

Current Gene Therapy Strategies for Colorectal Cancer

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Abstract: Colorectal cancer is one of the commonest cancers worldwide and despite improvements in surgery, radiotherapy and chemotherapy the overall five year survival is around 50%. Therefore, novel treatments need to be developed in order to add to the therapeutic armamentarium. Gene therapy is a promising new modality of treatment which can be used in combination with existing therapies. Current gene therapy strategies usually involve the use of interventional genetic techniques to enhance the immunological response to a tumour or to deliver cytotoxic agents to tumour cells. Such strategies can be used alone or in combination and are being explored in a number of clinical trials. More recently, attempts to correct some of the underlying genetic abnormalities in various cancers have been made. The genetic changes which lead to the development of colorectal cancer are well described and, therefore, may be amenable to correction. Replacement of tumour suppressor genes such as p53 has been shown to reverse phenotypic changes in animal models and has been licensed for human use in clinical trials. This technique appears to be safe in the small number of patients treated, thus far and several tumour responses have been demonstrated. Prophylactic treatment with tumour suppressor genes for individuals at high risk of developing colorectal cancer, such as those with familial adenomatous polyposis, may prove beneficial in preventing or delaying the onset of malignant change. The search for safer and more efficient gene delivery vectors continues since traditional adenovirus, retrovirus and plasmids are beset by problems of safety or efficiency. The ultimate gene delivery vector is likely to be a human artificial chromosome which would allow delivery of a large number of genes together with their controlling sequences. Colorectal cancer is a disease which can be attacked by a number of genetic mechanisms in order to kill tumour cells directly, prevent further growth and enhance the anti-tumour immune response. Clinical protocols need to move from the stage of small clinical trials to mass application and it is likely that improved gene delivery vectors will be necessary in order for this to occur.

INTRODUCTION

The use of interventional genetics for the treatment of human disease has moved from the realms of fantasy to clinical protocols and trials over the last 10 years. Initially gene therapy was envisaged as a type of gene replacement therapy whereby single genes would be introduced to correct a recessive single gene disorder. However, the scope of interventional genetics has been widened to include polygenic and multifactorial diseases such as cancer. The emphasis now is not on replacing defective genes with their "correct" version, but on providing useful new functions to cells by the introduction of therapeutic genes.

Cancer gene therapy encompasses a range of interventional genetic techniques for the treatment of malignant disease. The most widely applied of these techniques aim to introduce genes which will

enhance the host's immune response to a tumour. Newer strategies aim either to introduce cytotoxic "suicide" genes to kill tumour cells, or to introduce the protective multi-drug resistance (MDR) gene to protect bone marrow stem cells during ablative chemotherapy. Although cancer is due to multiple genetic abnormalities in a particular tumour gene therapy, strategies to "correct" one or more abnormality are starting to be explored. In general, these corrective gene therapy approaches involve either the introduction of tumour suppressor genes or the inactivation of proto-oncogenes.

Colorectal cancer is one of the commonest cancers in the Western world and most patients present with disease which is beyond the stage of cure by surgery. Adjuvant treatments such as radiotherapy and chemotherapy have long been used for patients with advanced local or systemic disease with some benefit. However, the five year survival for patients presenting with Dukes stage C disease (the single biggest group of patients) is still less than 50%. Therefore, new treatments such as novel chemotherapeutic agents, immunotherapy and gene therapy are all being explored. Gene

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therapy for colorectal cancer has reached the stage of clinical trials for small numbers of selected patients, usually those with disseminated disease. However, these new interventional genetic approaches face serious problems before they can offer a useful adjuvant role for the majority of patients with colorectal cancer.

IMMUNOTHERAPY

It has long been recognised that immunological mechanisms are important in the natural history of many cancers. Individuals with congenital or acquired immune deficiencies are known to be at high risk of developing a wide range of malignancies and in general tumours which elicit a marked host immune response are associated with an improved prognosis. Therefore, numerous attempts to harness such immune responses for therapeutic use have been made. Tumour associated antigens have been used as the targets of monoclonal antibody therapy and systemic treatment with immune modulators such as cytokines has been attempted. Many of these approaches have been hampered by difficulties in achieving therapeutic effects at the tumour site or by systemic side effects. Interventional genetic techniques are, therefore, being applied to the immunotherapy of colorectal cancer in an attempt to improve the targeting and efficacy of the immune response. These techniques aim to improve the host's immune response to a tumour by either non-specific mechanisms or specific immune responses.

