the direct DNA gene transfer method have a place in clinical gene therapy application. First, this form of gene transfer may be used effectively in cancer therapy in which transient expression in tumor cells may be sufficient to induce tumor cell destruction (e.g., insertion of cytokine or foreign HLA genes to enhance tumor immunogenicity) (8–12). Second, it might be useful for repeated injection into non-proliferative target tissues if permanent expression is not absolutely required (e.g., hormone production) (13). Third, the direct injection of DNA into cells of the skin might be a useful method for immunization. Animal studies have shown that the insertion of the DNA into skin cells with particle bombardment results in transient expression of the gene by the cell inducing a systemic immune response (14).

Table 1. Transfection vectors.

Method	Application in gene therapy		Transient (T) or Stable (S) Expression
	ex vivo	in vivo	
Viral	Yes (Y)	No (N)	
Retrivirus	Y	?	S
Adenovirus	Y	Y	T
Adeno associated virus (AAV)	Y	?	
Herpes virus	Y	Y	?
Vaccinia virus	Y	Y	T
Polio virus	Y	Y	T
RNA viruses	Y	Y	T
Non-viral			
Ligand DNA conjugates	Y	N	T
Adenovirus ligand DNA conjugates	N	Y	T
Lipofection	N	Y	T
Direct injection of DNA	N	Y	T
$CaPO_4$	Y	N	S

Recombinant virus vectors are so far the most commonly used method for clinical gene transfer protocols. Virus vectors are produced by replacing the viral genes required for the production of replication competent viruses with the genes desired for transfer, creating replication incompetent viruses. This switch of genetic material renders the virus unable to produce a productive viral infection while maintaining its ability to bind to the cell surface. A variety of different viruses are currently used. The **Moloney murine leukemia virus** (MoMLV) vectors has the advantages of high efficiency gene transfer, approaching 95% in some cell types in vitro and stable integration into the target cell genome. The potential disadvantage of retroviral vectors is the fact that they integrate randomly into the host cell genome. In other words, these vectors insert into a different place in one of the chromosomes in each cell. Random integration provides for the possibility that the integration event could occur within a gene that is absolutely required for normal cellular function or survival. In such a case, the transduced cell might be killed. However, a more serious theoretical problem with random integration may result if vector insertion results in the activation of an oncogene or inactivates a tumor suppressor gene. Either of these events could potentially contribute to the eventual transformation of that cell to a malignant phenotype. Induction of oncogenesis by this method is termed "insertional mutagenesis".

Among other virus vectors which are commonly used are the **Adenovirus vectors,** the **Adeno-associated virus (AAV) vectors** and the **Herpes simplex virus (HSV) vectors.** The adenovirus has a natural tropism for respiratory epithelium, the cornea and the GI tract. Therefore this vector system has been developed primarily for transfer of the cystic fibrosis transmembrane conductance regulator (CF TR) gene into airway cells as a treatment for cystic fibrosis. The primary advantage of adenovirus vectors are:

1) High-efficiency gene transfer regardless of the proliferative state of the tissue. Whereas the retrovirus will only insert its genes into dividing cells, the adenovirus vector will transfer genes into all cell types.

2) These viruses can be effectively delivered by an aerosol.

Table 2. Clinical protocols for the gene therapy of cancer, different approaches.

Major categories
1. Insertion of a cytokine gene into tumor cells in vitro; e.g. basically tumor cell vaccination with (IL-2, IL-4, TNF, GM-CSF, IF-G).
2. In situ injection of plasmids carrying a HLA gene into a tumor mass that is negative for the injected HLA gene; e.g. HLA-B7 gene into B7-negative melanomas.
3. Insertion of a suicide gene into tumor cells in situ with subsequent activation of the suicide mechanism; e.g. HSV-TK gene.
4. The use of tumor suppressor genes and/or anti-oncogenes; e.g. insertion of p53 gene in non-small cell lung carcinomas that are p53 defective.
5. The use of multidrug resistance (MDR) gene to protect bone marrow cells to allow higher-dose chemotherapy.

3) There is no concern about inducing malignancy. The disadvantage of the use of adenoviral vectors is that they appear to be able to infect nearly all cells, expressing their genes in each infected cell. This lack of discrimination could result in toxicity in normal surrounding cells. Furthermore, the repeated administration of the adenovirus may induce a specific antiviral immune response that will limit the effects of repeated administration of the therapeutic adenovirus vectors.

The Adeno-associated virus is considered "dependent" because it requires coinfection with another virus such as adenovirus or herpes virus to produce a productive infection. Integration of AAV is not known to be associated with any alterations in cell growth.

The Herpes simplex viruses have the unique advantage of being tropic for the central nervous system, establishing life-long latent infections in neurons. There is a two pronged approach for the treatment of CNS tumors with HSV (see below).

