Gene therapy in gastroenterology

Gene therapy can be defined as the introduction and expression of an exogenous gene in human cells for therapeutic benefit, and is conventionally restricted to human diseases associated with single gene defects. However, there are wider opportunities for genetic intervention and these include strategies to reduce or block gene expression as well as the introduction of non-mammalian genes. The potential role for genetic intervention extends from diseases caused by single gene defects, through severe viral infections to polygenic disorders such as diabetes mellitus and arteriosclerosis. The rapid progress in our understanding of some of the molecular mechanisms involved in the pathogenesis of cancer and metabolic disorders, coupled with the development of gene delivery vector technology, has stimulated us to consider novel genetic approaches to digestive diseases.

What are the possibilities for gene therapy in gastroenterology and how real are they?
Strategies for genetic intervention can be divided into five main groups. These comprise replacement or augmentation of gene expression, reduction of expression of genes by antisense or ribozyme technology, genetic prodrug activation, augmentation of immune responses, and polynucleotide vaccination. Some strategies can be achieved by ex vivo gene transfer into isolated human cells, which can then be reimplanted into the host, while others require delivery and expression of genes to target cells in vivo—a major challenge with current vector technology. In this review we consider important clinical applications within these categories and outline the directions of study that should lead to clinical trials in the near future.

Gene transfer technology
The field of gene delivery technology is advancing rapidly and there have been specific developments that could be translated into gene based therapies for gastroenterological diseases. For example ex vivo gene transfer methods have been studied extensively using hepatocytes obtained through liver biopsy, partial hepatectomy, and from specimens harvested for liver transplantation. Adult liver cells transiently undergo active proliferation permitting in vitro gene transfer even with vectors that require active cell division for entry and expression. Gene transfer may then be facilitated through a number of methods including viruses, liposomes, calcium phosphate cocrystallisation, particle bombardment, naked DNA injection, and electro-poration. The transfected cells are reintroduced into the host using, for example, a microcarrier system into the peritoneum, gel beads, hepatocyte coated cell support matrix implanted next to liver tissue, or into the spleen or portal circulation through direct injection.1

The spectrum of delivery systems for ex vivo gene transfer broadly applies also to the in vivo model. Although the transfer efficiency of liposomes is comparatively low, these lipids can be made comparatively easily to high chemical purity and have low immunogenicity, which may permit repeated administration. They have been used successfully with an in vivo model, by topical administration to epithelial cells both in the airways and the intestinal tract and also using the intravascular route. A recent study showed high efficiency transfer of the APC tumour suppressor gene in liposome complexes delivered to normal mouse colonic epithelium by rectal catheter infusion. Almost 100% of epithelial cells expressed the gene for up to four days consistent with the known rate of turnover of this tissue.2 Intravenous injection of a rat insulin gene expression vector in liposome complexes results in uptake primarily by the liver and spleen. Improvement in hepatocyte uptake can be achieved by incorporating lactosyl-ceramide into the phospholipid bilayer; this lactosyl-terminal asialoganglioside is specifically recognised by a receptor highly selective for hepatocytes.3 Many different lipid agents are now being explored for efficacy of DNA transfer and it seems probable that the composition of the complex will have to be optimised for different targets and different routes of administration.

Of the available methods of gene delivery, viruses have proved the most efficient thus far. Achieving viral gene transfer to specific organs for clinical application will be difficult, however, particularly as viral titres 10 to 1 to 1000 times higher than those usually attained (typically 10^6 infectious units per millilitre) will be necessary for in vivo strategies. There is now extensive experience with retroviruses whose main advantages include their small size, their ease of manipulation, with stable colinear integration with host genome. They are comparatively non-toxic and are efficient for gene transfer. Retroviruses persist in up to 5% of hepatocytes three months after injection of an
infected hepatocyte cell suspension into the portal vein after partial hepatectomy. The small intestinal epithelium is an attractive target for gene therapy because of its large surface area, easy accessibility, and the presence of stem cells with known locations. Although few studies have yet targeted the intestinal system directly, marker genes have been transferred to the epithelial surface using retroviral vectors in animal models. Clearly, unless the therapeutic or marker gene is transferred to the stem cells the rapid turnover of this specialised epithelium would seriously limit potential benefits of delivered genes. Retroviruses have a number of disadvantages, notably the requirement for cells that are actively dividing to permit viral DNA integration, the ability to carry only small DNA sequences, and a small but finite risk of causing insertional mutagenesis as a result of random integration.