Non-specific immune enhancement can be achieved by the introduction of genes for mediators such as interleukins, tumour necrosis factor and HLA antigens. Such approaches usually involve the transduction *in vitro* of resected tumour tissue with viral vectors carrying genes for cytokines and/or HLA antigens. These transfected cells are subsequently reinfused and allow local secretion of cytokines aiming to avoid the adverse effects of systemic cytokine administration. A number of cytokine gene therapy trials have been licensed in the USA and Europe which involve reinjection of tumour cells transduced with IL-2-containing retrovirus or adenovirus vectors [1]. Alternatively, adenovirus containing IL-2 can be directly injected into tumours - a small phase I/II clinical trial during which patients with unresectable intestinal adenocarcinoma were treated by intra-tumoural injection of an adenovirus:IL-2 construct at the time of surgery has been reported [2]. The first patients to be

treated showed increased expression of membrane bound IL-2 receptors and no toxicity events. One of the treated patients showed a positive tumour response with necrosis of the tumour mass.

Other cytokines such as IL-7 are also being investigated along with combinations of interleukins with HLA antigens or suicide genes. However, these approaches are costly and labour intensive, since cell cultures from each individual tumour must be established and then transduced with cytokine genes, but do guarantee matched antigens between the tumour and the vaccine. Newer strategies have attempted to use generic cytokine gene transduced cell lines as the vaccine. This approach assumes that tumour associated antigens are generic rather than specific to individual tumours. These allogeneic vaccines are not MHC restricted and therefore, economies of scale are theoretically possible. Individual cytokines have different properties which may be advantageous in their use for immunotherapy. For example IL-12 appears to induce a more prolonged anti-tumour response than IL-2 or cytotoxic gene therapy in an animal model [3]. Mice bearing experimentally induced colorectal tumours were treated by intra-tumoural injection of an adenovirus containing the mouse IL-12 gene. This treatment produced tumour regression and a prolonged survival compared to mice treated with a combination of IL-2 immunotherapy and HSVtk gene therapy.

T-cell immune responses appear to depend on antigen presentation in conjunction with costimulatory molecules of the MHC. Molecules such as HLA-B7 are essential to promote specific T-cell activation. Loss or reduction of expression of MHC class I molecules is a recognised feature of approximately 20% of colorectal cancers [4, 5]. In such circumstances specific T-cell responses to tumour associated antigens are impaired or absent. Such tumours, therefore, are poor stimulators of an immune response and may be capable of actively inducing a state of tolerance [6]. In order to make such tumours more "visible" to the immune system gene therapy strategies using costimulatory molecules are being investigated. A Phase I trial of immunotherapy for colorectal hepatic metastases using HLA-B7 gene transfer has recently been reported [7]. This protocol involved intra-lesional injection of HLA-B7 cDNA with liposomes in an attempt to elicit an improved T-cell dependent response to both the foreign MHC molecules and to improved recognition of tumour associated antigens. Fifteen patients were treated with no

significant toxicity but therapeutic benefit has yet to be determined.

The best known colorectal tumour associated antigen is carcinogenic embryonic antigen (CEA). This protein is expressed on colorectal cancer cells and has long been used as a target for immunotherapy. Gene therapy techniques involve vaccinating the patient with the cDNA for CEA in an attempt to generate a more effective and specific immune response than administering preformed anti-CEA monoclonal antibodies [8-10]. Intramuscular injection of a CEA nucleic acid "vaccine" has been shown to generate both cellular and humoral immune responses in dogs [11]. A phase I clinical trial of naked CEA cDNA in patients with metastatic CEA-expressing colorectal cancer has been completed [1, 12]. Preliminary results report no adverse inflammatory effects nor development of anti-DNA antibodies. Antibody responses to CEA and to the coadministered HBsAg control cDNA have been detected.

In an attempt to improve the immune response to CEA vaccination the Alabama group have investigated the co-delivery of CEA cDNA with the cDNA for a cytokine or co-stimulatory molecule. The cDNA for CEA and for B7-1 were administered either in separate plasmids or in a single plasmid with two independent expression cassettes. The latter led to an enhancement in anti-CEA antibody response and enhanced anti-tumour response in mice whereas delivery of the plasmids separately had no enhancing effect [13]. These results suggest that co-expression of CEA and a co-stimulatory molecule within the same cell is important. Prior administration of a plasmid encoding GM-CSF was also observed to augment anti-CEA antibody response and CEA-specific lymphoblastic transformation. Further work is needed to determine the optimum combination and timing of strategies to enhance the response to cDNA vaccination.

