Eur J Cancer, Vol. 26, No. 5, pp. 638–640, 1990.
Printed in Great Britain

0277–5379/90$3.00 + 0.00

Gene Therapy for Cancer

Christine Kinnon and Roland J. Levinsky

INTRODUCTION

RAPID advances in recombinant DNA technology make it likely that genetic manipulation and somatic gene therapy will become part of medical practice in this decade. In the case of the human cancers several possibilities arise for diagnosis, monitoring and treatment. Some have been shown to be effective whilst others are more speculative.

One example of the successful uses is that of γ-interferon recombinant gene products in the treatment of hairy cell leukaemia. Trials of other products such as interleukin 2 (IL2), interleukin 4 (IL4) and tumour necrosis factor (TNF), alone or in combination, in the treatment of several different cancers await completion. Recombinant products such as granulocyte colony stimulating factor and granulocyte/monocyte colony stimulating factor have been shown to ameliorate the granulocytopenia induced by the myelosuppressive chemotherapy regimens used for leukaemia, lymphoma and autologous transplantation for many solid tumours [1].

Where a disease susceptibility gene has been cloned, such as that for retinoblastoma [2, 3], or mapped, such as in colorectal carcinoma in the familial form of polyposis coli [4, 5], it may be possible to predict whether an individual has that genetic susceptibility by use of restriction fragment length polymorphism analysis.

Tumour infiltrating lymphocytes (TIL) may be expanded *in vitro* and, before reinfusion into the patient, labelled with a genetic marker (such as the neomycin resistance gene) to study their tumour homing and killing properties. The first investigation in man in which that gene was inserted into TIL cells with a retroviral construct has been completed [6]. Since TIL cells might recognize tumours and thus 'home' to sites of malignancy, their tumoricidal properties could be enhanced. Gene insertion that would either enhance local cytokine production (i.e. TNF, γ-interferon, IL2 or IL4) or deliver a therapeutic gene product such as a toxin (e.g. *Pseudomonas* exotoxin) is a promising area of research.

Theoretically, it should be possible to insert antisense genes (sequences that are complementary to the gene coding sequences) into tumour cells to switch off the expression of genes such as oncogenes [7]. Another possibility would be to use gene transfer techniques to purge malignant cells. For instance, targetted insertion of the thymidine kinase gene would render the malignant cells uniquely susceptible to drug therapy with gancyclovir. The difficulty here is how to target the gene in question to all the tumour cells *in vivo*. The prospect of tumour immunization based on gene therapy in order to recruit immune and inflammatory cells to the sites of malignancy is of more immediate potential since the infection could be performed *in vitro* and the cells, after injection back into the patient, would hopefully elicit an immune response against tumour associated antigens.

PRINCIPLES OF SOMATIC GENE THERAPY

Somatic gene therapy can be defined as the introduction of a functioning exogenous gene into a cell that either does not express its own copy of the gene or has a defective copy. In most cases the introduced gene would be in addition to the copy of the gene already present in the cell, which technically results in gene augmentation. A more difficult technique would be gene replacement whereby a defective gene is removed and replaced with a functional gene in the correct orientation and chromosomal location. Such gene replacement is not yet feasible, although a model for gene targeting involving homologous recombination is being developed [8]. This, however, involves introducing the gene into the germline and therefore passing it on to subsequent generations. With somatic gene therapy techniques the gene is introduced only into the somatic cells of the patient and not into the germline. Whereas it is unlikely that many ethical objections would be raised against somatic gene therapy, this would not be the case for germline therapy.

Candidate diseases for treatment by somatic gene therapy must meet the following criteria: (1) the disease must have a severe and predictable phenotype, a requirement met by most cancers; (2) the gene to be introduced must have been cloned; (3) expression of the gene product should not require too precise regulation nor particularly high levels of expression to overcome the defect; and (4) there must be a suitable delivery system for the implantation of the genetically modified cells.