Currently alternative viral vectors with potential advantages over retroviruses in specific applications are under development. Adenoviruses can infect non-dividing cells, can be concentrated to high titres and are comparatively high efficiency vectors. Adeno-associated viruses are ubiquitous and non-pathogenic in humans and can also infect non-replicating cells, but, like retroviruses and adenoviruses, are limited in the size of the foreign gene that can be inserted. This last problem may be overcome by the use of herpes simplex group viruses and possibly even vectors based on hepatitis B virus, which has potential additional advantages of hepatotropism and an ability to integrate with the host genome in vivo.

Cystic fibrosis

Replacement strategies for disorders resulting from a single gene defect are attractive candidates for gene therapy. Inheritance of two mutated copies of the cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome 7q22 causes this common autosomal recessive disorder. The CFTR functions primarily as a cyclic adenosine monophosphate activated chloride channel in epithelial cells. The CFTR gene has a complex mechanism of regulation and is expressed mainly in particular classes of epithelial cell. In humans these sites include certain cells of the lung, crypt cells in the ileum, duodenum and colon, the pancreatic ducts, and gall bladder. There is recent evidence of endogenous CFTR gene expression in intrahepatic biliary epithelial cells, which is consistent with clinical findings of cystic fibrosis induced biliary damage.

Despite recent molecular genetic information regarding the CFTR gene and its product, the exact cause of the mucus abnormalities in cystic fibrosis is unclear. The changes are not fully explained simply on the basis of loss or poor functioning of a small cAMP activated chloride conductance in the apical membranes of certain specialised epithelial cells. It is not clear how different mutations in the CFTR gene actually cause specific phenotypic presentations of cystic fibrosis.

Despite these problems substantial progress has been made with gene therapy strategies for cystic fibrosis. The four transgenic mice models available for study have severe intestinal disease with relative sparing of lung and pancreatic epithelia (unlike those in humans). Correction of the lethal intestinal defect has been shown by transfection of human CFTR by liposomal delivery in a vector under the control of rat intestinal fatty acid binding protein gene promoter. Treated mice survived for up to seven months, well beyond the expected four weeks of the control group, and also showed functional correction of ileal goblet cell and crypt cell hyperplasia and cAMP-chloride secretion. Gene therapy aimed at correcting lung abnormalities in human subjects has entailed the direct introduction of non-mutant CFTR cDNA into the epithelial cells of the respiratory tract in the hope that this will result in sufficient CFTR protein being made in these cells to correct the disease process. Vectors used so far include liposomes and adenoviruses, which each have their own drawbacks: the adenoviral route is proving relatively inefficient while immunological responses to adenovirus may limit its efficacy. Nevertheless initial data from two phase I clinical trials of cationic liposomal mediated CFTR delivery of a plasmid containing the CFTR cDNA to the nasal epithelium has resulted in over 20% correction of conductance abnormalities in nasal mucosal biopsies and overall the treatment was well tolerated. Attempts have also been made to transfer the CFTR gene into the biliary epithelium in vivo using adenoviruses. In one study, injection of the vector directly into the common bile duct during laparotomy resulted in gene expression in nearly all intrahepatic bile duct epithelial cells. Gene expression remained stable after 21 days in epithelial cells of small ducts. These results are encouraging particularly because gene delivery might be achieved by endoscopic retrograde cholangiopancreatography in the future.

α1 antitrypsin deficiency

Approximately 1% of the British population carry the PiZ defect caused by a point mutation in the α1 antitrypsin gene in chromosome 14, leading to low serum concentrations of α1 antitrypsin and predisposition to emphysema and liver cirrhosis. Savransky et al have proposed one approach to correcting this gene defect in human PIZZ GM2522 fibroblasts using the technique of targeted homologous recombination to the gene locus, replacing exon V of the abnormal gene with the exon V counterpart of a normal complementary DNA. Other groups have shown a similar effect in hepatoma cell lines and hepatocytes in rat liver in vivo using soluble carrier systems, which utilise the asialoglycoprotein receptors on hepatocytes. It is conceivable that in due course harvested human fetal hepatocytes identified as possessing the PiZ defect could be subjected to targeted homologous recombination in vitro and then reinfused into the portal vein.

