Principles and Applications of Gene Therapy in Colon Cancer

Rajaraman Durai, Shi Yu Yang, Alexander M Seifalian, Marc C Winslet

1) Academic Division of Surgical and Interventional Sciences, University College London 2) Royal Free Hampstead NHS Trust Hospital, London, United Kingdom.

Abstract

Colorectal cancer is one of the leading causes of mortality and morbidity. Mutations and aberration of some of the genes may lead to colon cancer. With advancing knowledge, more and more defective genes can be identified. Theoretically, correction of these defective genes, selective overexpression of certain genes may lead to not only prevention of cancer development but also regression of existing cancer. Carcinogenesis is a multistep process and more than one gene may be altered. At present, the success of gene therapy as a stand alone therapy is limited by poor expression and long-term non-expression of transferred genes, immunological effect on the viral vectors, viraemia, leukaemia and occasional deaths. Further experiments and trials are underway to modify the vectors and genes to make sure gene therapy is safe, effective and long-lasting before it can be used in day-to-day practice.

Keywords


Introduction

Colon cancer is the second commonest cancer in the European Union [1]. At least 50% of patients with colorectal cancer develop recurrences or metastases during their illness [2]. The cancer causes significant morbidity and mortality. There are approximately 50,000 to 100,000 genes in the human body. Different genes are active in different phases of the cell cycle. Defective genes may be associated with at least 30% of colon cancers. Some defective genes have been identified as responsible for familial colon cancers. Gene therapy involves introduction of genetic materials, which are nucleic acids, into the cells. The genetic material may be deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) which may help to replace or correct the malfunction due to defective genes. Gene therapy can be also performed to trigger an immune response or to produce a therapeutic substance. The main advantage of gene therapy is transfer of a particular gene to a specific group of mammalian or tumour cells [3] so that the desired effect will be localised and normal cells are spared. Cancer formation is often a multistep process and there may be point mutation, de-regulation or deletion of proto-oncogenes and anti-oncogenes which may be responsible for the development of cancer [4].

In 1990, Dr. W French Anderson performed for the first USA government-approved human gene transfer [5, 6] for a 4-year old girl with severe combined immunodeficiency syndrome. Although the patient was not cured, her condition improved and she acquired resistance to frequent colds. It stimulated interest in the scientific community about gene therapies. In the last 17 years, hundreds of genes have been transferred [7]. Although 1309 gene therapy trials have been conducted worldwide so far, only 45 gene therapies attained phase III level. Among the 1309 gene therapies, only 27.3% were performed in Europe. Currently, 11 gene therapies are undergoing trial for colon cancer in the UK. The aim of this review is to introduce the principles and applications of gene therapy in colon cancer to gastrointestinal trainees and practising physicians for them to understand.

Methods

Studies, case reports and review articles which were published between years 1958-2007 were identified by PubMed search using the keywords ‘gene therapy’, ‘colon cancer’ and ‘gene transfer’. Other resources include textbooks.

Methods of transferring genes into the mammalian cell

In general there are two ways of transferring genes.
Replication-deficient recombinant adenoviral vectors are predominantly used for colon cancer gene therapy, because they can be produced at a high titre and they readily infect a number of different cell types [14]. They also have a tropism for the liver and can infect both dividing and quiescent cells [15]. Therefore, these vectors are also useful for gene therapy involving liver metastases. Other viruses

Herpes simplex virus, which causes cold sores, can infect a wide range of dividing cells [16]. It has an advantage over other viruses. Whenever a patient shows an unwanted side effect from herpes simplex infection, it can be treated with antiviral agents such as acyclovir. Other viruses which may be useful as a vector include vaccinia virus, lenti virus and haemagglutinin virus of Japan.

Non-viral vectors

There are at least five non-viral methods of introducing DNA into the mammalian cell. They are calcium phosphate transfection [17], DEAE-dextran transfection, electroporation [18], liposome mediated transfection [19] and plasmid mediated gene transfer [20]. The first two procedures produce a chemical environment in which DNA attaches to the cell surface. The DNA is then endocytosed by unknown pathways.