Human gene therapy-ongoing clinical trials

The first human gene therapy experiment was initiated in 1990 for the treatment of a rare, congenital immunodeficiency disorder called adenosine desaminase (ADA) deficiency 1. ADA is an autosomal recessive disorder. Children afflicted with complete ADA deficiency will have a significant deficit in immune function. These children do not produce normal antibody responses to immunization with standard childhood vaccines, as a result, they are unable to completely resolve viral infections such as common respiratory or gastrointestinal infections. For ADA deficiency children without an identical matched marrow donor, ADA enzyme replacement therapy is currently used (15, 16). This successful experiments have provided a foundation for the new era of genetic healing, demonstrating that gene transfer can provide beneficial effects in patients. In the meantime many other diseases are treated by gene therapy. Hemophilia B (17), Gaucher disease (18), HIV-1 infection (19), respiratory manifestations of cystic fibrosis (20), Duchenne and Becker muscular dystrophy (21), and ornithine-transcarbamylase deficiency (22) among others, are currently targeted by gene transfer experiments.

Application of gene therapy to cancer

Major advances in our understanding of how cancer occurs have shown that cancer is a genetic disease resulting in the abnormal proliferation of cell clones. In general, a recessive mutation correlates with the loss of a function, such as a tumor suppressor gene, while a dominant mutation correlates with the gain of a function, such as an oncogene. This understanding of the genetic basis of cancer allows entirely new approaches for the treatment of cancer. For instance, the deletion of tumor suppressor genes could theoretically be corrected by the insertion of the normal copy of the gene. Likewise, the overexpression of an oncogene could be blocked at the genetic level by the insertion of an antisense gene that would bind to the oncogene, disabling its ability to express. In fact there is a wide variety of potential uses of gene therapy for the treatment of cancer, with more developing monthly (Table 2).

Another approach to the genetic therapy of cancer is gene transfer into T lymphocytes (23). Because T lymphocytes are critical for the prevention and elimination of tumors, investigators are growing lymphocytes from tumor biopsies for use as a delivery vehicle for genes directly to tumor deposits in situ. Investigators first wanted to determine whether tumor-derived T lymphocytes would find their deposits (24). These early studies, which involved gene marking of tumor infiltrating lymphocytes (TIL), demonstrated that the TIL may be "home" to metastatic melanoma tumor cell deposits. A similar trial is in progress in patients with renal cell carcinoma (25). In further series of experiments, researchers reasoned that the insertion of the tumor necrosis factor-α (TNF) cytokine gene or more recently the Interleukin 2 (IL-2) cytokine gene into TIL might increase their antitumor efficacy. TNF and IL-2 are proteins normally produced by activated macrophages and if infused in sufficient amounts in mice, it might contribute to the destruction of tumors. Unfortunately, the intravenous infusion of these cytokines in primates have significant, shock-like side effects, significantly limiting the maximum tolerated dose (26). The initial experiment with TNF gene-modified TIL attempts to use genetically altered T lymphocytes to deliver high concentrations of TNF directly to the tumor. The hypothesis is that following an intravenous (i.v.) infusion of TIL expressing a TNF retroviral vector, these TIL will migrate back to tumor deposits in the body, delivering a high concentration of TNF directly to the tumor with minimal systemic side effects compared to i.v. TNF therapy. The pace of this human experiment has been slowed due to the poor efficiency of gene transfer into human TIL and a down regulation of cytokine expression by the TIL.

The idea for the genetic modification of the patient's own tumor cells for use as a vaccine was initially received with some skepticism. Nonetheless, human clinical trials have already been initiated to study the immunobiology of this type of immunization systems in humans (27). These experiments are an attempt to immunize tumor-bearing patients specifically against their own tumor by genetically altering their tumor with one of a variety of genes that are expected to increase the host immune reactivity to the tumor. These "tumor vaccines" are produced by surgically removing the tumor from the body and making genetic alterations with in vitro insertion of TNF, IL-2, IL-4, IL-12, or transforming growth factor-β1 (TGF-β) cytokine genes in the tumor. Once the tumor has been shown to produce large quantities of the inserted gene product, the tumor cells are reinjected subcutaneously into the patient. Booster injections of cells are commonly administered at 2-week intervals. Melanoma, colorectal cancer, and renal cell carcinoma have been the primary focus of the tumor vaccine studies because they are believed to be more immunogeneic than other tumors and may therefore be more likely to respond to this type of therapeutic approach. Most protocols have used autologous tumor cells (27, 28), while a few centers are using allogeneic HLA matched tumor cells (11, 29).

The genetic modification of tumors in situ involved the direct injection of liposomes (DNA wrapped in lipid) (see above and our own experience below) and the in vivo gene transfer with murine retroviral vectors (30). Foreign antigens are being used to increase the immunogeneicity of melanoma tumor cell deposits using the direct injection of liposomes containing the HLA-B7 gene (31). The liposomes are taken up by the tumor cells by endo- and/or phagocytosis. The DNA then expresses the foreign HLA antigen (B7) transiently on the surface of the cancer cells. Animal studies have shown a significant increase in the antitumor immune response when some of the tumor cells express foreign antigens on the cell surface. This use of DNA-containing liposomes is the first use of non-viral-mediated in vivo gene transfer in humans. No untoward side effects were noted. This result suggests that the delivery of genes in situ by liposomes have great potential which convinced us to use the same technique for our own experiments (see below). In an effort to in-

crease the anti-tumor effect, the β2-microglobulin gene has been added to HLA-B7 in this gene transfer system.