Low density lipoprotein (LDL) receptor deficiency

Homozygous LDL receptor deficiency leads to familial hypercholesterolaemia, in which patients have six to eightfold increases in plasma LDL-cholesterol values associated with premature atherosclerotic disease and early death. This is a common disease resulting from a mutation in the gene for the LDL receptor affecting one in 500 of the population. LDL receptors are found on most tissues but it is hepatic expression of the receptor that is most crucial in cholesterol homeostasis. This condition may in many cases respond to drug treatment but transfer of LDL receptor genes to the liver may be a treatment option for the few subjects with intractable hypercholesterolaemia for whom the only treatment option is liver transplantation. Animal studies using the model for homozygous familial hypercholesterolaemia, the Watanabe heritable hyperlipidaemic rabbit, have shown successful ex vivo retroviral transfer of the receptor gene to 20% of cultured hepatocytes. Transduced cells then expressed receptor levels four or five times higher than normal hepatocyte controls, and after re-injection into the host liver, resulted in a lowering of total cholesterol value to 50–70% of its pre-treatment value, an effect that lasted over four months. Furthermore there are indications that clinical benefit may result from even partial correction of the total receptor defect, and clinical
trials are already underway in the United States using retroviral ex vivo gene transfer.

**Antisense DNA oligomer treatment: suppressing oncogene expression**

Antisense oligodeoxynucleotides are short (10–50 base) synthetic nucleotide sequences formulated to be complementary to specific DNA or RNA sequences. By the binding of these nucleotides to their targets, the transcription or translation of a single gene can be selectively inhibited by triggering RNAse H degradation of the target RNA and interfering with the processing of pre-mRNA. Examples of antisense oligomers with significant in vitro antiproliferative activity include those against c-myc in colorectal cell lines, c-myc in lymphoma lines, and bcr-abl in chronic myeloid leukaemia blast cells.

The ras oncogenes are an obvious potential target for antisense therapeutics as they are implicated in many solid tumours including more than 75% of pancreatic cancers and colorectal cancers. Inhibition of the ras signalling pathway modulates critical aspects of ras oncogene mediated transformation in whole cells. The resulting phenotypes include reduced anchorage dependent and anchorage independent growth and morphological reversion of the cells. Recently, reduced biological aggressiveness and loss of anchorage independent growth was reported in experiments using homologous recombination to target k-ras. Using antisense oligonucleotides to target regions of k-ras mRNA there are some antiproliferative effects in human pancreatic cancer cell lines, but there is wide variability of response rates in different cell lines and little evidence of sequence specificity. Moreover, there is no correlation of antiproliferative effect with reduction in amounts of k-ras protein expressed and these agents theoretically directed against cellular genes act instead through unpredictable sequence independent mechanisms. Similar findings have been reported for oligonucleotides designed against c-myc and c-myc sequences.

**Antisense DNA oligomer treatment: suppressing hepatitis B virus expression**

Using asialo-orosomucoid coupled with poly-L-lysine, both single and double stranded DNA have been delivered specifically to hepatocytes by targeting their asialoglycoprotein receptors. An antisense oligodeoxynucleotide complementary to the polyadenylation signal for human hepatitis B virus was introduced into HepG2 hepatoma cell line by Wu et al. This cell line had been transfected with a complete human hepatitis B viral genome and secreted infectious virions into the culture medium. At 24 hours and seven days after exposure to the antisense sequence, HBV DNA values were 80% and 95% lower than controls respectively. Furthermore, using similar carrier systems, two separate antisense sequences injected into Peking ducks (an animal model for HBV infection) resulted in suppression of viral replication by over 90% compared with controls.

**Replacing defective tumour suppressor genes**

In cell culture malignant properties can often be reversed by inserting normal tumour suppressor genes. The difficulty for corrective strategies lies in delivering actively expressed vectors to every single tumour cell of an established and possibly disseminated cancer in vivo. The same argument may not apply for prophylactic therapy to replace gene function in cells of patients with inherited abnormalities of tumour suppressor genes, where it may not be necessary to correct the constitution of every cell to significantly reduce the risk of cancer development within the lifetime of a person.

Normal (wild type) p53 is involved in the control of cell cycle progression as well as in arresting replication to permit repair in DNA damaged cells. It may also be involved in restricting precursor populations by mediating apoptosis or programmed cell death. Abnormal or mutant p53 protects the accumulating gene rearrangements and chromosomal abnormalities and has been associated with virtually every sporadically occurring malignancy including gastrointestinal tumours and hepatocellular carcinoma. There is experimental evidence showing the benefits of correcting p53 abnormalities. Replacement of wild type p53 using retroviral expression vectors in both human lung cancer cell lines with mutant or deleted p53 in vitro and in the nu/nu mouse model of orthotopic human lung cancer resulted in suppression of the malignant phenotype.