Electroporation uses an electric field to open up pores in the cell. The DNA presumably diffuses into the cell through the pores. So this technique can be used in any cell type. Electrogene therapy is an in vivo application of electroporation where naked plasmid DNA is injected and electric pulses are delivered directly to the tissue [18]. However, the efficiency of this method in vivo is still lower than virally mediated gene transfer. Electrically-assisted gene transfer is advantageous in clinical situations because of lack of immunogenicity, easiness to prepare large quantities of plasmid DNA, reproducibility and the availability of electro-pulsators for human use [18].

In liposome-mediated transfection, liposomes containing cationic and neutral lipids mediate the transfection of DNA. The advantages of this method include: a) is easy to prepare; (b) ability to inject large lipid:DNA complexes;
Gene therapy in colon cancer

and (c) low immunogenic response [19]. The mechanism of gene transfer by this method is poorly understood.

Plasmids can also be used for transferring DNAs. They are extra chromosomal DNA molecules which are self replicating. Escherichia coli (E.Coli) carry plasmids which offer resistance against antibiotics, heavy metals and obscure bacteriophages. Replication of these plasmids may or may not require plasmid-coded proteins and may or may not be synchronised with the cell cycle. Some of these plasmids can be freely transferred from one bacterium to another. Artificial plasmids have been constructed in laboratories since 1970, with fragments of DNA and naturally occurring plasmids. All these plasmids have three common features. They all have a replicator, a selectable marker and a cloning site. A replicator is a stretch of DNA that contains the site at which DNA replication begins. A selectable marker is an ab gene encoding resistance to some antibiotics. The cloning site is a restriction endonuclease cleavage site into which foreign DNA can be introduced without interfering with the plasmids' ability to replicate. Uptake of plasmid DNA can be enhanced by using a hand-held Swiss jet injector, which uses pressurized air to force small volumes (3-10μl) of naked DNA into targeted tissues [21]. The process by which plasmids are introduced into E.coli is known as transformation. Transformation is a very important tool in recombinant DNA technology. The addition of new genes to a recipient cell introduces a heritable modification in the recipient cell’s phenotype. Plasmids targeting liver can be injected into veins [22].

Genetic changes and colon cancer carcinogenesis

Numerous mutagenic events can occur throughout the colon cancer development including loss of heterozygosity in tumour suppressor genes such as APC, MCC, DCC, p53 and K-ras [23]. Colon cancer tumorigenesis is a step-wise process (Fig. 2) [24] in which mutations accumulate over time and oncogenes are activated while tumour suppressor genes are deactivated. Colon cancer mechanisms are discussed as common and alternate pathways.

Common pathway: Tumour suppressor genes make proteins that suppress tumour formation by limiting the cell growth. Mutations involving the tumour suppressor genes could result in a loss of their ability to restrict tumour growth [25,26]. Vogelstein et al (Fig 1) found that mutations involving a tumour suppressing gene called the adenomatous polyposis coli (APC) gene resulted in colon cancer [27]. Normally, APC binds to b-catenin and phosphorylates it. But when mutations occur, b-catenin interacts with transcription factors instead of stimulating growth. At present the transgene expression after gene therapy is short lived, therefore its role is limited in familial adenomatous polyposis where the colon cancer risk continues for life. P53 is another tumour suppressor gene which blocks the cell cycle and stimulates apoptosis. It is altered in more than 80% of colorectal cancers.