CYTOTOXIC GENE THERAPY

Over the last five years the most exciting developments in cancer gene therapy have come in the field of cytotoxic gene therapy. This approach allows the administration of a non-toxic prodrug which is converted to an active toxic metabolite by a specific enzyme in the target cells. The gene encoding a specific enzyme can be introduced into the target tumour cells by a variety of gene transfer vectors. Examples of prodrug and enzyme pairs

which have been investigated for use in cancer gene therapy are listed in Table I.

The most widely used drug:enzyme pair is herpes simplex virus thymidine kinase (HSVtk): ganciclovir. The non toxic prodrug ganciclovir is phosphorylated by HSVtk but not by human thymidine kinase to the toxic triphosphate form. Ganciclovir triphosphate is incorporated into the DNA of dividing cells causing chain termination and cell death [14]. In order to achieve tissue or tumour specificity different selective mechanisms can be incorporated into either the vector or the construct used to deliver the HSVtk gene. Retroviral vectors will only infect dividing cells and this property has been used to advantage in the treatment of malignant brain tumours which consist of actively dividing cells in a background tissue of terminally differentiated cells. Stereotactic local injection of retrovirus containing HSVtk therefore ensures that only the tumour cells are transduced with HSVtk and are therefore susceptible to treatment with ganciclovir. The surrounding non malignant brain cells contain only the human form of thymidine kinase which is not able to convert ganciclovir to its toxic metabolite. This strategy has been used in several clinical trials for the treatment of malignant brain tumours [15-19]. Initial results showed tumour regression and improved survival, however tumour recurrence is now proving to be a problem in some early survivors.

One of the major mechanisms for the efficacy of cytotoxic gene therapy treatments is the so called "bystander effect". This is the phenomenon by which small molecules, such as active drug metabolites, are able to pass between cells so that untransfected cells are subject to the same effects as those expressing the transgene. It has been shown that experimental tumours can be completely eradicated by cytotoxic gene therapy when only a proportion of the cells have taken up and expressed the relevant gene [17, 20-22]. Preclinical studies have shown that the HSVtk: ganciclovir combination can be used to treat peritoneal carcinomatosis derived from colorectal cancer [23]. Affected rats were treated with repeated HSVtk injections followed by ganciclovir treatment. Approximately 40% of animals showed long term disease free survival. Failure to respond or recurrent disease was associated with low expression of the transgene mRNA.

The enzyme: drug pair cytosine deaminase: 5-fluorocytosine (5FC) is being used in a phase I clinical trial for the treatment of metastatic

Table-1

Enzyme	Prodrug	Active Metabolite	Clinical trials
Alkaline phosphatase	Phenolmustard phosphate Doxorubicin phosphate Mitomycin phosphate Etoposide phosphate	Phenolmustard Doxorubicin Mitomycin Etoposide	
Azoreductase	Azobenzene mustards	Phenylenediamine - mustards	
-glucuronidase	Phenolmustard-glucuronide	Phenolmustard	
-lactamase	Vinca-cephalosporin Phenylenediamine-mustard- cephalosporin	4 desacetylvinblastine-3- carboxyhydrazide Phenylenediamine-mustard	
Carboxypeptidase G2	Benzoic acid mustard glutamates	Benzoic acid mustards	
Cytosine deaminase	5-Fluorocytosine	5-Fluorouracil	Breast cancer Colon cancer
DT diaphorase	5-(Aziridine-1-yl)-2,4- dinitrobenzamide(CB 1954)	5-(Aridizin-1-yl)-4- hydroxyl-amino-2- nitrobenzamide	
Plasmin	Peptidyl-p-phenylene-diamine- mustard	Phenylenediamine-mustard	
Thymidine kinase (herpes simplex virus)	Ganciclovir	Ganciclovir triphosphate	Brain tumours Haematological malignancies Head & neck cancer Melanoma Multiple myeloma Ovarian cancer Prostate cancer

colorectal cancer [24]. This trial includes patients with two or more liver metastases (biopsy proven) from colorectal cancer, who are scheduled for laparotomy for either resection or other procedure. Adenovirus vector containing the gene for cytosine deaminase is injected under computed tomography control into one metastasis and the patient subsequently treated with oral 5FC. At laparotomy the treated and control metastases are then removed in order to assess clinical response and look for evidence of viral gene transfer. Results of this clinical study have yet to be published.