Furthermore, there was also evidence that the combination of restoration of p53 function and sequential administration of the cytotoxic drug cisplatin was synergistic in reducing the malignant behaviour of these cell lines, clearly important findings that may influence approaches to adjuvant treatment for cancer.

There could also be a role for replacement therapy for p53 in patients with Barrett’s oesophagus, a condition that may respond poorly to medical treatment. Adenocarcinoma arising in Barrett’s oesophagus is often preceded by mucosal dysplasia. Several groups have found an association between p53 mutation and adenocarcinoma related to Barrett’s oesophagus. In addition, recent reports suggest that p53 dysfunction may participate in the progression from dysplasia to carcinoma, and that there is a correlation between presence of mutant p53 and increasing dysplastic features. For those patients with histological evidence of progressive dysplasia who are unfit for surgery and refractory to treatment with acid suppression, correction by insertion of wild type p53 using submucosal endoscopic injection of retroviral or adenoviral suspensions may be an alternative treatment. Clearly such a strategy will require considerable improvement in gene delivery systems and targeting mechanisms for the preneoplastic cells, as well as more sophisticated understanding of transcriptional control to permit appropriate expression.

The identification of the APC gene participating in familial adenomatous polyposis affecting the large and small intestine, and the DNA mismatch repair gene families (hMSH2, hMLH1, hPMS1, and hPMS2) involved in hereditary non-polyposis colorectal cancer syndrome will stimulate further interest in the replacement of tumour suppressor gene function in stem cells in organs at risk of cancer development. However, the paucity of knowledge concerning the temporal and spatial control of expression of these genes will delay the application of such prophylactic gene therapy.

**Inserting drug activating ‘suicide’ genes**

There are many examples of genes preferentially expressed in tumours compared with normal tissues. Genetically directed enzyme prodrug therapy (GDEPT) exploits the differences in gene expression between different cell types to increase the specificity of cell destruction by coupling to promoters of differentially expressed genes to prodrug activating enzymes. Examples of useful promoters include carinoembryonic antigen in colorectal cancer, ERBB2 in breast and pancreatic cancer, and prostate specific antigen in prostate; the most widely used prodrug activating enzymes are cytosine deaminase (which converts
5-fluorocytosine to the cytotoxic 5-fluorouracil) and herpes simplex virus thymidine kinase (which converts ganciclovir to toxic phosphorylated derivatives). The system is designed so that significant transcription of the enzyme gene is activated only in tumour cells by linking the enzyme gene to transcriptional control elements (a 'molecular switch') selective for a particular tumour or tissue type. Significant antitumour effects from conversion of 5-FC to 5-FU have been seen in colorectal cancer cell lines transduced with the cytosine deaminase gene under a constitutive promoter. There was also significant regression of tumour volume even when as little as 2% of the tumour mass contained cytosine-deaminase expressing cells. The last effect was also seen when a CEA-HSVTK construct was used to transduce CEA expressing pancreatic cancer cells, which were then engrafted into severe combined immune deficient mice. When ganciclovir was given there was a significant reduction in tumour size even when only 10% of the cells expressed HSVTK. This important feature of the GDEPT system is probably due to a so called 'bystander effect' attributed to transfer of the activated dominant enzyme into a cell in which it is expressed, but which is not itself an antitumour target. There has been some success in targeting tissue types such as the gastrointestinal epithelium and pancreas using the carinoembryonic antigen gene promoter, and also targeting tumour specific transcriptional activation using the α fetoprotein promoter in hepatocellular carcinoma.

**Augmentation of the immune response and cancer vaccines**

The promise shown by systemic high dose immunotherapy to treat cancer in murine studies has not been fulfilled in human trials except in a few cases of renal cell carcinoma and melanoma. Indeed the initial dose regimens were extremely toxic. It may be more important to deliver a small dose of cytokine at a specific site rather than high doses systemically, which has led to two approaches to cytokine based gene therapy that augment naturally occurring immune response to malignancies. The first approach entails the insertion of cytokine genes into cultured tumour infiltrating lymphocytes ex vivo. This subset of T cells is critical for the prevention and elimination of tumours, and their antitumour efficacy improves with the transfer of the genes for tumour necrosis factor and interleukin 2. ‘Cancer vaccines’ constitute the second approach and entail the induction of cytokine expression in the tumour cells so that T cells recognition of tumour antigens is enhanced. Immunostimulatory cytokine genes are transduced into tumour cells ex vivo, the tumour cells are irradiated to eliminate malignant activity and reintroduced into the host. Cytotoxic T cells recognise tumour specific antigens presented on the surface of these cells. They are induced by the local secretion of the transferred cytokine gene product to expand, target, and destroy cancer cells. In addition to cytokines, a number of other genes are also capable of inducing an antitumour response including allogeneic HLA (human leucocyte antigen) genes and costimulatory molecules such as the B7 family, B7.1 and B7.2. B7 is expressed mainly on antigen presenting cells and serves as costimulatory signals for T cells, by interacting with its ligands CD28 or CTLA-4.