Alternate pathway: Hereditary non polyposis coli (HNPPC), sometimes called Lynch syndrome, accounts for approximately 5-10% of all colorectal cancer cases. Several genes have been identified that are linked to HNPPC. Mutations in the MLH1, MSH2, and MSH6 genes are the most frequent cause of HNPPC [28,29]. Although multiple genes have been linked to HNPPC, most families with HNPPC have only one mutated gene. Genetic testing is available for the MLH1, MSH2, and MSH6 genes. The HNPPC genes are part of a group of genes called mismatch repair genes. They make proteins which repair DNA mistakes that occur when cells divide. If one of these genes has a mutation, then DNA mistakes cannot be repaired, leading to damaged DNA and an increased risk of cancer. The risk of colorectal cancer in families with HNPPC is 70-90%, which is several times the risk in the general population. Women with HNPPC also have an increased risk of cancers of the uterus, ovaries, stomach, small intestine, kidney and breast [30].

MYH associated polyposis: This is a recently identified inheritable bowel cancer in which a person needs two faulty copies of the MYH gene to be at an increased risk of cancer.

Fig.2 Vogelgram showing molecular mechanisms involved in the pathogenesis of colorectal cancer. Small adenoma increase in size under the influence of growth controlling pathways. Large adenomas become cancerous under the influence of various genes and growth factors including IGF.
Gene therapy approaches

The gene therapy approaches currently being employed can be classified into five major categories [14, 31, 32]. They are: (1) Enzyme/prodrug systems (suicide gene therapy) [33, 34]; (2) Gene correction (tumour suppressor gene replacement therapy and oncogene inactivation) [35]; (3) Immune-gene therapy; (4) Drug resistance gene therapy and (5) Miscellaneous. Table 1 provides a current summary of colorectal cancer gene therapy trials in the UK.

Basis of gene therapy and its application in colorectal cancer

Virus-directed enzyme-prodrug therapy (VDEPT): VDEPT is the transfer of genes encoding bacterial or viral or fungal enzymes into tumour cells which can convert inactive prodrug into short lived toxic metabolites, limiting the toxic effects to the tumour cells (Table 2) [34, 36]. This form of therapy is also known as suicide gene therapy. Examples include: (1) Thymidine kinase and ganciclovir [37]; (2) Cytosine deaminase and 5-fluorocytosine [38, 39]; (3) Thymidine phosphorylase and 5 Fluoro uracil (5 FU) [40]; and (4) Nitroreductase and the prodrug CB1954 [41]. One of the major advantages of this form of therapy is the so called ‘bystander effect’ [42]. This is a phenomenon by which small molecules, such as an active drug metabolite, are able to pass between cells via gap junctions so that untransfected cells are also affected. Thymidine kinase of herpes simplex can phosphorylate ganciclovir, which can inhibit DNA polymerase leading to cell death. An animal experiment on colorectal cancer demonstrated profound bystander effects when the combination of thymidine kinase/ganciclovir was used [37]. Surprisingly, when herpes simplex virus thymidine kinase gene therapy was used alone without ganciclovir, it increased tumour growth and Cox-2 expression [43]. In an in vivo experiment, when monocyte chemoattractant protein gene therapy was used along with thymidine kinase/ganciclovir, it augmented the antitumor effects [44].

There is a new technique called double enzyme/prodrug therapy. Genes representing two enzymes such as CD and uracil phosphoribosyl transferase can be used along with 5-FC. This form of suicide gene therapy works better than single enzyme CD/5-FC model [45]. There is an interesting novel technique where cytokines can be combined with enzyme/prodrug therapy. When granulocyte macrophage colony stimulating factor gene transfer was used along with thymidine kinase/ganciclovir system, it resulted in complete inhibition of liver metastases [46]. Intraperitoneal route can be a route of administration for certain form of gene therapies. Yeast CD gene when administered intraperitoneally was found to increase the enzymatic expression in liver metastases [47]. Yeast CD transgene expression is superior to bacterial enzyme. Gene correction: Cancer may result when there is an imbalance between proto-oncogenes and tumour suppressor genes. Gene corrective therapy is aimed at reversing some of the genetic abnormalities by either introducing a tumour suppressor gene or inactivating proto-oncogene by an anti-sense method [48]. Tumour suppressor gene TP53 is the

