The development of new treatment strategies for oesophageal cancer

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Gene therapy is the introduction of new genetic material with resulting therapeutic benefit.

The current status of cancer gene therapy and the general principles of its application in gene therapy and oesophageal carcinoma focuses on how these principles can be applied to oesophageal cancer.
INTRODUCTION

Over the last decades, enormous progress has been made in the understanding of basic molecular genetics. This is particularly true for oesophageal adenocarcinoma with its well defined premalignant lesions. It is now generally accepted that oesophageal adenocarcinoma develops via a sequence of genetic changes in which the normal squamous cell epithelium is replaced by columnar epithelium (Barrett's oesophagus) due to longstanding gastro-oesophageal reflux disease (GORD).\(^1\) The metaplastic Barrett's epithelium is associated with an increased risk of malignant degeneration via low-grade and high-grade dysplasia into adenocarcinoma.\(^2\) Insights into the mechanism of pathological processes during malignant degeneration in combination with the synergistic progress in recombinant DNA technology, have stimulated efforts to develop therapeutic approaches to prevent or correct the genetic changes in oesophageal malignancies and optimize the limited current treatment strategies for this aggressive disease.

In general, gene therapy can be described as the introduction and expression of an exogenous gene into human cells with resulting therapeutic benefit. It was originally developed for inherited disorders with well defined single gene defects. The first human gene transfer study (pioneered by Anderson Blease in 1990) attempted to correct adenosine deaminase deficiency in patients with severe combined immune deficiency disorder (SCIDD).\(^3\) In a clinical trial, patient T lymphocytes were genetically modified in the laboratory (ex vivo) and so far this is the only study with good long-term results. Nowadays, the largest proportion of gene therapy targets cancer with 608 of the 918 (66%) registered clinical trials being for various malignancies (Table 1). In oncology, gene therapy can be defined as the introduction of DNA into cells (either neoplastic or normal) in order to eliminate malignant cells.\(^4\) This can be achieved by modulating the immune response to a tumour (genetic immunotherapy), reversing the malignant process by correcting genetic abnormalities (gene replacement therapy), or inducing malignant cell death (suicide gene therapy or viral oncolysis). Another possibility is to enhance a tumour's responsiveness to conventional treatment strategies such as chemotherapy and radiotherapy, or to protect normal tissue by introduction of genetic material that renders resistance to toxic effects of such treatment (drug resistance gene therapy).\(^5\)

A number of strategies have been developed to accomplish cancer gene therapy. These approaches include immunotherapy, gene corrections, and cytotoxic gene therapy.\(^5,6\) However, despite substantial progress in the field, wide clinical application and success have not been achieved.\(^7\) In cancer gene therapy, the main problem is efficient gene delivery. At present, both
TABLE 1
Clinical trials for cancer gene therapy registered at the official trial site of the Journal of Gene Medicine until January 31, 2004 (http://www.wiley.co.uk/genetherapy/clinical/).

<table>
<thead>
<tr>
<th>Malignant disease</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>127</td>
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</tr>
<tr>
<td>Prostate</td>
<td>78</td>
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</tr>
<tr>
<td>Breast</td>
<td>65</td>
<td>10.7</td>
</tr>
<tr>
<td>Head and neck</td>
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<td>9.4</td>
</tr>
<tr>
<td>Lung</td>
<td>53</td>
<td>8.7</td>
</tr>
<tr>
<td>Ovarian</td>
<td>45</td>
<td>7.4</td>
</tr>
<tr>
<td>Renal</td>
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<td>4.4</td>
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<tr>
<td>Leukaemia</td>
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<td>3.5</td>
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<tr>
<td>Colorectal</td>
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<td>3.1</td>
</tr>
<tr>
<td>Cervical</td>
<td>14</td>
<td>2.3</td>
</tr>
<tr>
<td>Bladder</td>
<td>7</td>
<td>1.2</td>
</tr>
<tr>
<td>Brain/neuroblastoma</td>
<td>6</td>
<td>1.0</td>
</tr>
<tr>
<td>Solid tumour</td>
<td>6</td>
<td>1.0</td>
</tr>
<tr>
<td>Hepatocellular</td>
<td>4</td>
<td>0.7</td>
</tr>
<tr>
<td>Gastric</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>Oesophageal</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>Other</td>
<td>75</td>
<td>12.3</td>
</tr>
<tr>
<td>Total</td>
<td>608</td>
<td>100</td>
</tr>
</tbody>
</table>

Transduction efficiency and selectivity are far from optimal. Another obstacle is the lack of good study models to analyse novel vectors for gene therapy. This overview is intended to describe the general principles of cancer gene therapy and specifically reviews the present status of developments in gene therapy for oesophageal cancer.

GENE DELIVERY METHODS

To introduce new genetic material into malignant cells, vectors are used. The gene of interest must be inserted into a vector adjacent to a promoter that induces transcription. Then the construct must be packaged and delivered to the specific target cells, transcribed and expressed in high enough concentration to exert its effect. The transfer of transgene constructs into living cells can be accomplished using non-viral or viral vectors.
TABLE 2
Vectors used in clinical trials for cancer gene therapy as registered at the official trial site of the Journal of Gene Medicine until January 31, 2004 (http://www.wiley.co.uk/genetherapy/clinical/).