There are currently two phase I clinical trials using immuno-gene therapy for colorectal cancer. In San Diego, Sobel et al are transducing irradiated autologous colon cancer cells to express interleukin 2 using a retrovirus and re-injecting the modified cells into the patient. At the Mayo clinic, Rubin et al are attempting to treat hepatic metastases by direct injection of B7-1 cDNA using liposomes.

Cytotoxic T cells depend on antigen being presented in the context of self class I MHC molecules, whereas T helper lymphocytes require activation by antigen presented in the context of self class II MHC molecules. Aberrant expression of the MHC is a common feature of gastrointestinal cancers. Class I antigens are frequently lost, while class II expression is often upregulated. Polynucleotide vaccination (in contrast with conventional vaccines consisting of peptides, whole tumour cells or tumour cell lysates) has great therapeutic potential in that delivery of genes that express unique oncoproteins such as k-ras or p53 endogenously within a cell may result in an MHC class I CD8+ response and proliferative activation of cytotoxic T cells, rather than a less effective class II CD4+ response. This may be a further means of breaking down immunotolerance to tumours, which could lead to the generation of tumour specific responses. Mutated forms of the ras proto-oncogene, in particular, contain potentially antigenic T cell epitopes specific for the malignant phenotype. Certain k-ras mutations that produce new peptides including consensus binding motifs could result in increased immunogenicity as a result of the increased HLA-C recognition produced. For the immune system, Sendai virus vectors have been used to deliver suicide genes to the tumour cells. These would be rendered non-infectious. For therapy, an adenovirus vector (by which a virus can be rendered non-infectious) could be used to deliver therapeutic genes that will cause the tumour cell to self-destruct. In this way there will be an advantage as the tumour cells will be killed with an effect against the tumour, whereas the viral vector is self-limited. This approach is currently being studied in a phase I clinical trial in Europe.

**Future directions**

There is no shortage of ideas and applications for genetic intervention in human disease, but there are serious limitations not only with the efficiency and targeting of the present generation of gene transfer vectors but also with our incomplete understanding of transcriptional control. These fields are under intense investigation and real advances are likely in the next decade.

In the short-term further useful improvements are possible from manipulation of the RNA and DNA viruses with which we are already familiar. Development of high efficiency viral packaging systems and refinement of the purification and concentration processes can be expected to improve the titres of viral vectors to values that could permit gene transfer by systemic administration. Targeting of delivery should be possible by incorporating single chain antibodies to cell surface antigens or ligands for transmembrane receptors into the envelope or penton coat proteins of retroviruses and adenoviruses respectively, and there are encouraging signs that this approach can be successful. In the intermediate term, we expect the arrival of 'designer
vectors' incorporating the most useful elements from both viral and synthetic systems and these could be varied depending on the particular application. For instance, the inverted terminal repeats of adeno-associated virus that mediate stable chromosomal integration could be combined with a backbone of another vector with a large insert capacity such as herpes virus or a bacteriophage. Integration might be enhanced by packaging a functional recombinase enzyme with the gene expression construct in liposomal complexes targeted to a particular class of cells with an antibody. In the longer term, basic research into the structure and organisation of mammalian chromosome should enable the construction of artificial chromosomes, which could carry whole clusters of genes with their natural control elements into cells.

The control of gene transcription is extremely complicated and, even for the most intensely investigated systems such as the globin genes, our understanding is still fragmentary. While most protocols presently use strong viral promoters to drive expression of recombinant cDNA copies of therapeutic genes, future work must be directed to defining the genomic elements that enable temporal and spatial control of expression through a lifetime. The identification of locus control regions that can insulate gene clusters from interference by surrounding genetic influences has been an important step, and many investigators are now working to understand how the promoter and enhancer/silencer elements of a gene interact with structures within the nucleus. Advances in this area will require parallel developments in the sophistication of vector design before they can be translated into practice.

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