Interventional genetics and cancer treatment

Gene therapy predicted to join the therapeutic menu within the decade

The discovery of oncogenes and tumour suppressor genes has opened new avenues for exploring the control of cellular growth in health and disease. The protein products encoded by many of these genes have been characterised—some in considerable detail—and the evidence is now compelling that their abnormal expression is related to the development of human cancer. Can any of this be harnessed for therapeutic gain?

Systemic treatment of cancer is bedevilled by the similarity of tumour cells to normal cells, at least under most physiological conditions. Currently available chemotherapy is often highly toxic and, sadly for patients with many common tumours, simply ineffective. Although new agents, often discovered empirically, are entering clinical trials, there is little sign of classic pharmacological approaches providing a breakthrough.

The prospect of genetic intervention is much more appealing as it gets to the core of the problem. Thanks to rapid advances in recombinant DNA technology, we now have a range of sophisticated vectors that can deliver genetic information to cells in vivo. Most protocols currently use replication defective retroviruses, which can very efficiently achieve stable integration into human somatic cells. Gene transduction with these agents is essentially restricted to dividing cells, but although this could limit applications for other genetic disorders, it should not restrict gene therapy for cancer, where the target population of malignant cells is expanding.

Other viral vectors have features that may be useful for delivering genes in particular circumstances. For instance, large segments of DNA can be successfully cloned in place of the early region 1 genes of replication deficient adeno virus. Such altered viruses can transduce non-replicating cells, including differentiated epithelial cells of the respiratory tract and elsewhere, and this has made them the chosen vectors for genetic therapy of cystic fibrosis. Other delivery systems include liposomes and the promising technique of receptor mediated delivery of nucleic acids.

Two interventional genetic approaches might be exploited to block expression of genes associated with cancer. Antisense technology uses sequences of nucleic acid (DNA or RNA) complementary to a specific target messenger RNA to block the production of proteins selectively. The second approach uses ribozymes that are RNA sequences with the capacity to cleave target RNA selectively. These may be incorporated as cassettes in antisense vectors and may be even more effective in abrogating expression of target genes. Such approaches have proved effective in reverting the transformed phenotype of malignant cells in vitro, but several problems remain before clinical application would be feasible.

Most of the 38 protocols approved for genetic therapy are being carried out in the United States on patients with cancer. Britain has lagged behind, and the committee for the approval of protocols proposed by the Clothier report has only just been announced by the Department of Health. Several British groups have protocols ready for testing cancer with genetic therapy.

Four approaches are being investigated. The first, virally directed enzyme prodrug therapy, involves the delivery of suicide or "Trojan horse" vectors to tumours and certain normal cells. This entails coupling a promoter region of a gene expressed in cancer cells with a non-mammalian enzyme that can activate a prodrug to a cytotoxic agent. The selectivity may not need to be absolute—for example, if the promoter is specifically activated in breast epithelial tissue it may be possible to produce a "genetic mastectomy," effectively destroying all normal as well as malignant breast cells.

The transcriptional regulatory sequences of carcinoembryonic antigen (implanted in colorectal cancer), tyrosinase (melanoma), c-erb B2 (breast, pancreatic, and gastric cancer), and a fetoprotein (hepatoma) have been coupled to the genes for several drug activating enzymes. One of the most versatile enzymes is cytosine deaminase, found in fungi and bacteria, which converts the antifungal drug flucytosine to the much more toxic fluorouracil. Better understanding of the secrets of transcription control may lead to new selective vectors.

The second approach is to use lymphocytes that home in on tumours as vehicles for delivering a biological payload to the tumour. Tumour infiltrating lymphocytes can be harvested from tumour biopsy specimens, grown in vitro, and infected with suitable retroviruses containing human cytokine genes. Lymphocytes that have been successfully transduced (that is, they express the genes carried by the virus) are selected and their population expanded for reintroduction into the patient. Tumour necrosis factor, interferon alfa, and interleukin 2 have already been expressed and secreted in high concentrations within certain tumours. These agents are toxic when given systemically, and so producing them at high concentration within the tumour is an attractive goal. As yet, however, there is little evidence that cytokine secreting tumour infiltrating lymphocytes perform any more effectively than the parent cells.
Modifying the immunogenicity of tumour cells by inserting genes encoding either cytokines or the products of the major histocompatibility complex can circumvent the problem of the weak immunogenicity of many animal tumour systems. Encouraging results have been obtained by expression of the IL-2 gene in a mouse colonic cancer model and of the IL-4 gene in a mouse renal cancer model. Many clinical studies of genetic immunomodulation are currently under way, particularly in those diseases in which powerful immune responses have been detected, such as melanoma and renal cell carcinoma.