Table 1. Currently experimented gene therapy trials in the UK*

<table>
<thead>
<tr>
<th>Phase</th>
<th>Gene used</th>
<th>Vector used</th>
<th>Methodology applied</th>
</tr>
</thead>
<tbody>
<tr>
<td>I(Pilot)</td>
<td>CEA</td>
<td>Vaccinia</td>
<td>Post vaccination CEA peptide challenge in combination with 5-fluorouracil and folinic acid</td>
</tr>
<tr>
<td>I</td>
<td>Oncofetal Antigen</td>
<td>Vaccinia</td>
<td>Evaluation of the safety, bio distribution and efficacy of trovax in patients with metastatic</td>
</tr>
<tr>
<td>II</td>
<td>Oncofetal Antigen</td>
<td>Pox</td>
<td>Trovax in colorectal cancer patients undergoing surgery for resectable liver metastases</td>
</tr>
<tr>
<td>I</td>
<td>Granulocyte macrophage colony stimulating factor</td>
<td>Herpes simplex</td>
<td>Gene therapy for bowel cancer that has spread to the skin</td>
</tr>
<tr>
<td>I</td>
<td>Oncofetal Antigen</td>
<td>Vaccinia</td>
<td>TroVax in conjunction with chemotherapy in patients with metastatic colorectal cancer</td>
</tr>
<tr>
<td>I/II</td>
<td>CEA</td>
<td>Naked plasmid DNA</td>
<td>DNA vaccination with a CEA/pDOM fusion gene in patients with carcinoma expressing CEA</td>
</tr>
<tr>
<td>II</td>
<td>Oncofetal Antigen</td>
<td>Vaccinia</td>
<td>Immunologically evaluating ST4-MVA (TroVax) in patients undergoing surgical resection of colorectal liver metastases</td>
</tr>
<tr>
<td>II</td>
<td>Carcinoembryonic Antigen /MUC-1</td>
<td>Vaccinia &amp; Pox</td>
<td>Randomised trial assessing Anti-CEA, Anti-MUC-1 Vaccination ± Chemotherapy ± GM-CSF after Surgery in Stage II colorectal cancer</td>
</tr>
<tr>
<td>I</td>
<td>Oncofetal Antigen</td>
<td>Vaccinia</td>
<td>Safety and Immunology evaluation of TroVax produced by the Baxter synthetic route in patients with stage IV colorectal carcinoma</td>
</tr>
<tr>
<td>I</td>
<td>CEA &amp; CD3</td>
<td>Retrovirus</td>
<td>Transfer of autologous tumour antigen-specific T cells with pre-conditioning chemotherapy and intravenous IL2 in patients with advanced CEA positive tumours</td>
</tr>
<tr>
<td>I</td>
<td>IL-2</td>
<td>T cells</td>
<td>Transfer of autologous tumour antigen-specific T cells with pre-conditioning chemotherapy and intravenous IL2 in patients with advanced CEA positive tumours</td>
</tr>
</tbody>
</table>

*Courtesy: (http://www.wiley.co.uk/genmed/clinical)
Carcinoembryonic antigen (CEA) is often expressed by colorectal cancers. Immunizations with dendritic cells (DC) transfected with RNA encoding tumour antigens, can stimulate tumour antigen-specific immune responses in vitro and in animal models. In a Phase II study [60] on patients with resected hepatic metastases of colon cancer, the safety and feasibility of administering DC transfected with CEA mRNA was assessed. There were no major side effects. Nine out of 13 patients showed relapse at a median of 122 days. Immunologic response was found in biopsies of DC injection sites and peripheral blood samples.