Murine fibroblasts that are producing retroviral vectors (retroviral vector producer cells or VPC) are directly implanted into growing brain tumors in human patients. The gene being transferred into the surrounding brain tumor cells is the herpes simplex-thymidine kinase (HS-tk) gene, which confers a sensitivity to the anti-herpes drug, ganciclovir or GCV (Cymevene®, Grünental, Austria). GCV is phosphorylated by the HS-tk enzyme within the cell. Other cellular kinases convert ganciclovir-monophosphate to the triphosphate form which results in cell death. This retroviral-mediated in vivo gene delivery system selectively targets proliferating cells. Since the CNS is relatively quiescent, brain tumors became a logical choice as a first application (32). The direct injection of HS-tk vector producing cells into the tumor mass so results in selective killing of tumor cells without damage to surrounding normal tissues. Thus, the first protocol for human gene therapy with the above mentioned approach for patients with recurrent glioblastoma multiforme is under investigation in Austria (personal communication). These brain tumor gene therapy experiments represent the first use of virus-mediated in vivo gene transfer in humans.

The therapeutic use of enzymes linked to a CEA-promoter gene which can activate pro-drugs into their biologically active forms is discussed in a review article by *Mullen* (33). In this article the author reports about uses of metabolic suicide genes which can convert a relatively non toxic pro-drug in a highly toxic agent. Cells genetically transduced to express such genes essentially commit metabolic suicide in the presence of the appropriate pro-drug.

And already there are further strategies of cancer treatment modalities on the horizon. This exciting gene therapy experiments are going to be challenged by a new series of experiments with **"Anti-sense and Triplex-DNA"** therapies. Both strategies are aiming to stop the production of pathogenic proteins by either blocking transcription (triplex) or translation (antisense) during protein biosynthesis (34).

There is common agreement in the medical setting that preventive medicine is to be regarded with highest priority. In certain families, genetic abnormalities passed along in the family are known to predispose individuals to cancer. For instance, some forms of colon cancer are clearly genetically based (35). In future, retroviral vectors could be used to insert stable genes into mammary epithelial cells by inducing proliferation of the tissue by hormonal manipulation in these high risk patients. Further clarification of the genetic basis of cancer will certainly allow the possibility for a variety of genetic manipulations that may function to prevent the onset of malignancy. Just as for therapy, the potential uses of gene transfer for the prevention of cancer are seemingly unlimited.

Original Scientific Paper

From the Department of General and Trauma Surgery, Heinrich-Heine-University, Düsseldorf, Germany

Recurrent Nerve Palsy and Hypocalcemia After Surgery of Benign Thyroid Diseases

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Key-words: Thyroid surgery – benign thyroid diseases – recurrent laryngeal nerve palsy – hypoparathyroidism – permanent outcome.

Schlüsselwörter: Schilddrüsenoperation – gutartige Schilddrüsenerkrankungen – Nervus-recurrens-Parese – Hypoparathyreoidismus – Spätergebnisse.

Summary: Background: A critical analysis of early and late postoperative complications is necessary to assure the quality of surgery for benign thyroid diseases. The 2 major complications are palsy of the recurrent laryngeal nerve and hypoparathyroidism. Yet, long-term and follow up studies, as well as pre and post operative investigations are rather scarce.

Methods: 3246 patients operated on for benign thyroid diseases between 4/86 and 12/93 were retrospectively screened and analyzed for early postoperative recurrent laryngeal nerve palsy and hypoparathyroidism. Permanent laryngeal nerve paralysis and hypocalcemia was investigated by sending questionnaires to these patients and their physicians.

Results: 88 patients (2.7%) had early postoperative laryngeal nerve palsy. 58 (1.78%) of them recovered completely, reducing the cases of permanent paralysis to 30 patients (0.92%), 22 of which had proven (0.68%) permanent recurrent laryngeal nerve paralysis. The 8 questionable cases (0.24%) could not be evaluated.

Hypoparathyroidism necessitating calcium and/or vitamin D-treatment for more than 2 years was present in 18 patients (0.6%), which were without symptoms under this medication.

Conclusions: Dissecting the recurrent laryngeal nerve and visualizing the parathyroid glands during surgery for benign thyroid diseases decrease nerve paralysis and hypoparathyroidism to a permanent prevalence of less than 1%.

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Rekurrensparese und Hypoparathyreoidismus – Komplikationen der Chirurgie gutartiger Schilddrüsenerkrankungen

Zusammenfassung: Grundlagen: Die Qualität der Chirurgie gutartiger Schilddrüsenerkrankungen wird u. a. an der Häufigkeit postoperativer Komplikationen gemessen. Die beiden wichtigsten Komplikationen sind hierbei die Nervus-recurrens-Parese und der Hypoparathyreoidismus. Insgesamt gibt es jedoch nur wenige Langzeitstudien, deren Zahlen über Rekurrensparesen und Hypoparathyroidismus auf nachprüfbaren Untersuchungsergebnissen beruhen.

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