Perhaps the most exciting of future possibilities for genetic intervention in cancer is the direct manipulation of oncogenes or tumour suppressor genes. Although we recognise that cancer is a multigene disorder (with five or six genetic changes needed to induce malignancy), some genes may be critical at particular stages and reversal or blockade of a single event may be therapeutically useful. Mutant oncogenes of the ras family are found in about a third of all cancers and as many as 80% of pancreatic tumours. This family presents an obvious target for antisense attack, and a protocol to use a retroviral delivery system for antisense treatment in adenosarcoma of the lung has just been approved in the United States.

The restoration of tumour suppressor gene function has proved an effective strategy for reversing the malignant phenotype in vitro and recently in several animal systems using retinoblastoma gene constructs. Unfortunately we do not yet have good methods to control the quantity of protein produced, and overproduction could adversely affect normal tissue. Indeed, transgenic mice with an extra copy of the RBl gene are consistently smaller than normal.

Once the entire human genome has been mapped and sequenced it will become amenable to manipulation. Replacing new genes for old—homologous recombination—is now feasible in animal cells, but the technology requires a great deal of refinement before such a feat could be achieved in human somatic cells. Transgenic animals with deleted or mutant tumour suppressor genes provide an elegant model for correction of inherited predispositions to cancer by gene transfer either into particular tissues or into the whole organism.

All new technologies carry risks. Gene therapy is emotive, with potential hazard not only to the patient but also to staff and the environment. Considerable effort has gone into evaluating the risks and developing ingenious methods to reduce them. Thus disabled viruses that cannot reproduce themselves, suicide marker genes that can destroy cells containing the relevant vector when required, and close examination of clinical protocols by national ethical and scientific committees all help to limit potential problems. A decade ago it would have been inconceivable that we would now know enough about the molecules of cancer to consider genetic intervention. We believe that within 10 years cancer centres will be offering gene therapy as an effective systemic treatment along with surgery, radiotherapy, and chemotherapy.

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Fluoridation in Britain today

Water companies are getting in the way

The recent decision by Welsh Water not to continue fluoridating water in Gwynedd means that the water fluoridation schemes established as part of the Ministry of Health’s demonstration study in 1955-6 on Anglesey and in Watford and Kilmarnock have now all stopped operating. That the schemes provided benefits seems undeniable. For example, after the burgh council stopped the Kilmarnock scheme Professor John Mansbridge of the University of Edinburgh continued to monitor the dental health of children in Kilmarnock and in the control community of Ayr. He showed that five years later the dental health of children in Kilmarnock had deteriorated. At baseline in 1956 the average 4 year old had 7.1 decayed, missing, or filled teeth. By 1961, after five years of fluoridation, the equivalent figure was 3.0; in 1962 fluoridation stopped, and by 1968 the number of decayed, missing, or filled teeth had reverted to 6.1. Throughout the period 1956 to 1968 the equivalent figure in 4 year old children in the control town of Ayr remained between 6.9 and 7.2.

The Anglesey scheme operated for over 30 years, albeit rather intermittently for the past few years. Thomas and Kassab recently reported on the substantial benefits of fluoridation for the dental health of women aged up to 32 years who had lived continuously on Anglesey and who were attending St David’s Maternity Hospital between July 1986 and July 1987 for their confinement. The dentist recording the dental condition of both the women from Anglesey and the control group from the non-fluoridated Gwynedd mainland was blind to women’s residential status; those living in the fluoridated area had 30% fewer caries. The fluoridation plant in Watford, also showing signs of age, stopped operating...