The possibility of using gene therapy techniques for the treatment of genetic diseases in man has been much discussed [9–11]. The most likely initial candidate diseases are those that involve single gene defects and that are currently amenable to

Correspondence to C. Kinnon.
C. Kinnon and R.J. Levinsky are at the Hugh Greenwood Department of Immunology, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, U.K.

treatment by bone marrow transplantation. These diseases are generally characterized by a genetic defect expressed in a cell series derived from the pluripotential bone marrow stem cell. Introducing a functional gene into these cells should result in the permanent correction of the gene defect. Indeed, studies in mice have shown that genes can be stably introduced into bone marrow stem cells by retroviruses and expression in the differentiated progeny can be detected for variable periods [12–14].

Diseases that are currently treated by bone marrow transplantation include those in which the defect may manifest as a complete or partial lack of functioning cells of a particular lineage (as in some of the immunodeficiency diseases) or the result of aberrent or defective synthesis of a particular enzyme (as in the purine salvage pathway disorders) or of a vital transport molecule (as in the haemoglobinopathies). In these situations the defective gene could be augmented or replaced by its cloned functional equivalent. In addition, the insertion of a functioning gene into a bone marrow stem cell may provide an exogenous source of enzyme in all haematopoietic lineages and potentially correct the defective gene in conditions such as the lysosomal storage diseases. In many other diseases where the organ providing the molecule is more remote and the activity of the function is localized, the possibility of infecting other target organ tissues, such as hepatocytes, endothelial cells, kerotinocytes, lung epithelium and possibly fibroblasts, will need to be explored. In the context of the correction of genetic diseases, applications have now been made to the U.S. Recombinant DNA Advisory Committee to start treating patients with severe combined immunodeficiency due to adenosine deaminase (ADA) deficiency with retroviral vectors to insert the ADA gene into the patient's lymphocytes before reinfusion [15].

TECHNIQUES OF GENE INSERTION

There are two types of methods for introducing cloned genes into cells: namely, physical and viral [16]. Physical methods include coprecipitation of DNA with calcium phosphate, the use of polycations or lipids to complex the DNA, cell fusion techniques with liposomes, erythrocyte ghosts or protoplasts, microinjection and electroporation. While large numbers of cells can be treated at once (except by microinjection) these techniques have the disadvantage that the efficiency of integration is low and multiple copies of the DNA are usually integrated.

Viruses have often been used to introduce DNA into cells in culture; the first expression vectors utilized the DNA tumour viruses, SV40 and polyoma [17]. More recently, work in this area has focused on the murine retroviruses, Moloney leukaemia virus and myeloproliferative sarcoma virus. Retroviruses have several features that make them suitable for use as vectors for somatic gene therapy. The production of progeny virus is non-lytic, leading to the establishment of permanent, viral producing cell lines that give high titre virus for infection of target cells [18]. The *gag*, *pol* and *env* genes, required for viral encapsidation, can be supplied *in trans*, which means that replication defective retroviruses can be produced that are capable of only one round of infection, technically eliminating the problem of constant reinfection of cells. Any infective retroviruses that did arise in the patient should, however, be efficiently neutralized by complement present in the patient's sera. Retroviral vectors can infect up to 100% of target cells and the DNA is usually integrated as a single copy. Once integrated into the host genome the provirus acts as a cellular gene in that it is faithfully passed on to progeny cells.

However, there are several disadvantages to using retroviruses as vectors for gene therapy. Perhaps most obvious is the fact that retroviral vectors can only encode up to about seven kilobases of introduced DNA, which in most cases is probably not sufficient to encode a full-length gene and its appropriate control sequences. Loss of control sequences may lead to inappropriate tissue expression and low levels of expression of the gene product. Several problems can arise because of the non-specific nature of the integration event when the gene is randomly inserted into the genome. These include non-expression of the introduced gene or insertional mutagenesis, whereby a normally active gene is silenced or a silent deleterious gene may become activated.

Further disadvantages include the possibility of recombination between the exogenous retroviral vector and endogenous human retroviruses, which could potentially produce infective virus in the patient. Interactions between endogenous retroviruses and retroviral vectors can give rise to phenotypic alterations such as pseudotyping and phenotypic mixing even without genetic recombination events; mixed viral phenotypes have been observed in a human T cell line infected with both Moloney leukaemia virus and human immunodeficiency virus-1 [19]. Furthermore, a murine proviral transcript has been identified in a human cell line which had previously been infected with defective retroviral vectors that had been propagated in the producer cell line as pseudotypes [20]. Expression of such exogenous proviruses in this system could also give rise to the production of replication-competent viruses. These potential problems suggest the need to develop new packaging cell lines from species that have fewer endogenous retroviruses, such as birds.