An alternative selective mechanism for cytotoxic gene therapy is to use tissue- or tumour-specific promoters so that the transgene is only expressed in tumour cells and not in the

surrounding tissue. For instance, the cERB2 promoter has been used in a clinical trial of cytotoxic gene therapy for metastatic breast cancer [25-27] since cERB2 is overexpressed by the majority of breast tumours. Similarly for colorectal cancer the CEA promoter can be used to improve specificity of gene expression in metastatic lesions within the liver in mice [28]. The CEA promoter is inactive in hepatocytes but active in colon-derived malignant cells. This selectivity can be demonstrated by comparing the effects of two adenovirus constructs carrying the gene for HSVtk with either a CEA promoter or the ubiquitously activated cytomegalovirus (CMV) promoter. The Ad.CEA-tk construct allowed expression of HSVtk within the metastatic tumours while the Ad.CMV-tk construct led to widespread

expression of HSVtk within both the metastases and the surrounding liver. However, the CEA promoter is much less effective than CMV leading to a 30% reduction in HSVtk expression despite increasing the dose of adenovirus. This balance between selectivity and effectiveness epitomises the problems faced by many gene therapy strategies. A similar strategy has been reported using the cytosine deaminase (CD) gene and treatment with 5-fluorocytosine (5FC) for the treatment of mice with colorectal cancer xenografts [29]. When the CD gene was under the control of a CEA promoter the tumour regression and survival rates were improved with a lower incidence of bone marrow suppression compared with those treated with CD under the control of an SV40 promoter.

DRUG RESISTANCE GENE THERAPY

One of the major limitations of current chemotherapy regimes is the bone marrow toxicity associated with these drugs. However it is well recognised that subpopulations of tumour cells are resistant to particular chemotherapeutic agents and continue to grow in a selective manner in the presence of such drugs. These cells contain specific genes which render them resistant to particular compounds. One such is the multiple drug resistance (MDR1) gene which confers resistance to vinca alkaloids (vinblastine, vincristine), anthracyclins (adriamycin, daunorubicin), etoposide and paclitaxel [30, 31]. Strategies to introduce MDR1 into human bone marrow stem cells have therefore been devised and clinical trials have been licensed in advanced breast cancer and haematological malignancies. Bone marrow stem cells are transduced *ex vivo* and reinfused into patients receiving chemotherapy with one or more of the above drugs [32-34]. Difficulties have been encountered in achieving prolonged expression of the MDR1 transgene in the treated patients which has limited the clinical benefit of this elegant approach to cancer gene therapy [34].

CORRECTIVE GENE THERAPY

In simplistic terms, the development of malignancy occurs due to imbalance in the normal cellular complement of proto-oncogenes and tumour suppressor genes. It has long been recognised that tumours are characterised by activation of proto-oncogenes and/or loss or inactivation of tumour suppressor genes. In the

case of colorectal cancer numerous genetic abnormalities have been described and it seems to be the cumulative effect of several changes which allows tumour development rather than an abnormality of one particular gene [35, 36]. Corrective gene therapy aims to reverse some of these genetic abnormalities by either the introduction of tumour suppressor genes or inactivation of proto-oncogenes by antisense technology. Initially this type of approach was dismissed as a non-starter due to perceived difficulties both practically and intellectually. However over the last few years some of these problems have been overcome experimentally and a small number of phase I/II clinical trials of corrective gene therapy have been licensed.

One of the major problems with this type of approach is the choice of gene. Most established tumours exhibit numerous genetic abnormalities and so the concept of correcting only one gene may be flawed. However there is experimental data to show that introduction of normal p53 gene into cancer cell lines which exhibit multiple genetic changes can reverse some of the phenotypic features and growth disturbances in these lines [37]. Similarly the introduction of normal functional retinoblastoma (RB) tumour suppressor gene can restore normal growth in cell lines derived from sarcomas and retinoblastomas [38].

Because of its pivotal role in the control of cell turnover p53 has been the obvious candidate tumour suppressor gene for use in corrective gene therapy strategies. Numerous groups have administered p53 by various vectors into tumour cells in culture and have demonstrated reduced cell proliferation and reduced potential for inducing tumours in animal models [37, 39-41]. Colorectal cancer develops due to the accumulation of a number of genetic changes which appear to occur in a fairly predictable order [35, 36] with loss or inactivation of p53 being one of the commonest early events. Therefore p53 replacement is an attractive strategy for the treatment of colorectal cancer. Animal studies have been performed in order to demonstrate the benefits and safety of such an approach [41-44]. In these studies adenovirus expressing p53 was injected into tumour nodules of metastatic colorectal cancer in mice resulting in inhibition of tumour growth. Similar results have been demonstrated in animal models bearing ovarian [45] or prostatic [46] adenocarcinomas, glioblastoma [47] and squamous carcinomas of the head and neck [48, 49].

