Science, medicine, and the future

Gene therapy

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In the eight years since the first human gene transfer experiment, in which a patient with malignant melanoma received genetically modified autologous T cells,[1] about 30 gene therapy companies have been launched, three major gene therapy journals have been established, more than 200 human gene therapy protocols have been approved, and over 2000 patients have received gene therapy. As yet, however, only a handful of patients with rare conditions have benefited from gene therapy, and early optimism about this new treatment has given way to disillusionment. In this article I look at the current status of gene therapy research and discuss the key problems that must be overcome to realise the enormous potential of gene therapy to treat not just single gene disorders but many common diseases such as arthritis, cancer, hypertension, atherosclerosis, diabetes, and asthma.

Scope of gene therapy

Gene therapy is a term that can be applied to any clinical therapeutic procedure in which genes are intentionally introduced into human somatic cells. There are two main approaches: in vivo gene therapy, in which genes are delivered directly to target cells in the body, and ex vivo gene therapy, in which the target cells are genetically modified outside the body and then reimplanted (fig 1).

Gene transfer into human cells is not a new concept: viral genes are introduced into human cells when a viral vaccine is administered to protect against disease. However, the clinical goals of gene therapy are different and often depend on the prolonged survival of genetically engineered cells or tissues in the patient. The key technologies that allowed gene therapy were the methods by which cellular genes could be isolated (cloned), manipulated (engineered), and transferred into human cells. The efficient transfer and expression of therapeutic genes in human cells is accomplished by inserting them into vectors (see box). The function of the vectors is to protect the therapeutic genes and to transport them safely into the nuclei of the target cells, where they can finally be decoded (expressed) to produce a therapeutic protein. Vectors allow the stable genetic modification of cells, tissues, and organs to restore deficient functions in genetic disease, to generate tissues with entirely new properties, and to create transplantable tissue factories for the controlled release of therapeutic proteins.

Possible future developments

Improved manipulation of human cells and tissues outside the body will greatly facilitate implantation of genetically engineered products

Innovative approaches to gene therapy will be applied to common diseases of the cardiovascular, respiratory, gastrointestinal, and nervous systems

Future vectors will deliver genes more accurately and efficiently and will allow better long term control of gene expression

Considerable resources will be needed for producing customised vectors and for harvesting, processing, culturing, genetically modifying, and reimplanting cells and tissues, and these will therefore be concentrated in regional gene therapy centres

It is still often assumed (incorrectly) that the aim of gene therapy is to modify genetically defective sperm, ova, or zygotes to remedy genetic disease, or even to create genetically perfect human beings. In fact, gene therapy is solely concerned with introducing genes into somatic cells and has nothing to do with genetic modification of the human germline, which is not allowed in any country.

Correcting genetic disease

Much of the early gene therapy research focused on single gene disorders, of which more than 4000 are known. However, current vectors are designed only to deliver genes and cannot remove or replace defective genes whose products may contribute to disease pathology (such as the abnormal haemoglobin S that causes red cell damage in sickle cell anaemia). In addition, efficient gene transfer to most affected target cells may be required for therapeutic benefit, and this may be impossible with current gene delivery systems. Because of these limitations, research efforts have concentrated on a few genetic deficiency diseases such as cystic fibrosis, haemophilia, and severe combined immune deficiency in which the target cells are relatively accessible and disease pathology is entirely