Activation of cytotoxic lymphocytes and natural killer cells, using cytokines such as IL-2 and IL-12 which can be transferred directly into tumour cells, could result in an anti-tumour effect [61]. Systemic administration of interleukins is limited by their side effects. In vivo experiments using intratumoral IL-2 gene therapy showed regression of tumours in 76% of mice [62]. In a Phase I/II clinical trial on patients with unresectable colon cancers who were treated with intra-tumoral injection of an adenovirus-IL-2 at the time of surgery, showed that one patient’s tumour expressed increased numbers of membrane bound IL-2 receptors. Another patient showed necrosis of the tumour mass. In a study on mice with experimentally induced colorectal tumours, adenovirus containing mouse IL-12 gene was injected into the tumour and it showed tumour regression and prolonged survival [63].

**Table II. Enzyme-Prodrug gene therapy.**

<table>
<thead>
<tr>
<th>Prodrug</th>
<th>Enzyme(gene)&amp; metabolite</th>
<th>Mechanism of action</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ganciclovir</td>
<td>Thymidine kinase, Ganciclovir triphosphate</td>
<td>Inhibition of DNA polymerase</td>
<td>Works better when 5FU is added</td>
<td>[71]</td>
</tr>
<tr>
<td>5 Fluorocytosine</td>
<td>Cytosine deaminase, 5 Fluorouracil</td>
<td>Inhibition of thymidylate synthase</td>
<td>Yeast CD gene causes increased expression than bacterial CD gene</td>
<td>[38,39]</td>
</tr>
<tr>
<td>5'-deoxy-5-fluorouridine</td>
<td>Thymidine phosphorylase, 5-Fluorouracil</td>
<td>Inhibition of thymidylate synthase</td>
<td>In vivo experiment showed tumour suppression of subcutaneous cancers</td>
<td>[40]</td>
</tr>
<tr>
<td>CB1954</td>
<td>Nitro-reductase, bifunctional alkylation agent</td>
<td>Formation of DNA cross links</td>
<td>Effective even in non-proliferating cancer cells. Use of oncolytic agent has been shown to be effective in therapy.</td>
<td>[104]</td>
</tr>
</tbody>
</table>

Most commonly mutated gene in human cancer [35, 49] which is present in 20-69% of colorectal cancers. Its product, known as ‘p53’, normally regulates the cell cycle and repairs abnormal DNAs. If the DNA cannot be repaired by p53, it causes growth arrest or apoptosis [50]. Loss of p53 leads to uncontrolled and aggressive cellular growth. In a phase I controlled trial, an adenovirus encoding wild type TP53 gene was delivered by hepatic artery infusion to 16 patients with TP53 mutated colorectal liver metastases [51]. The side effects were fever and transient derangement in liver function. Although the gene was expressed in subsequently resected tumours, there was no significant change in radiological appearance of the tumours. It supports the fact that lower levels of p53 result in cell cycle arrest whereas higher levels cause apoptosis [52]. Another gene known as K-ras is also often mutated in colorectal cancer [53, 54]. It encodes for a protein called p21 which is involved in cell signal induction and the control of cell proliferation. P53 gene up-regulates p21 and it results cellular arrest but when P53 is incompetent of inducing p21 then it results in apoptosis [52, 55]. Reduced p21 has been correlated with metastatic colon cancer. The K-ras mutations can be detectable in DNA purified from the stool [56]. In vitro experiments showed increased apoptosis when K-ras overexpressing colon cancer cells were transfected with apoptotic genes (bax, caspase-8 and PKG) [53]. The function of mutated genes can be inhibited by the use of anti-sense oligonucleotides with specific sequences that bind to complementary mRNAs and prevent their translocation. Anti-sense Bcl-2 has been shown to potentiate apoptosis in lymphoma [57] and anti-sense K-ras has been shown to suppress growth of colon cancer cells [58].

