Gene therapy for cancers

Targeting 'cancer genes'
The prospect for genetic manipulation of human disease has stirred excitement both in the scientific and clinical community. A view of cancer as a 'genetic' disease which is often refractory to treatment makes it a prime target for such an approach. In the mendelian sense, cancer as a genetic disease is a rare event. The number of individuals with a single gene disorder and associated predisposition to cancer are relatively small, but include patients with retinoblastoma where 40-45% of cases are attributable to an inherited abnormality; and the Li-Fraumeni syndrome, where a single mutation may lead to the development of a variety of tumours including soft tissue sarcomas, premenopausal breast cancer, and adrenocortical carcinoma.

The genetic basis for increased risk of tumour formation come from inherited mutations in tumour suppressor genes which frequently encode important proteins involved in cell cycle regulation or growth. Tumour suppressor genes are exciting targets for genetic therapy as the presence of one functioning allele appears to protect against tumour development. Moreover, the restoration of a single copy of a tumour suppressor gene may suppress the malignant phenotype. Retroviral mediated transfer of the RB1 gene into retinoblastoma cell lines results in inhibition of the neoplastic phenotype both in vitro and in vivo. This is manifest as a reduction in growth rate and clonogenicity of cell lines and tumour formation in nude (immunosuppressed) mice.1

The majority of childhood malignancies arise in individuals with no obvious cancer predisposition, however, and result from an accumulation of new mutational events. In these sporadic tumours it is possible to identify a number of genes which exert their effect in a dominant fashion (so called oncogenes). The persistence of a single wild type allele is insufficient to counteract the effect of the transforming mutation and requires a different biological approach to silence its action. Unfortunately, direct repair of aberrant genes through homologous recombination is beyond current technical capacity. Even when DNA is introduced experimentally by microinjection into the nucleus, the efficiency of recombination is approximately 10^-2 to 10^-3.2 3 This is too low for any meaningful effect in a malignant cellular population.

An alternative approach is to use antisense oligonucleotides. These compounds have been developed to take advantage of the specific base pairing that take place between complementary strands of DNA and RNA. Through steric hindrance of mRNA or enzymatic cleavage of RNA hybrids, protein synthesis is prevented.4 5 Early clinical targets include the fusion protein produced from a tumour specific translocation in chronic myeloid leukaemia – the oncogene BCR-ABL,6 and cell survival signals such as the protein bcl-2.7 In vitro studies have shown that antisense oligonucleotides directed against either of these oncogenes have some antitumour effect and clinical studies are focused on testing oligonucleotides for purging bone marrow of leukaemic cells.

Translating these compounds into pharmaceutical products will require considerable development. Increasingly, stable oligonucleotides are being produced that are resistant to nuclease digestion by chemical modification of the phosphodiester backbone. However, such changes can result in lowered affinity and the production of significant non-specific cytopathic effects.8 9 The challenge of producing useful compounds is the object of much research activity that should bear fruit in the next decade.

Although direct manipulation of gene products is an interesting approach to tumour control, it is constrained by the complex biology of many tumours. Malignant transformation frequently results from the cooperation of multiple oncogenes as well as loss of suppressor functions. Consequently much more research is required to identify the pivotal events in maintenance of the malignant phenotype before suitable targets in many paediatric tumours can be identified.

Because of these problems a number of groups are concentrating on transferring genes into cancer cells which aim to increase the therapeutic index of existing treatment strategies. These can be broadly divided into increasing the host effector mechanisms for tumour rejection (immunotherapy), tumour expression of enzymes which will selectively activate prodrugs to cytotoxic anabolics, and the development of strategies to protect normal tissues from the cytotoxic effects of chemotherapy.

Immunotherapy by genetic modulation
The bulk of current clinical trials in this area involve immunotherapy and stem from observations that a range of cytokines and subpopulations of lymphocytes are capable of mediating tumour regression in human tumours.10-12 It is assumed that the cytokines expand and
enhance the ability of activated lymphocytes to reject tumour populations. The value of this type of cancer treatment is limited by both side effects and the small range of conditions that appear to be responsive. It is proposed that local expression of cytokines through genetic manipulation of tumour cells will reduce the systemic side effects of intravenous cytokine therapy and may act as a more potent recruiting source for tumour specific cytotoxic lymphocytes. A number of experiments have indicated that lymphocytes activated in this way are capable of rejecting both distant (non-transduced) tumours as well as a subsequent challenge with non-transduced tumour,

13,14 and suggests that activated lymphocytes may be primed to recognise common tumour related antigens expressed on distant metastases.

Clinical studies rely on the removal of tumour, ex vivo transduction with a gene coding for a specific cytokine, irradiation of the tumour population, and reimplantation into patients. The only paediatric tumour that is currently being studied with this approach is neuroblastoma. It is too early to assess whether this form of immunomodulation will be of benefit in either increasing the efficacy of cytokine mediated tumour rejection or in expanding the range of paediatric cancers which may be sensitive to this approach.