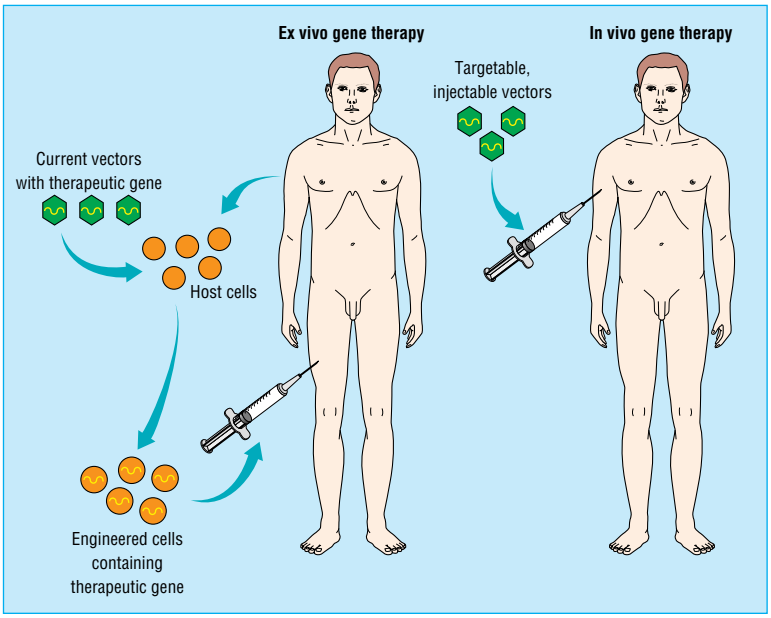

Fig 1 Gene therapy strategies. With current vectors, cells are usually genetically modified outside the body before reimplantation (ex vivo gene therapy). Targetable injectable vectors would facilitate the direct delivery of genes to cells and tissues in the patient (in vivo gene therapy)

attributable to the lack of a normal protein. However, methods for replacing or repairing defective genes are being explored, and there are good long term prospects for viable approaches against other genetic diseases.

Vectors in gene therapy

Essential components

1. A therapeutic gene that codes for a therapeutic protein—This can come from the genome of a human, another animal, a plant, a bacterium, or a virus and may code for any type of protein

2. A regulatory element to control the expression of the therapeutic gene—Depending on the choice of regulatory element, gene expression can be high or low level, specific to certain cell types, or even continuously variable and responding to local environmental factors such as the partial pressure of oxygen or the concentration of a drug

3. A gene delivery vehicle that can efficiently and accurately deliver the therapeutic gene with its associated regulatory element into the target cells

Classification of vectors

Synthetic (non-viral)

- Uptake of DNA into mammalian cells is facilitated by condensing it with lipids, peptides, proteins, inactivated virus particles, or crystals of calcium phosphate
- DNA can also be coated onto microprojectiles and fired into the nuclei of target cells by a gene gun

Viral

- Essentially a virus from which the viral genes have been removed to allow insertion of therapeutic genes and is usually incapable of replicative spread
- Generally give much higher efficiencies of gene delivery than non-viral vectors
- Any virus can provide the basis for a vector in principle, but retroviral and adenoviral vectors have been most widely used
- Retroviral vectors, in contrast to adenoviral and plasmid-based vectors, integrate their DNA into the chromosomal DNA of the target cells, ensuring gene transfer to the target cell progeny

Cell and tissue engineering

Tissue engineering involves the use of biological or synthetic materials in combination with (genetically modified) mammalian cells to create functional transplantable tissues.[2] This has enormous potential for treating trauma, burns, degenerative diseases, and other types of organ failure. One example of this approach is the genetically engineered skin fibroblast implant, or neo-organoid, which is at an advanced stage of preclinical development. Neo-organoids are made from cultured skin fibroblasts that have been stably modified with a gene of interest by means of a retroviral vector (fig 2). The genetically modified fibroblasts are dissociated from their culture dish, mixed with collagen, and then layered onto polytetrafluoroethylene (PTFE) fibres that have been coated with collagen. Retracted lattices then form, in which the fibroblasts are packed around a PTFE backbone. These structures can be implanted into the peritoneal cavity of the host, where they acquire a vascularised pedicle and secrete the product of the transferred gene for long periods. Neo-organoids have been used successfully in experimental animals for sustained delivery of erythropoietin,[3] to reverse the anaemia of chronic renal failure, and for the correction of lysosomal storage diseases.[4]

The inclusion of biological or synthetic materials is not always needed to generate an engineered tissue. For example, gene transfer techniques can be used to generate vascular grafts with anticoagulant properties, cellular grafts resistant to HIV infection, stem cell grafts resistant to myeloablative chemotherapy, and T cells reprogrammed to recognise cancer cells.

Controlled delivery of therapeutic proteins

Genetically modified cells can be used as a depot from which a therapeutic protein is steadily released. The protein may be released locally or systemically or may remain anchored to the surface of the cells that produce it. Moreover, by linking the therapeutic gene to a regulatory element that is responsive to a drug, it is possible to use an oral drug to control the level of gene expression. When mice were transplanted with muscle cells that had been transduced with an erythropoietin gene controlled by a promoter regulated by tetracycline, the release of erythropoietin into the blood stream could be controlled by dosing the animals with tetracycline.[5] Gene therapy can therefore provide an attractive method for the controlled administration of various therapeutic proteins such as monoclonal antibodies, interferons, and haemopoietic growth factors.