**Immune-gene therapy:** Immunological mechanisms are important for the elimination of cancer by the human body. Individuals with immune deficiencies, such as HIV infection, are at a high risk of developing cancers. Cancer cells are recognized and destroyed by CD8+ cytotoxic T cells and natural killer cells. But many cancer cells escape this immune mediated destruction by exhibiting loss of HLA class I antigens. A phase I trial of immunotherapy for colorectal metastases using intra-lesional injection of HLA-B7 cDNA with liposome on 15 patients who were HLA-B7 negative, did not show any therapeutic benefits [59]. This HLA-B7 gene transfer resulted in production of HLA-B7 protein in at least 50% of patients, however it did not show therapeutic benefits. It means the cancer molecular mechanisms are much more complex. Carcinoembryonic antigen (CEA) is often expressed by colorectal cancers. Immunizations with dendritic cells (DC) transfected with RNA encoding tumour antigens, can stimulate tumour antigen-specific immune responses in vitro and in animal models. In a Phase II study [60] on patients with resected hepatic metastases of colon cancer, the safety and feasibility of administering DC transfected with CEA mRNA was assessed. There were no major side effects. Nine out of 13 patients showed relapse at a median of 122 days. Immunologic response was found in biopsies of DC injection sites and peripheral blood samples.

**Drug resistance gene therapy:** The main limiting factor for patients undergoing chemotherapy is bone marrow toxicity. There is a gene called multiple drug resistance gene (MDR1) which may confer the bone marrow resistance to vincristine, anthracyclins and paclitaxel [66]. This gene therapy is still in an experimental stage.

**Chemo-gene therapy:** The susceptibility of cancer cells to chemotherapy is enhanced by combining them with gene therapies. This is a new development in gene therapy which is known as chemo-gene therapy. It means administering a chemotherapeutic agent such as 5 FU in conjunction with a gene such as Flt3L to have a synergistic effect on cancer [67]. Flt3L is a haematopoietic factor which stimulates immune cells which may kill cancer cells. Another
combination of interest is wild type p53 and 5FU/Cisplatin [68, 69]. The synergism which results from the combined gene therapy and chemotherapy is much more efficient and may eradicate the tumour. Selective overexpression of alpha-interferon in colon cancer cells greatly enhances their susceptibility to 5 FU [3, 70]. Some cancer cells may be resistant to chemotherapeutic agents. This can be overcome by combining gene therapy and chemotherapy. Recent in vivo experiments show that suicide gene therapy using thymidine kinase/ganciclovir system when used with thymidylate synthase inhibitors increases the survival of mice with colon cancer xenografts [71]. When TRAIL gene and actinomycin D are administered together, they suppress the metastatic liver tumours [72]. Inhibition of IGF receptor greatly enhances the apoptotic response of cancer cells when exposed to chemotherapy [73]. Adenovirus-mediated transfer of caspase-8, an enzyme involved in apoptosis, has been shown to increase the sensitivity of colon cancer cells to 5FU [74]. Bax protein overexpression has also been shown to potentiate the effect of chemotherapeutic agents [75].

**Obstacles in gene therapy**

There are several hurdles in gene therapy. Cancer cells can develop further mutations when we try to correct the existing genetic defect [76]. Viral uptake by tumour cells may not be sufficient enough to cause the desired effect. Prior radiotherapy has been shown to increase the viral uptake by various cancer cells [77]. Replication competent virus has been shown to improve gene transfer. Viral systemic toxicity and the short lived nature of gene therapy can be a problem [78]. Systemic dissemination of virus during intratumoral administration may be reduced by mixing them with alginate [9]. Alginate can also be used for prolonging the effect of gene therapy. When fibroblasts transfected with IL-12 gene were enclosed in alginate microspheres, they showed sustained release of IL-12 which resulted in the reduction of colon cancer which were adjacent to them [79]. Occasional death has been reported in intra-arterial viral vector infusion [80]. Hepatic artery injection of viral vector can cause fever, rigor and fatigue. The effects of viral dissemination may be solved by selecting vectors such as herpes simplex which may respond to antiviral therapy. Direct injection of virally mediated gene therapy may result in a poor distribution of a gene throughout the tumour. This can be overcome by the use of the oncolytic virus in addition to standard gene therapy [81]. When bacterial enzyme CD was used for suicide gene therapy, it was not efficient in the conversion of 5-FC into 5-FU. Use of yeast CD has been found to be superior [82]. Leukaemia and death from viral dissemination are serious problems which have to be looked into before practising gene therapy on a routine basis.