It is possible that safer, more efficient, higher titre viral vectors, capable of encoding larger pieces of DNA, may be developed based on other viruses, including vaccinia, adeno-associated virus, herpes and bovine papilloma viruses.

GENE THERAPY TO ENHANCE CANCER TREATMENT

In terms of the use of gene therapy for the treatment of cancer the first study of retroviral vectors as tumour markers *in vivo* has been completed [15]. This study involved reinfusing the patient's IL2 stimulated T lymphocytes after the cells had been infected *in vitro* with retroviruses carrying the neomycin resistance marker gene. This allows the TIL cells to be tracked in the patient as they 'home' to the tumour, where it is hoped that they will attack the tumour cells.

Many secreted growth factors and lymphokines have now been cloned, which has given rise to the use of recombinant gene products in the treatment of cancer and in the future these genes may be introduced into tumour cells by somatic gene therapy techniques. Indeed, several mouse models for this sort of therapy have now been established [21–23]. However, the design of these studies usually involves gene insertion into tumour cells and seeding the tumour into either nude or syngeneic mice. From these reports, it is unclear whether the alterations in the antigenic profiles of the tumours alone are sufficient to generate autologous immune responses, thereby causing rejection of the tumours. As yet there is no clear evidence that the increase in cytokine production by the enhanced expression of the appropriate genes in the tumour cells has any direct effect on the growth and proliferation of tumour

infiltrating and killing lymphocytes. Indeed, the potential of this type of system may be one of tumour immunization and the use of cytokine gene upregulation should possibly be reserved for augmentation of TIL therapy.

Although many cancers have a genetic basis, few cancer 'causing' genes have been identified. Perhaps the most notable are the cancer suppressor genes which, when their function is disrupted, are responsible for causing cancers (e.g. retinoblastoma and Wilms' tumour). The gene for retinoblastoma has been cloned [2, 3] and a candidate gene for the Wilms' tumour locus has now been identified [24, 25]. These cancers are presumed to arise as a result of mutations affecting the expression of both alleles of the relevant wild-type gene. Therefore, by restoring functional expression of the relevant gene, it is postulated that the cancer phenotype might be suppressed and even possibly reversed. There is some support for these ideas from *in vitro* experiments in which the normal retinoblastoma gene was introduced into tumour cells in tissue culture using retroviral vectors and some of the transformed characteristics of the cells were apparently lost [26]. However, it is difficult to see how this approach could be applied to the treatment of patients with these diseases since any remaining tumour cells would have a growth advantage over the genetically modified cells.

It is clear that the potential for genetic manipulation in cancer therapy is vast. We have outlined a few of the potential applications but much of this work is still at the stage of animal research. Targeting the gene into the cell is still one of the major obstacles and until these problems have been overcome, gene therapy for cancer will remain an armchair exercise.