Strategies for local release of therapeutic proteins at specific locations are also being explored. For example, local release of anti-inflammatory proteins from genetically modified cells in inflamed joints is being tested in clinical trials for treating rheumatoid arthritis.[6] Similar approaches are being considered for other inflammatory conditions such as asthma.

Destruction of unwanted cells

Gene therapy can be used in different ways to destroy unwanted cells such as cancer cells or HIV infected cells. One strategy is to introduce a drug susceptibility gene ("suicide" gene) into target cells so that they are selectively killed when the appropriate drug is subsequently administered.[7] A wide variety of strategies are being explored to eliminate cancer cells. Examples include the use of personally customised DNA vaccines encoding the patient's own cancer-specific antigens, genetically modified tumour cell vaccines expressing cytokine genes or foreign histocompatibility genes (to enhance the immune response to the cancer cells), the infusion of genetically modified T lymphocytes expressing engineered T cell receptors that enhance their ability to recognise and kill tumour cells, and the direct delivery of genes encoding cytotoxic proteins to tumours (or their proliferating blood vessels).[8]

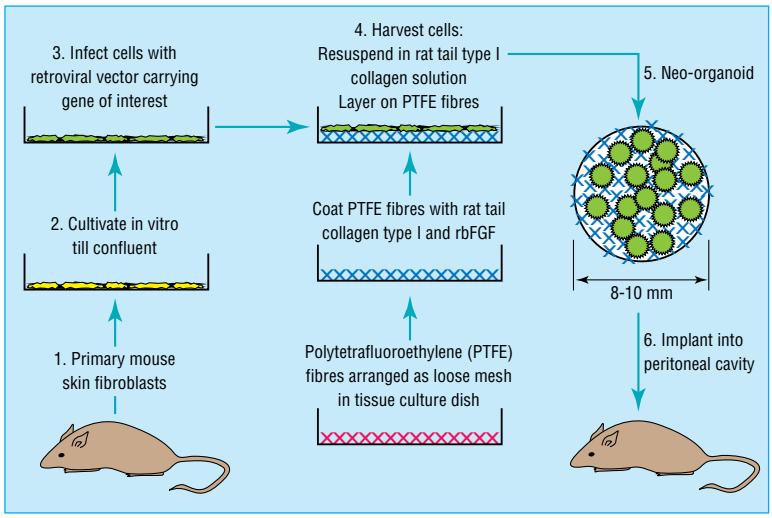

Fig 2 Construction of a genetically engineered tissue implant (neo-organoid)

Current status of gene therapy

At the start of 1997, more than 200 gene therapy protocols had been approved and more than 2100 patients had received gene therapy, over 1700 of these in the United States.[9] The diseases most often treated with gene therapy are cancer (68%), AIDS (18%), and cystic fibrosis (8%), and several different vector systems have been approved for use in human clinical trials (56% used retrovirus, 24% non-viral, 10% adenovirus, 5% poxvirus, and 1% adeno-associated virus).

Most trials of gene therapy for cancer fall into the category of destruction of unwanted cells through inducing an antitumour immune response or by using suicide genes. As expected for phase I clinical trials of new anticancer treatments, therapeutically beneficial responses have not generally been seen, although there have been occasional promising results. Several trials of treatment for cystic fibrosis have shown that normal copies of the defective CFTR gene can be delivered to airway epithelium and that this corrects the defect in chloride transport at a cellular level. With current vectors, however, the efficiency of gene transfer to small airway epithelium is still much lower than is needed for real therapeutic benefit.[10 11]

Perhaps the high point of gene therapy research was treatment of severe combined immune deficiency secondary to adenosine deaminase deficiency by reinfusing genetically corrected autologous T cells into two affected children[12]: the first patient to be treated has had a full and sustained recovery of a whole range of immunological parameters and is now able to lead a normal life. A second clinical success occurred with recipients of allogeneic bone marrow transplants who had recurrent malignancies.[13] T cells from the original bone marrow donor can mediate regression of the malignancy in this situation, but they can go on to inflict serious damage to normal host tissues. A suicide gene (thymidine kinase) was therefore introduced into the donor T cells, making them susceptible to ganciclovir, before they were infused into the patients so that they could be eliminated after the tumours had regressed to prevent further damage.