**New molecular targets for gene therapy**

Genes that are involved in apoptosis are important areas for gene therapy. Bcl-XL, a protein involved in apoptosis, is overexpressed by colon cancer cells. In vivo experiments show promising results for using adenovirus-mediated RNA interference therapy targeting Bcl-XL on colon cancer [83]. BiK protein belongs to the proapoptotic Bcl-2 family. Mutant BiK gene has been shown to prolong the survival of mice with carcinomatosis peritoneii [84]. One of the enzymes regulating apoptosis known as signal-regulating kinase-1, when inhibited, has been shown to cause tumour regression [85]. Similarly, Bax gene therapy has been shown to induce an intense apoptosis [86].

Growth factors are important targets for gene therapy. NK4 is an antagonist of the hepatocyte growth factor which may be useful in disseminated peritoneal cancer [87]. The epidermal growth factor receptor (EGFR) suppression can cause apoptosis. An in vitro study using LoVo cells showed that suppression of EGFR can be a potential target for gene therapy [88]. Overexpression of inhibitory binding protein such as insulin-like growth factor binding protein-4 can increase apoptosis of colon cancers [89, 90]. Cellular adhesion molecules are another target for gene therapy. An adhesion molecule called CD-44 when interfered, results in regression of cancer [91].

Heat shock proteins are potential targets. One such protein called Hsp90beta is phosphorylated when exposed to a chemotherapeutic agent 5FU. Its use needs further evaluation [92]. Tissue plasminogen activator (tPA) has been shown to influence liver metastases. Gene therapy utilising tPA improved the survival of mice with liver metastases [93]. Chemokines can suppress the growth of cancers. A chemokine called fractalkine has been shown to reduce the growth of cancer cells [94]. Mutations of gap junction connexin 43 gene is frequently found in colon cancer which needs exploring [95]. Cyclin dependent kinase 4 (CD K4) is a protein which is involved in regulation of cell cycle. Antisense inhibition of CDK4 in cell culture caused increased apoptosis and a decrease in cellular proliferation [96].

TNF-related apoptosis-inducing ligand (TRIAL) is a new member of the tumour necrosis factor super family. It induces apoptosis of tumour cells, but not normal cells [97]. It has been shown to increase apoptosis of colon cancer cells [98]. Inhibition of phosphoinositide 3-kinase (PI3K/Akt) by RNA interference sensitizes resistant colon cancer cells to TRAIL-induced cell death through the induction of TRAIL receptors [99]. Cancer cells may acquire resistance to TRAIL gene therapy [100].

Short interfering RNA (siRNA) targeting VEGF has been shown to reduce colon cancer proliferation [101]. In vivo experiments show Angiopoietin-1 overexpression in colon cancer cells which leads to a decrease in tumour growth [102]. Endostatin is a protein which inhibits endothelial proliferation. Gene therapy utilising endostatin has decreased angiogenesis and has been shown to have a direct inhibitory effect on some cancer cells [103].

**Conclusion**

Gene therapy is useful for cancers which are associated with altered expression of genes. With advancing knowledge, more and more genes are identified. The exact role in preventing
and curing colon cancer with gene therapy is still at a primitive stage. There is no doubt that chemo-gene therapy, which is a combination gene transfer and chemotheraphy, will play an important role in advanced cancers. Several gene therapy trials are underway worldwide. Based upon the results of these trials, gene therapy may be used either as a sole therapy or as an adjuvant to other modalities of treatment.

Conflicts of interest

None to declare.

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