Pergamon

European Journal of Cancer Vol. 30A, No. 8, pp. 1047–1049, 1994
Elsevier Science Ltd
Printed in Great Britain
0959–8049/94 $7.00+0.00

0959-8049(93)00220-7

Comments and Critique

Gene Therapy for Cancer

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INTRODUCTION

RECOMBINANT DNA technology has resulted in the recognition of a number of molecular mechanisms that control growth and differentiation. The targeting of these pathways for therapy is sometimes known as molecular therapeutics and this is rapidly becoming the fifth modality of anticancer treatment, joining surgery, radiotherapy, chemotherapy and immunotherapy. Cytotoxic and molecular therapies both act at a 'molecular' level, but with the former, sites of action are chemically defined, whereas molecular therapy either utilises or targets specific DNA or RNA sequences. Targeting RNA with complementary nucleotides is known as antisense or oligonucleotide therapy whereas gene therapy alters the genome by ablation, addition, substitution or augmentation of specific DNA sequences.

There is no agreed classification of gene therapy, but the terms 'direct' and 'indirect' are often used. Direct gene therapy involves altering a DNA sequence that is responsible for malignant transformation or its maintenance. The ablation of oncogenes or the addition of absent tumour suppressor genes are examples of this strategy. Indirect therapies are those that result in growth control or tumour cell death via an intermediate step which is initiated by the expression of the inserted DNA sequence, e.g. stimulating host anti-tumour immune responses, altering tumour angiogenesis or the insertion of prodrug targets, so-called gene-directed enzyme prodrug therapy (G-DEPT). An alternative classification depends on target cell type: direct gene therapy then refers to changes in the tumour cell genome, and alterations to non-transformed host cells, e.g. lymphocytes or endothelial cells, are indirect therapies.

STRATEGIES

The design of any new treatment involves the identification of a specific pathway, the means to alter it and a method by which it can be targeted. The specific strategic issues that need to be addressed when designing a gene therapy protocol are summarised in Table 1. First, it is necessary to identify the type of molecular intervention that is appropriate: direct strategies are those that replace mutant or absent tumour suppressor genes, like p53, with the wild type sequence [1] or add DNA sequences expressing specific antisense transcripts. More available interventions involve the addition of so-called suicide genes (G-DEPT) which produce enzymes that convert non-toxic drugs into cytotoxic compounds, and the production of such enzymes within the genetically-modified cells results in site-specific cytotoxicity. Examples of suicide enzymes are herpes simplex virus-derived thymidine kinase (HSV-TK) and cytosine deaminase which convert the non-toxic drugs ganciclovir and 5-fluorocytosine to the toxic products ganciclovir-triphosphate and 5-fluorouracil, respectively [2, 3]. An unexplained advantage of this strategy is seen with ganciclovir, the so-called 'bystander effect', whereby cells adjacent to those expressing the enzyme are also killed [4]. Other similar strategies include the insertion of sequences coding for toxins such as ricin, diptheria toxin or pseudomonas. Bone marrow has been the target of a number of protocols, in particular, the introduction of the drug resistance gene *mdr*-1 [5] into haemopoetic cells to allow dose escalation of chemotherapeutic agents without having to resort to haemopoetic rescue techniques. Gene therapy techniques have also been applied to bone marrow 'marking' experiments in high-dose chemotherapy studies to assess whether a relapse is from the 'host' or the graft. The role of bone marrow purging or a comparison between two purging methods can also be assessed by splitting a marrow harvest and inserting two different reporter (marker) genes, e.g. the retroviral markers LNL-6 and GIN-a, into each aliquot and at relapse probing the malignant cells for the marker genes [6]. Gene-directed immunotherapeutic strategies are based on our current knowledge of the host–tumour immune reaction and involve inserting sequences encoding tumour-specific or -associated antigens [7], idiotypic

Table 1. Strategic considerations

Intervention
Tumour suppressor gene
G-DEPT
Toxin
Immune response
Target cell
Tumour
Lymphocyte
Fibroblast
Keratinocyte
Hepatocyte
Muscle cell
Delivery
In/ex vivo
Local/systemic
Receptor-targeted (e.g. antibody or asialogycoprotein-linked)

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Received 16 Mar. 1994; accepted 27 Apr. 1994.

determinants [8], co-signalatory molecules [9], MHC antigens [10] or cytokines such as interleukin-2 [11], interferon-γ [12] and tumour necrosis factor [13]. Many of these approaches are essentially vaccination protocols and are designed to augment the host's defective response to the presence of a tumour. These are mainly concerned with the treatment of melanoma and renal cell carcinoma, both being immunogenic tumours, although one study of particular interest in patients with low-grade non-Hodgkin's lymphoma involves the intramuscular injection of idiotypic sequences.