1. Vadhan-Raj S, Buescher S, Broxmeyer E *et al.* Stimulation of myelopoiesis in patients with aplastic anemia by recombinant human granulocyte–macrophage colony-stimulating factor. *N Engl J Med* 1988, **319**, 1628–1634.
2. Friend SH, Bernards R, Rogelj S *et al.* A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. *Nature* 1986, **323**, 643–646.
3. Lee W-H, Bookstein R, Hong F, Young L-J, Shew J-Y, Lee EY-HP. Human retinoblastoma susceptibility gene: cloning, identification, and sequence. *Science* 1987, **235**, 1394–1399.
4. Bodmer WF, Bailey CJ, Bodmer J *et al.* Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature* 1987, **328**, 614–616.
5. Leppert M, Burt R, Hughes JP *et al.* Genetic analysis of an inherited predisposition to colon cancer in a family with a variable number of adenomatous polyps. *N Engl J Med* 1990, **322**, 904–908.
6. Kasid A, Morecki S, Aebersold P *et al.* Human gene transfer: characterization of human tumor-infiltrating lymphocytes as vehicles for retroviral-mediated gene transfer in man. *Proc Natl Acad Sci USA* 1990, **87**, 473–477.
7. McManaway ME, Neckers LM, Loke SL *et al.* Tumour-specific inhibition of lymphoma growth by an antisense oligodeoxynucleotide. *Lancet* 1990, **335**, 808–811.
8. Capecchi MR. Altering the genome by homologous recombination. *Science* 1989, **244**, 1288–1292.
9. Anderson FW. Prospects for human gene therapy. *Science* 1984, **226**, 401–409.
10. Kantoff PW, Freeman SM, Anderson FW. Prospects for gene therapy for immunodeficiency diseases. *Annu Rev Immunol* 1988, **6**, 581–594.
11. Friedmann T. Progress toward human gene therapy. *Science* 1989, **244**, 1275–1281.
12. Dick JE, Magli MC, Huszar D, Phillips RA, Bernstein A. Introduction of a selectable gene into primitive stem cells capable of long-term reconstitution of the hemopoietic system of W/Wv mice. *Cell* 1985, **42**, 71–79.
13. Keller G, Paige C, Gilboa E, Wagner ER. Expression of a foreign gene in myeloid and lymphoid cells derived from multipotent haematopoietic precursors. *Nature* 1985, **318**, 149–154.
14. Dzierzak EA, Papayannopoulou T, Mulligan RC. Lineage-specific expression of a human β-globin gene in murine bone marrow transplant recipients reconstituted with retrovirus-transduced stem cells. *Nature* 1988, **331**, 35–41.
15. Anonymous. News Article. *Nature* 1990, **344**, 483.
16. Sambrook J, Fritsch EF, Maniatis T. Expression of cloned genes in cultured mammalian cells. In: *Molecular Cloning; a Laboratory Manual*, 2nd edn. Cold Spring Harbor, Cold Spring Harbor Press, 1989, 16:1–16:73.
17. Rigby PWJ. Expression of cloned genes in eukaryotic cells using vector systems derived from viral replicons. *Genetic Eng* 1982, **3**, 84–134.
18. Cone RG, Mulligan RC. High-efficiency gene transfer into mammalian cells: generation of helper-free recombinant retrovirus with broad mammalian host range. *Proc Natl Acad Sci USA* 1984, **81**, 6349–6353.
19. Lusso P, Veronese FDM, Ensoli B *et al.* Expanded HIV-1 cellular tropism by phenotypic mixing with murine endogenous retroviruses. *Science* 1990, **247**, 848–852.
20. Scadden DT, Fuller B, Cunningham JM. Human cells infected with retrovirus vectors acquire an endogenous murine provirus. *J Virol* 1990, **64**, 424–427.
21. Tepper RI, Pattengale PK, Leder P. Murine interleukin-4 displays potent anti-tumour activity *in vivo*. *Cell* 1989, **57**, 503–512.
22. Watanabe Y, Kuribayashi K, Miyatake S *et al.* Exogenous expression of mouse interferon γ cDNA in mouse neuroblastoma C1300 cells results in reduced tumorigenicity by augmented anti-tumor immunity. *Proc Natl Acad Sci USA* 1989, **86**, 9456–9460.
23. Fearon ER, Pardoll DM, Itaya T *et al.* Interleukin-2 production by tumor cells bypasses T helper function in the generation of an antitumor response. *Cell* 1990, **60**, 397–403.
24. Gessler M, Poustka A, Cavenee W, Neve RL, Orkin SH, Bruns GAP. Homozygous deletion in Wilms tumours of a zinc-finger gene identified by chromosome jumping. *Nature* 1990, **343**, 774–778.
25. Call KM, Glaser T, Ito CY *et al.* Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumour locus. *Cell* 1990, **16**, 509–520.
26. Huang H-J S, Yee J-K, Shew J-Y *et al.* Suppression of the neoplastic phenotype by replacement of the RB gene in human cancer cells. *Science* 1988, **242**, 1563–1566.