Future developments

Making better vectors

Poor performance of vectors continues to be the major limiting factor in gene therapy. Most clinical activity has focused on ex vivo protocols, in which target cells are genetically modified outside the body and reimplanted. With current vectors, however, the efficiency of gene transfer to the explanted cells is usually too low for real benefit. Also, expression of the therapeutic genes in the transduced cells is usually inadequately regulated, often varying greatly from cell to cell and declining rapidly with time. Even with increased efficiency of gene transfer and better control of gene expression, ex vivo gene therapy will remain cumbersome and costly, and much research is therefore focused on developing high titre, targetable, regulatable, injectable vector systems that will permit highly efficient and accurate transfer of genes to target tissues in vivo.

There are exciting advances in enhancing vector stability, reducing vector immunogenicity, developing successful strategies for targeting vectors, regulating gene expression, developing new lentiviral vector systems, and developing strategies for repeated administration of immunogenic vector particles.[14–16] However, since gene therapy vectors are complex structures, it is unrealistic to expect any one research group to optimise every component of a vector system. Effective collaboration will be the key to success.

Ensuring adequate resources and standards of work

The clinical testing of new approaches in gene therapy and their subsequent integration into routine clinical practice will require considerable resources. In particular, ex vivo approaches, tissue engineering, and the construction of personally customised vectors are highly dependent on the availability of appropriate facilities and trained staff.

Regulatory requirements are becoming more stringent for treatments involving ex vivo culture and genetic modification of cells. Facilities for gene therapy should therefore be built to a high specification with respect to microbiological containment and should

operate under the rigorous discipline of Good Manufacturing Practice. Associated facilities will be required for cryopreservation of the genetically modified cells and for extensive laboratory tests to assure their quality. When components of the gene therapy vector are to be customised for individual patients, as in the idiotypic DNA vaccination trial recently conducted in Cambridge,[17] there will be an additional requirement for a dedicated laboratory for customising vectors and an associated production facility where they can be manufactured.

Safety and ethical issues are obviously of general concern, and it is critically important that gene therapy trials should not go ahead unless the risks of harm to patients, carers, relatives, and the general population have been duly considered by the appropriate regulatory bodies. The risks of inadvertent gene transfer to the human germline must also be minimised.

Conclusions

The scope for clinical benefit from gene therapy is enormous, limited only by the poor performance of currently available vector systems. However, there are many new and improved systems for gene delivery under development, and these will both strengthen existing efforts and facilitate new strategies. Although the timescale of future developments is not predictable, with adequate resources to support the necessary translational research, the advent of useful gene therapy products seems inevitable.