New genetic material may be delivered to cells *in vivo* or *ex vivo*, locally or systemically, and the cell type to be targeted needs to be carefully defined because it may not always be necessary to target the tumour cells themselves. This is particularly the case with immunotherapy protocols, but applies to any strategy that results in the expressed product exerting a paracrine or distant effect. Local delivery may be an effective way of treating a specific organ, e.g. intrahepatic artery infusion, and this can be used in conjunction with other targeting techniques, such as those exploiting organ-specific receptors. Methods include coupling the DNA sequence to a monoclonal antibody [14] or, in the case of hepatocyte targeting, asialogycoprotein [15]. Cells in which *ex vivo* transfer has taken place can be returned to their tissue of origin, e.g. bone marrow cells to bone marrow, or transplanted to a different anatomical site. New genetic material can be inserted into cells that do not normally express that particular gene, e.g. keratinocytes can be used to express G-CSF. In cytokine gene therapy protocols, the cell type engineered to produce the cytokine may, however, be crucial. For example, recent work in mice suggests that vaccines comprising tumour cells secreting interleukin-2 are more effective than when fibroblasts are used to secrete interleukin-2 [16]. Target cell specificity can be achieved by tissue-specific promoters which can be inserted into the gene constructs resulting in site-specific gene expression. The best example of this is the tyrosinase promoter which is only activated in melanocytic tissue [17]. This promoter has been inserted upstream from the interleukin-2 gene in one particular construct with the result that interleukin-2 will only be expressed in melanocytic cells. Finally, consideration must be given to the nature of the genetic material to be inserted and its appropriateness for the protocol, e.g. native DNA for direct injection into tumours or muscle, retroviral vectors for *ex vivo* insertion, liposomal vectors for intravascular delivery.

METHODOLOGY

Strands of naked DNA can be directly injected into tissue, but the efficiency of gene transfer is low, except in the case of muscle. A variety of methods can be used to increase the efficiency of DNA uptake by cells and these can be broadly divided into physical, chemical and biological methods (Table 2). Physical methods include direct injection [18], microinjection [19], high velocity microprojectiles [20] and electroporation, which involves the passage of small electric currents through cell suspensions in the presence of the gene to be inserted [21]. Calcium phosphate precipitation [22], liposomes [23] and DEAE-dextran [24] are chemical methods, and biological methods use viruses, mainly retroviruses [25], adenoviruses [26], adeno-associated viruses [27] and the herpes simplex virus [28]. When viruses are used as vectors, the sequence for insertion is substituted for the viral genome, resulting in a recombinant virus. In the case of the most developed system, retroviral vectors, the viruses have been heavily modified, with the sequences encoding viral proteins removed, leaving only the sequences that control expression (LTR regions). Adenoviruses and herpes simplex virus are useful vectors because, unlike retroviruses, they do not require the target cells to be actively dividing before they will infect them. They are, therefore, particularly appropriate when targeting cells which have a slow proliferative rate, e.g. cells of the central nervous system. However, they can contaminate the environment and are potentially infectious. Retroviruses are perhaps the safest vectors, producing a stable integration in the genome with a predictable product, and they can also be modified to remove their ability to become replication competent ('helper virus') and hence infectious. Assays to exclude the presence of helper virus in a retrovirally-derived construct are a very important safety issue.

Table 2. Methodologies for gene insertion

Physical
Direct injection
Microinjection
Microprojectiles
Electroporation
Chemical
Calcium phosphate
DEAE-dextran
Liposomes
Asialo glycoprotein
Biological
Retrovirus
Adenovirus
Parvovirus
Herpes simplex virus

A major problem with all the targets described above is the observation that, in many experiments, the gene eventually becomes lost or expression no longer occurs. This is presumably due to failure to integrate the gene into the stem cell population so that as cells differentiated and die, the sequence is lost. However, even when there is evidence that the stem cell has been infected, the progeny sometimes fail to express the gene. The reasons for this are unclear, but presumably it is due to changes in the regulatory mechanisms of transcription as cells mature. This loss of expression need not necessarily be a barrier to sucessful treatment because provided that the target cells are easily available, the technology for insertion reproducible, and the method of delivery acceptable, patients can be treated on several occasions.