Early human trials of p53 gene therapy have been licensed and small numbers of patients recruited. The first major trial of p53 gene therapy was reported in 1996 when nine patients with lung cancer in relapse were treated by Roth and colleagues [50]. Wild-type p53 in a retroviral vector with a β -actin promoter was introduced by direct intra-tumoural injection. Three patients showed clinical tumour regression and in three tumour growth stabilised. Induction of apoptosis was observed in six patients and there was no toxicity and no evidence of viral shedding. A trial of adenovirus-mediated p53 gene therapy for head and neck squamous cell carcinoma has been performed at the MD Anderson Centre [51]. One patient with resectable disease showed a complete response and of the seventeen patients with unresectable disease two showed partial responses, six stable disease and nine progressive disease. No significant toxicity was observed.

Ten patients with metastatic colorectal cancer have been treated with p53 adenovirus via hepatic artery infusion with no severe toxicity and only minor fever and flu-like symptoms [41] during dose escalation studies. Similar p53 gene therapy protocols are being investigated for other tumour types. In one trial fifteen patients with liver tumours (nine hepatocellular carcinoma and six colorectal liver metastases) were treated by direct intra-tumoural injection of p53 and liposomes. Four of the nine hepatocellular carcinomas showed reduction in tumour volume and fall in serum human alpha-fetoprotein measurement but there was no objective response in the group with colorectal liver metastases [52, 53]. The mechanism of action in the tumours which responded to p53 treatment has not been fully elucidated. It has been observed that the introduction of p53 does induce apoptosis but whether this is in individually transfected cells or whether there may be a bystander effect on neighbouring cells has not been determined. Tumour regression may also be due to immunological events if the p53 construct or the products of apoptosis are able to induce an immune response. HCC are known to contain many lymphocytes and in the treated patients in this trial there was evidence of many CD-8 lymphocytes.

Corrective gene therapy may find a role as a potentiator of other treatments rather than as a definitive therapy in its own right. Interesting observations have been made showing the effect of gene therapy on response to existing anticancer treatments. For instance it has been shown that the

introduction of p53 cDNA into lung cancer cells increases their sensitivity to cisplatin [54]. Similarly wild type p53 expression in colorectal cancer cells has been shown to enhance their sensitivity to ionising radiation, and combined p53 and radiation treatment reduced the growth of colorectal cancers in nude mice more than either treatment alone [55, 56]. These experiments indicate some of the ways in which p53 gene therapy may prove useful in combination with other anticancer treatments in the future.

Most of the p53 gene therapy trials to date have used either plasmid DNA or adenovirus to achieve gene transfer. Viral gene transfer is more efficient but there are thought to be risks of immunological reactions to the adenoviral components and the possibility of producing neutralising antibodies so that repeated treatments would be ineffective. However there is experimental data to suggest that repeated p53:adenovirus administration can produce tumour regression in an animal model without causing significant immunological problems [57].

One of the major conceptual problems with corrective gene therapy is the inability of current gene delivery vectors to target all at-risk cells. The theory of "one hit - one kill" has been generally accepted [58] leading to the dismissal of corrective gene therapy as an unrealistic proposition within the scientific community. Moreover it has been assumed that the malignant cells within a treated tumour which remained untransfected would retain a selective growth advantage and therefore outgrow the treated cell population leading to enhanced tumour growth. However *in vitro* gene transfer experiments suggest that far higher proportions of cells can be transfected than was previously thought even with traditionally "inefficient" vectors such as liposomes [59, 60].

It is not clear whether it is necessary to transfect every cell within a tumour in order to obtain a clinical effect. The bystander effect which has been described for cytotoxic gene therapy may be applied to gene therapy by any mechanism since small molecules will pass to neighbouring cells. The clinical responses to VDEPT are far more marked than would have been expected in the absence of a bystander effect. If a similar effect is seen with the products of corrective gene therapy strategies then the limitation of "one hit one kill" will no longer be applied. Furthermore it may not be necessary to target every cell within a tumour in order to produce therapeutic benefit. Current

chemotherapeutic drugs and radiotherapy protocols do not a 100% tumour cell killing but do produce significant clinical responses and improved tumour-free survival.

ANTISENSE

Antisense oligodeoxynucleotides (ODNs) are specific sequences which bind to complementary mRNA and prevent its translation. Therefore antisense ODNs directed against oncogenes are being investigated as potential cancer gene therapy tools. These ODNs prevent translation of genes encoding signal transduction proteins including *k-ras*, *fos* and *c-myc*. Most attention has been devoted to anti-*ras* treatment which has produced a reduction in cell proliferation and tumourigenicity [61-64]. However antisense ODNs exhibit various non-specific effects which are common to all ODNs (reviewed in [65]). Therefore observed changes in cell proliferation may be due to non-specific binding to other proteins [66-68], interference with mechanisms of viral infection [68-70], and direct effects on cell turnover [71, 72].