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1 Rosenberg SA, Aebersold P, Cornetta K, Kasid A, Morgan AA, Moen R, et al. Gene transfer into humans: immunotherapy of patients with advanced melanoma using tumor infiltrating lymphocytes modified by retroviral gene transduction. *N Engl J Med* 1990;323:570-8.
2 Vacanti CA, Mikos AG. Letter from the editors. *Tissue Eng* 1995;1:1-2.
3 Naffakh N, Henri A, Villeval JL, Rouyer-Fessard P, Moullier P, Blumenfeld N, et al. Sustained delivery of erythropoietin in mice by genetically modified skin fibroblasts. *Proc Natl Acad Sci U S A* 1995;92:3194-8.
4 Moullier P, Bohl D, Heard J-M, Danos O. Correction of lysosomal storage in the liver and spleen of MPS VII mice by implantation of genetically modified skin fibroblasts. *Nat Genet* 1993;4:154-9.
5 Bohl D, Heard J-M. Modulation of erythropoietin delivery from engineered muscles in mice. *Hum Gene Ther* 1997;8:195-204.
6 Evans CH, Mankin HJ, Ferguson AB, Robbins PD, Ghivizzani SC, Herndon JH, et al. Clinical trial to assess the safety, feasibility, and efficacy of transferring a potentially anti-arthritic cytokine gene to human joints with rheumatoid arthritis. *Hum Gene Ther* 1996;7:1261-80.
7 Freeman SM, Whartenby KA, Freeman JL, Abboud CN, Marrogi AJ. In situ use of suicide genes for cancer therapy. *Semin Oncol* 1996;23:31-45.
8 Zhang J, Russell SJ. Vectors for cancer gene therapy. *Cancer Metastasis Rev* 1996;15:385-401.
9 Marcel T, Grausz JD. The TMC worldwide gene therapy enrollment report, end 1996. *Hum Gene Ther* 1997;8:775-800.
10 Gill DR, Southern KW, Mofford KA, Seddon T, Huang L, Sorgi F, et al. A placebo-controlled study of liposome-mediated gene transfer to the nasal epithelium of patients with cystic fibrosis. *Gene Ther* 1997;4:199-209.
11 Porteous DJ, Dorin JR, McLachlan G, Davidson-Smith H, Davidson H, Stevenson BJ, et al. Evidence for safety and efficacy of DOTAP cationic liposome mediated CFTR gene transfer to the nasal epithelium of patients with cystic fibrosis. *Gene Ther* 1997;4:210-18.
12 Blaese RM, Culver KW, Miller D, Carter CS, Fleisher T, Clerici M, et al. T lymphocyte-directed gene therapy for ADA-SCID: initial trial results after 4 years. *Science* 1995;270:475-80.
13 Bonini C, Ferrari G, Verzeletti S, Servida P, Zappone E, Ruggieri L, et al. HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. *Science* 1997;276:1719-24.
14 Friedmann T. Overcoming the obstacles to gene therapy. *Sci Am* 1997;276:80-5.
15 Felgner P. Nonviral strategies for gene therapy. *Sci Am* 1997;276:86-91.
16 Cosset F-L, Russell SJ. Targeting retrovirus entry. *Gene Ther* 1996;3:946-56.
17 Hawkins RE, Russell SJ, Marcus R, Ashworth LJ, Brissinck J, Zhang J, et al A pilot study of idiotypic vaccination for follicular B-cell lymphoma using a genetic approach. *Hum Gene Ther* 1997;8:1287-99.

Memorable patients

The social admission and the considerate consultant

Fifteen years ago I was a house officer on one of the first medical units to be integrated between general and geriatric medicine. The "take" had been busy and on the post take ward round I had several interesting cases to present to the professor. One of the least interesting was an 80 year old woman I had seen briefly the evening before. I told the professor that she was an elderly lady who was really a social admission. She had been living with her family who were having difficulty coping, and she had been admitted temporarily while her social circumstances were being sorted out.

I saw the registrar wince as the great man's face clouded momentarily. He turned to the patient and with a few well chosen questions obtained a history of considerable recent weight loss and a disordered swallow, which had recently progressed to almost complete dysphagia. He turned to me and remarked that perhaps we should do a barium swallow to confirm the diagnosis of carcinoma of the oesophagus. He explained that he did not use the label "social admission" as it was so often misleading.

I was chastened, and throughout my training in medicine for the elderly the memory of my own inadequate assessment of the case and the professor's masterful display of clinical acumen have remained with me.

Three years ago, during one of my first post take ward rounds as a senior lecturer in medicine for the elderly on an integrated medical unit, the senior house officer presented a case to me of an elderly woman, who, she said, was a social admission because her relatives were no longer able to cope with her at home. I turned to the patient and established that she had become a burden on her family ever since her legs had started to become weak. The lower limb weakness had progressed so that she was no longer able to support her weight and had taken to her bed. I turned to the house officer and remarked that if ever I lost the use of both my legs then I would try to remember to call a social worker for help with my problem. The house officer promptly burst into tears and was inconsolable for several minutes.

I have now learnt two lessons from my patient with oesophageal carcinoma. The first is that careless assessment of older patients and unthinking application of convenient social labels to their medical admission is a mistake. The second, learnt more recently, is that you should treat junior colleagues with as much patience and respect as mislabelled older patients. To do less may be to miss an opportunity to inspire a colleague to consider a career in your specialty.

Stuart Parker, *senior lecturer in medicine for the elderly, Leicester*

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