ETHICS

Ethical considerations concern four areas: patient selection, scientific validity, safety and cell type to be engineered. The questions of patient selection and scientific validity of a particular protocol have to be considered for any new treatment. Broadly, in cancer gene therapy protocols, the ethical considerations for both these issues are no different from those governing other experimental treatments. However, the safety issue is very different and needs to be assessed in terms of risk to the patient, the environment and health care workers. The issue of safety for the patient is similar to that encountered in new drug trials, the main problem is that of the danger to staff and public, especially when viral vectors are used. There is also the danger of insertional mutagenesis, although this is not a new problem in cancer therapy since second malignancies are induced by both chemo-

and radiotherapy. However, there is concern over gene transfer to the germ line, particularly when DNA or viral vectors are administered systemically. Patients should be advised to use contraception during the period of treatment and possibly for 1 year afterwards. Finally, there is the question of which cells can be engineered, somatic or germ line. Germ line therapy involves the insertion of genetic material into sperm, ovum or very early embryos. This question needs to be constantly addressed because in animals the approach is feasible and in humans the strategy might be desirable for certain inherited conditions, such as metabolic diseases and haemoglobinopathies. It may also be a relevant strategy for families with inherited cancer syndromes. However, so far governments have stated unequivocally that no germ line gene therapy studies are to be performed.

In the U.K. ethical and safety guidelines for gene therapy were laid down by a Department of Health Committee chaired by Sir Cecil Clothier (1992), which has now been superseded by the Gene Therapy Advisory Committee. Workers proposing to start gene therapy trials are advised to involve local hospital/institute safety committees early in the design of any trial, and in the U.K., the Health and Safety Executive needs to be kept informed. No trial can begin without the consent of the licensing authority, local ethics committee and Gene Therapy Advisory Committee in the U.K. Finally, as with all studies, responsibility for patient indemnity must be clearly defined and this can be a difficult problem. Well-established contingencies within research institutions are unlikely to cover gene therapy. In our experience, we have found the Cancer Research Campaign (CRC) extremely supportive, and they have adopted our own and other U.K. protocols, providing appropriate indemnity cover. The order in which the various permissions for a gene therapy study should be sought is not well defined. Our advise is to get all local safety and ethical permissions first, then proceed to external protocol review by a body such as the CRC, and finally to send the protocol to the relevant licensing and regulatory government agencies (the Medicines Control Agency and the Gene Therapy Advisory Committee in the U.K.).