Advances in understanding the process of apoptosis have allowed various genes to be identified which may be targets for antisense gene therapy in the future. One gene which has been explored in this regard is *Bcl₂*, an anti-apoptotic mediator. Antisense *Bcl₂* has been shown to potentiate apoptosis in lymphoma cell lines [61-64, 73-75] and in SCID mice inoculated with lymphoma cells [76]. A clinical trial of antisense *Bcl₂* in non-Hodgkin lymphoma has been reported [77] - two of the nine treated patients showed tumour regression and in another two there was a reduction in the number of circulating lymphoma cells. In future it seems likely that other genes within the apoptotic pathways such as the *Bax* genes or the caspases will be explored in a similar manner. It may be that achieving a balance between overexpression of pro-apoptotic genes and antisense inhibition of anti-apoptotic genes can be used either to destroy malignant cell populations or to protect bone marrow stem cells in patients undergoing chemotherapy.

PROPHYLACTIC GENE THERAPY

The genetic basis of colorectal carcinogenesis has long been recognised but over the last 12 years specific genes have been identified [35] leading to Fearon and Vogelstein's seminal description of the

genetic changes occurring during the adenoma-carcinoma sequence [36]. It may therefore be possible to target gene therapy at an earlier stage either to prevent the development of adenomata or to prevent adenoma to carcinoma progression.

The hereditary disorder familial adenomatous polyposis (FAP) exemplifies the adenoma carcinoma sequence *par excellence*. Due to a germline mutation in the adenomatous polyposis coli (APC) gene patients develop multiple intestinal polyps one or more of which invariably becomes malignant unless prophylactic surgery is undertaken. The APC gene is a tumour suppressor gene which was identified [78] and sequenced [79] and consists of approximately 8.5kb of cDNA. It has been proposed that replacement of wild type APC at high levels at an early stage may prevent or delay the onset of malignancy [60]. It is debatable whether such an intervention should be aimed at primary prevention of adenoma formation or at prevention of adenoma to carcinoma progression. In vitro transfection of the APC gene into colonic epithelial cells has been shown to be efficient [60] and associated with suppression of expression of the endogenous mutated APC sequence [80]. It has also been shown that human APC can be expressed in rats [60] and mice [81] after delivery of a "gene: liposome enema". Despite the use of liposomes as the delivery vector expression of the transgene could be detected up to three weeks after treatment in the latter study. This suggests that a dosing schedule could be devised which is not too onerous for patients who need repeated treatments. Studies are currently in place to assess whether this corrective strategy has any therapeutic benefit in animal models of FAP [82]. Although prophylactic surgery remains the mainstay of management for individuals with FAP there are three clinical situations where a novel adjuvant treatment is needed. Patients who have undergone colectomy are at risk of developing rectal cancer but some may be unsuitable for surgery to remove the rectum. These patients are currently kept under surveillance and may receive treatment with non steroidal agents. It may prove possible to add topical gene therapy to current treatment in order to delay or prevent the development of rectal cancer. Duodenal cancer and desmoid disease are two of the major causes of death in FAP patients after they have undergone colectomy. None of the currently available treatments are very effective in these situations and therefore if prophylactic gene therapy could be shown to be safe it may prove useful as a preventative treatment in these areas.

COMBINATION STRATEGIES

Most of the Phase I cancer gene therapy trials have shown that interventional genetics is safe but none of these approaches has been very effective in terms of clinical benefit or prolonged life expectancy [83]. Therefore combinations of different modalities of treatment are being investigated in order to determine whether enhancement of anti-tumour effects can be achieved.

Protocols which combine cytotoxic and immunological gene therapy are being explored in an attempt to improve tumour cell killing. One small study of rats with disseminated intraperitoneal colon carcinoma has shown improved survival in the group treated with a combination of liposomes and two plasmids encoding HSVtk and IL-2 followed by ganciclovir, compared to groups receiving either HSVtk:ganciclovir or IL-2 only [84]. A similar combination of HSVtk and IL-2 in adenoviral vectors has been used in the treatment of mice with hepatic metastases from colon carcinoma [85]. The groups treated with Ad.HSVtk:ganciclovir alone and Ad.IL-2 and Ad.HSVtk:ganciclovir in combination both showed necrosis and regression of the metastases. However it was only the latter group which developed systemic anti-tumour immunity against re-challenge with inoculations of further tumour cells.