Activation of prodrugs

Enzyme activation of prodrugs also has at its heart an attempt to diminish the side effects of treatment and to expand the range of agents available for tumour control. The principle of this approach is to target genes to tumour sites which code catalytic enzymes and which can locally activate systemically administered prodrugs.15 When the genes are delivered by viral vectors the process is known as ‘viral delivered enzyme prodrug therapy’ (VDEPT). Although the concept is simple, tumour selective targeting presents enormous challenges. In clinical situations such as brain tumours, where a tumour is fatal because of local expansion, direct instillation of a vector system containing the gene of interest may be sufficient for tumour control. The principle has been demonstrated in glioblastoma where rats bearing xenografts of tumour have had the thymidine kinase gene introduced into tumour through stereotactic implant of retroviral producers.16 The retrovirus spreads through the tumour population carrying and integrating the gene into dividing cells. Intravenous administration of ganciclovir results in local tumour necrosis, and this treatment is now the focus of a phase I clinical study in high grade gliomas. Unfortunately, the number of clinical situations where this approach is relevant is limited. Often patients relapse because of failure to control metastatic disease and the systemic delivery of vectors containing genes may not be sufficiently specific or efficient to achieve the desired aim.

To overcome concerns of specificity a number of groups are looking at harnessing tissue specific transcriptional regulatory sequences for gene activation (TSTRS). For example α fetoprotein is only expressed in fetal tissue and in germ cell and liver tumours such as hepatoblastoma. The transcription of this gene is under the control of TSTRS which can be isolated and coupled to genes of interest. Huber and colleagues have used this approach to obtain specific gene expression in liver tumour and a similar approach has been successfully demonstrated with respect to the tyrosinase promoter in melanoma and the carinoembryonic antigen promoter for colonic carcinoma.17-19 The problems with this approach lie, as do those based around targeting tumour suppressor genes, in the development of effective vector systems which are capable of efficient tumour transduction. Because of the difficulties in attaining such a goal in vivo, many researchers continue to focus on ‘biological niche’ therapy, where ex vivo manipulation of tissue may provide some therapeutic gain.

Reducing unwanted toxicity targeting normal tissue

Targeting normal tissues includes the manipulation of bone marrow where incorporation of specific genes may protect against the cytotoxic effects of chemotherapy. These may be genes such as the MDR gene which codes for a membrane pump (not unlike the cystic fibrosis transporter) and which exports cytotoxic agents from the cell.20 Whether such an approach offers any clinical advantage over alternative techniques for haematopoietic support during chemotherapy such as haematopoietic growth factors or mobilised peripheral blood progenitor populations remains to be seen.

However, it is also important to consider toxicities which although not immediately life threatening have delayed or chronic effects. Such considerations influence the use of anthracyclines (myocardial damage), ifosfamide and cisplatin (renal toxicity), and certain nitrosoureas (progressive lung fibrosis). While cytotoxicity of haematopoietic stem cells would be interesting, protection of other tissues such as lung or kidney would be a therapeutic breakthrough. Issues here centre on understanding mechanisms of tissue damage and how they may be modulated. Intervention in the process of tissue injury will require the development of in vivo techniques for tissue specific gene delivery. A major research effort is going into the development of flexible vector systems which are capable of use in vivo and revolve around modification of viruses with natural tissue tropism for human cells (for example, retroviruses will integrate widely into cycling cells,21 adenoviral vectors have some respiratory specificity,22 and herpes related vectors are being studied for neuronal gene transfer). As yet there are minimal data on how some of these systems will operate in vivo. Clinical experience with retroviral mediated gene transfer is limited to ex vivo manipulation of human cells as modified murine retroviruses are rapidly inactivated by human comple-

ment.23 Other retroviruses with a different natural host range such as the feline leukaemia viruses may prove to be less sensitive and are the subject of study by a number of research groups.

Additionally, a further generation of viral vectors are already being developed. In pseudotype vectors, virions bearing the genome of one virus are encapsidated by the envelope proteins of another.24 This approach has the capacity for manipulating viruses which may be capable of efficiently transferring and expressing genes but have a restricted host range. In a similar vein retroviral vectors have also been engineered to express surface antibody directed against a cell surface receptor.25 Consequently it would seem likely that a range of vectors will become available for in vivo use with differing levels of operational specificity. How useful any particular vector will be will clearly depend on the therapeutic end point. To successfully target tumour suppressor genes, cellular differentiation signals or protective functions, vectors will be needed that are both efficient and capable of sustaining gene expression. If the clinical end point is cell death, specific rather than stable gene expression will be needed, and gene linkage to TSTRS will assume greater importance. These considerations imply the development of tailor made systems for each biological intervention and tumour type. In paediatric cancer where approximately
60% of patients are cured, careful targeting of conditions as well as genes is required.

LINDA LASHFORD

Phillips Institute for Cancer Research,
Christie Hospital NHT Trust,
Wilmslow Road,
Manchester M20 9BX

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