1. Kaneko Y, Tsukamoto A, Kurokawa K. Suppression of hepatoma cell growth by enhancing p53 gene expression. *Human Gene Therapy* 1992, **3**, 612.
2. Culver KW, Ram Z, Wallbridge S, Ishii H, Oldfield EH, Blaese RM. *In vivo* gene transfer with retroviral vector-producer cells for treatment of experimental brain tumours. *Science* 1992, **256**, 1550–1552.
3. Mullen CA, Kilstrup M, Blaese RM. Transfer of the bacterial gene for cytosine deaminase to mammalian cells confers lethal sensitivity to 5-fluorocytosine: a negative selection system. *Proc Nat Acad Sci USA* 1992, **89**, 33–37.
4. Caruso M, Panis Y, Gagandeep S, Houssin D, Salzmann JL, Klatzmann D. Regression of established macroscopic liver metastases after *in situ* transduction of a suicide gene. *Proc Natl Acad Sci USA* 1993, **90**, 7024–7028.
5. Hanania EG, Fu SQ, Zu Z, Hegewisch-Becker S, Andreeff M, Deisseroth AB. Progeny of long-term culture initiating cells transduced with an MDR-1 safety modified retrovirus display high levels of p-glycoprotein and taxol resistance. *Cancer Gene Ther* 1993, **1**, 4.
6. Brenner MK. Gene transfer into human hematopoetic progenitor cells: a review of current clinical protocols. *J Hematother* 1993, **2**, 7–17.
7. Severinson L, Burrascano M, Ronlov G, *et al.* Retroviral vectors encoding human papillomanvirus type 16 E7 protein against challenge with E7 expressing tumours. *Human Gene Ther* 1992, **3**, 2.
8. Hawkins RE, Winter G, Hamblin TJ, Stephenson FK, Russell SJ. Genetic approach to idiotypic vaccination. *J Immunother* 1993, **14**, 273–278.
9. Townsend SE, Allison JP. Tumour rejection after direct constimulation of CD8+ T cells by B7-transfected melanoma cells. *Science* 1993, **259**, 368–370.
10. Hui K, Grosveld F, Festenstien H. Rejection of transplantable AKR leukaemia cells following MHC DNA-mediated cell transformation. *Nature* 1984, **311**, 750–752.
11. Gansbacher B, Zier K, Daniels B, Cronin K, Bannerji R, Gilboa E. Interleukin-2 gene transfer into tumour cells abrogates tumourogenicity and induces protective immunity. *J Exp Med* 1990, **172**, 1217–1224.
12. Gansbacher B, Bannerji R, Daniels B, Zier K, Cronin K, Gilboa E. Retroviral vector-mediated γ-interferon gene transfer into tumour cells generates potent and long lasting antitumour immunity. *Cancer Res* 1990, **50**, 7820–7825.
13. Rosenberg SA, Anderson WF, Blaese M, *et al.* The development of gene therapy for the treatment of cancer. *Ann Surg* 1993, **218**, 455–464.
14. Roux P, Jeanteur P, Piechaczyk M. A versatile and potentially general approach to the targeting of specific cell types by retroviruses: application to the infection of human cells by means of major histocompatibility complex class I and class II antigens by mouse ecotropic murine leukemia virus-derived viruses. *Proc Natl Acad Sci USA* 1989, **86**, 9079–9083.
15. Wu GY, Wilson JM, Shalaby F, Grossman M, Shafritz DA, Wu GH. Receptor-mediated gene delivery *in vivo*. Partial correction of genetic analbuminaemia in nagase rats. *J Biol Chem* 1991, **266**, 14338–14342.
16. Tsai SC, Gansbacher B, Tait L, Miller FR, Heppner GH. Induction of anti-tumour immunity by interleukin-2 gene-transduced mouse mammary tumour cells versus transduced mammary stromal fibroblasts. *J Nat Cancer Inst* 1993, **85**, 546–553.
17. Vile RG, Hart IR. *In vitro* and *in vivo* targeting of gene expression to melanoma cells. *Cancer Res* 1993, **53**, 962–967.
18. Wolff JA, Malone RW, Williams P, *et al.* Direct gene transfer into mouse muscle *in vivo*. Science 1990, **247**, 1465–1468.
19. Capecchi MR. High efficiency transformation by direct microinjection of DNA into cultured cells. *Cell* 1980, **22**, 479–488.
20. Johnston SA. Bolistic transformation: microbes to mice. *Nature* 1990, **346**, 776–777.
21. Potter H. Electroporation in biology: methods, applications, and instrumentation. *Analyt Biochem* 1988, **174**, 361–373.
22. Graham RL, van der Eb AJ. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 1973, **52**, 456–467.
23. Felgner PL, Gadek TR, Hom M, *et al.* Ringold GM, Danielsen M. Lipofection: a highly efficient, lipid-mediated DNA transfection procedure. *Proc Natl Acad Sci USA* 1987, **84**, 7413–7417.
24. Lopata MA, Cleveland DW, Sollner-Webb B. High level transient expression of a chloramphenicol acetyl transferase gene by DEAE-dextran mediated DNA transfection coupled with a dimethyl sulfoxide or glycerol shock treatment. *Nucleic Acids Res* 1984, **12**, 5707–5717.
25. Roux P, Jeanteur P, Piechaczyk M. A versatile and potentially general approach to the targetting of specific cell types by retroviruses. *Proc Natl Acad Sci USA* 1989, **86**, 9079–9083.
26. Levrero M, Barban V, Manteca S, Ballay A, Balsamo C, Avantaggiati ML. Defective and non-defective adenovirus vectors for expressing foreign genes *in vitro* and *in vivo*. *Gene* 1991, **101**, 195–202.
27. Lebkowski JS, McNally MM, Okarma TB, Lerch LB. Adeno-associated virus: a vector system for efficient introduction and integration of DNA into a variety of mammalian cell types. *Mol Cell Biol* 1988, **8**, 3988–3996.
28. Fink DJ, Sternberg LR, Weber PC, Mata M, Goins WF, Glorioso JC. *In vivo* expression of β-galactosidase in hippocampal neurons by HSV-mediated gene transfer. *Human Gene Ther* 1992, **3**, 11–20.

Acknowledgement—Dr M. Collins is supported by a grant from the Cancer Research Campaign.