An alternative combination of suicide and cytokine genes, HSVtk and GM-CSF, administered via adenovirus has been proved to increase survival in mice with colorectal liver metastases [86]. In this study mice with metastatic colon carcinoma were treated with HSVtk gene therapy alone, or with IL-2 immunotherapy, or with IL-2 and GM-CSF in combination. Adenoviral vectors were used throughout. Treatment with HSVtk alone caused tumour regression which was prolonged by the addition of IL-2. However all animals developed recurrent liver metastases within a few months. In the group treated with HSVtk, IL-2 and GM-CSF survival was prolonged with several animals tumour free at 4 months.

Various other combinations have been explored for the treatment of tumours in animal models. For example reinjection with tumour cells co-expressing IFN- and HSVtk followed by ganciclovir caused regression of both the treated tumour and tumours at distant sites. HSVtk:ganciclovir alone failed to produce distant

responses [87]. This study adds to the mounting evidence that an immunological approach is necessary in order to produce a systemic anti-tumour response.

It has become apparent that gene therapy provides only a marginal improvement in tumour inhibition when used alone and therefore it is essential to investigate its use in conjunction with other modalities of treatment. In the case of colorectal cancer this usually involves gene therapy strategies with chemotherapy particularly 5-fluorouracil. Mice bearing tumour nodules of colorectal cancer were treated with intra-peritoneal 5-fluorouracil and intra-tumoural p53-adenovirus while control animals received either of the treatments alone [44]. At doses of 5FU and adenovirus which were subtherapeutic when used alone, the combination produced significant tumour reduction due to an apparently enhanced anti-tumour effect.

NEW APPROACHES

None of the current gene transfer vectors is ideal for therapeutic use - in general viral vectors are beset by safety considerations while non-viral vectors are less efficient in cell targeting and gene delivery. Therefore, the development of new gene delivery vectors is an exciting area of research. Various viruses such as herpes simplex virus (HSV), lentiviruses and haemagglutinin virus of Japan (HVJ) have been studied in both *in vitro* and *in vivo* gene transfer experiments. Each has individual properties which can be harnessed for particular therapeutic use but all have potential safety problems. Therefore, conjugate vectors which combine the safety features of non-viral vectors with the efficiency of viruses are being developed. For example adenovirus complexed with polylysine and transferrin can be used to deliver DNA. The transferrin binds to specific transferrin receptors, the complex is internalised by receptor mediated endocytosis and the adenovirus enhances endosomal lysis to release the DNA into the cytoplasm and thus escape lysosomal enzyme digestion.

HERPES SIMPLEX VIRUS

Herpes simplex virus (HSV) has been investigated as a potential gene transfer vector since it can infect a wide range of dividing cells. Wild-type HSV infection leads to a cascade of viral gene expression resulting in production of

multiple virions and cell death, and HSV vectors can cause cell death [88]. These features have been utilised in an attempt to target metastatic colorectal cancer cells within the liver in an animal model [89]. The HSV vector used in this study, hrR3, is defective in the gene for ribonucleotide reductase and therefore can only replicate in cells which contain this enzyme ie. actively dividing cells. Therefore hrR3 replicates in cells which are actively dividing such as metastatic carcinoma cells but not in the relatively quiescent hepatocytes. In this study the reporter gene lacZ was contained in the hrR3 and administered to mice with metastatic colorectal cancer. LacZ expression was demonstrated in 101 of 105 liver metastases with minimal expression in surrounding liver tissue. Tumour cell killing was also demonstrated *in vitro*. This combination of gene transfer properties and oncolytic activity makes HSV a useful candidate as a vector for cancer gene therapy strategies.

VP22

The VP22 protein is part of the tegument of type 1 herpes simplex virus (HSV1) and has been shown to have intercellular trafficking properties [90]. In both infected and transfected cells VP22 trafficks from the cytoplasm to neighbouring cells by a process which requires the presence of actin in the cytoskeleton. VP22 then accumulates in the nucleus of these cells. The process allows great expansion of infectivity since microinjection of one cell results in VP22 trafficking to up to 200 neighbouring cells. Since this phenomenon was first described efforts have been made to harness VP22 as a potential gene delivery vector. It has been used to deliver the reporter gene green fluorescent protein (GFP) and the "suicide" gene HSVtk *in vitro* [91, 92]. VP22 potentially appears to be a useful gene delivery vector since the VP22 fusion complexes do not seem to be size restricted, multiple cells are infected following transfection of a single cell and can be used to treat cells which are resistant to viral transduction.

HUMAN ARTIFICIAL CHROMOSOME

The ultimate gene delivery vector is likely to be a human artificial chromosome (HAC) which would allow the transfer of large quantities of DNA, possibly multiple genes in their genomic forms with controlling sequences. Such an element would replicate independently at cell division and therefore once-only treatment would

be possible. Yeast artificial chromosomes (YACs) are well established and it thought that a HAC is likely to require the same essential DNA constituents as a YAC ie. telomeres, a centromere and origins of replication (reviewed in [93, 94]). Much work has been done to try to characterise these elements in human chromosomes but only the telomeres are well understood. Telomeres consist of DNA and protein and act to protect the end of chromosomes during cell division. Telomeric DNA consists of approximately 5-20kb of TTAGGG repeats and the length of the telomeres is controlled by the enzyme telomerase and by the telomere repeat binding protein TRF1. The best characterised constituent of centromeres is alpha satellite DNA, a 171bp repeat which is organised into tandem arrays. Origins of replication have not been well characterised in human chromosomes. The first HAC was described in 1997 when a first generation human mini-chromosome was constructed by the combination of PCR-generated telomeric DNA with arrays of alpha satellite DNA, a marker gene and human DNA sequences [95]. These mini HACs were stably maintained in culture for up to six months and marked an exciting development in interventional genetics.

GENE REPAIR

Since the lack of a perfect gene delivery vector is one of the major problems of current gene therapy strategies, a novel approach aimed at gene repair rather than replacement has been considered. RNA enzymes (ribozymes) can recognise RNA transcripts and produce specific cleavage and splicing [96]. This technique has been used shorten the trinucleotide repeat expansion of myotonic dystrophy [97] and to convert sickle -globin mRNA to -globin transcripts [98]. The efficiency of such conversions is currently approximately 5-10% but for diseases in which severity is quantitatively related to the lack of a particular protein then even this low level of correction may be beneficial.

Over the last five years understanding of the mismatch repair (MMR) genes has improved and the enzymes involved in these pathways have been utilized in attempts at therapeutic gene repair (reviewed in [99]). This system is aimed at correcting point mutations or other short "mistakes" in the DNA sequence by means of an RNA:DNA chimera. The chimera is constructed by placing a DNA sequence containing the "correct" base sequence between two RNA

sequences. This chimera, therefore, produces a mismatch with the native strand because the DNA is read as an "incorrect" sequence. This allows excision and repair using the MMR pathways. This chimera-directed targeted gene repair produces very variable results with a correction rate of approximately 2%. Although this type of corrective genetic intervention is very experimental at present, it does hold the potential to have clinical application in diseases where specific causative mutations have been identified.

CONCLUSIONS

Any review of gene therapy for colorectal cancer is out of date even before publication but I have attempted to describe some of current approaches to this type of treatment. The case for the development of novel adjuvant treatments is well established because of the poor five year survival rates of patients presenting with colorectal cancer. Most of the currently licensed trials involve the use of interventional genetics to enhance the host's immune response to a tumour or to deliver cytotoxic drugs to tumour cells. Different vectors are being investigated in an attempt to improve gene delivery and ensure safety. Viral based vectors are able to target and enter host cells very efficiently and, therefore, the established vectors are mainly derived from adenovirus or retrovirus. One of the limitations of adenoviral vectors has been their inability to replicate after transfection but recent work has led to the development of replication-competent adenoviruses. Replication holds the potential for recombination with wild-type adenoviruses and therefore raises safety considerations. Other viruses such as HSV or lentiviruses which have their own particular tropisms will allow vectors to be tailored to specific target tissues. Ultimately the best gene delivery vector is likely to take the form of a HAC which would allow the stable transfer of multiple genes in their genomic form.

Corrective gene therapy in the form of replacement of tumour suppressor genes or inactivation of proto-oncogenes with antisense is likely to be superseded by gene correction strategies. As our understanding of the MMR pathways improves it is likely that targeted gene repair will become technically easier and more efficient. Gene correction may dispense with the need for gene delivery vectors and therefore remove many of the potential safety considerations which restrict interventional genetics at present.

At present gene therapy is limited to clinical trials in small numbers of patients with advanced disease or who have relapsed despite conventional treatments. The tumour load in these patients is likely to be extremely large and therefore current protocols are unlikely to show an overall survival advantage but the best that can be expected is a degree of tumour regression or improvement in surrogate end points such as markers of cell turnover. Although individual clinical responses have been reported in several trials, gene therapy has yet to make a major impact on the treatment of colorectal cancer. Providing that safer and more efficient gene delivery systems can be developed, gene therapy can then be extended to patients as part of their initial treatment or even as a prophylactic measure. The future of gene therapy for colorectal cancer presents exciting scientific challenges and will raise important ethical questions as more complex interventions become possible.

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