<table>
<thead>
<tr>
<th>Vector</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>181</td>
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</tr>
<tr>
<td>Adeno-associated virus</td>
<td>3</td>
<td>0.5</td>
</tr>
<tr>
<td>Retrovirus</td>
<td>126</td>
<td>20.7</td>
</tr>
<tr>
<td>Pox virus</td>
<td>52</td>
<td>8.6</td>
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<tr>
<td>Vaccinia virus</td>
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<td>4.9</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
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<td>4.1</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>Measles virus</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>Naked/plasmid DNA</td>
<td>85</td>
<td>14.0</td>
</tr>
<tr>
<td>Lipofection</td>
<td>69</td>
<td>11.3</td>
</tr>
<tr>
<td>RNA transfer</td>
<td>10</td>
<td>1.6</td>
</tr>
<tr>
<td>Gene gun</td>
<td>5</td>
<td>0.8</td>
</tr>
<tr>
<td>Adenovirus + retrovirus</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>Pox virus + vaccinia virus</td>
<td>10</td>
<td>1.6</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
<td>1.0</td>
</tr>
<tr>
<td>Total</td>
<td>608</td>
<td>100</td>
</tr>
</tbody>
</table>

Non-viral vectors
Non-viral gene transfer systems include the direct tissue injection of DNA which can be enhanced by electroporation. Another way to enhance gene transfer is to use reagents that allow DNA transfer across the cell membrane like liposomes, peptide delivery systems, or polymer vectors. All non-viral methods have a high level of safety since they are considered non-toxic and non-immunogenic. However, efficiency of gene transfer is lower than can be achieved when using viral vectors. Furthermore, the limited expression period of the introduced DNA and the absence of any mechanism to maintain the introduced DNA within the cell can be another disadvantage. Therefore, the use of non-viral approaches seems to be limited as vectors for cancer gene therapy.

Viral vectors
Viral vectors are used in the majority of gene therapy studies due to their higher efficiency of gene transfer compared to non-viral techniques. Viral vectors can be either DNA or RNA based. DNA viral vectors include
adenoviruses and adeno-associated viruses (AAVs), and RNA viral vectors are retroviruses (for example the lentivirus derived from human immunodeficiency virus). Each vector system has its own advantages and disadvantages which determine their suitability for different clinical applications. Selection is based on the maximum permissible transgene size, transfection efficiency, maximum viral titre attainable, tendency to provoke inflammatory and immune response, persistence of gene expression, ability to transduce non-dividing cells, target cell-specificity, and impact on the host genome.

Adenoviruses are non-enveloped linear double-stranded DNA viruses. Uptake of the adenoviral particles involves binding of the viral fiber knob with cell receptors and integrins, followed by internalization via receptor mediated endocytosis. The viral genome does not integrate into the host genome but instead replicates as episomal elements in the nucleus of the host cell with no risk for insertional mutagenesis. The adenoviral genome can accommodate up to a 30 kb DNA insert. Adenoviral vectors can efficiently deliver genes to a wide variety of dividing and non-dividing cell types, but for the most part transfections are transient. A significant problem associated with adenoviral vectors is initiation of an immune response and the associated generation of neutralizing antibodies that prevents subsequent treatment with the vector.

AAV vectors are non-enveloped single-stranded DNA human parvoviruses that are dependent on a helper virus, adenovirus or herpes simplex virus, to proliferate. They are also capable of infecting both dividing and non-dividing cells. Although AAV was thought to integrate into the genome, current data indicate that this vector mostly remains in an episomal form. Still the large concatemers formed by this vector do result in prolonged expression. In contrast to adenoviruses, they do not cause a significant host inflammatory response. A significant limitation of AAVs as vectors is that the viral genome can only accept DNA inserts up to 4.7 kb in length.

Retroviruses are single-stranded RNA viruses that attach to a cell surface receptor via an envelope surface protein, followed by receptor mediated endocytosis. The outer envelope protein coat is shed and the viral genome undergoes reverse transcription to form a double-stranded DNA intermediate. The viral nucleoprotein complex then enters the cell nucleus and integrates randomly into the host genome. An advantage of this vector is that the permanently integrated viral genome can be transmitted to the progeny of the transduced parent cell resulting in sustained transgene expression, but transfections occur with low efficiency in non-dividing cells. Another disadvantage is the potential risk for insertional mutagenesis due to the random integration. An overview of the various vectors used in clinical cancer gene therapy trials is given in Table 2.
STRATEGIES FOR CANCER GENE THERAPY

The various strategies for cancer gene therapy used in clinical trials are summarized in Table 3.

### TABLE 3
Gene therapy approaches used in clinical trials for various cancers as registered at the official trial site of the Journal of Gene Medicine until January 31, 2004 (http://www.wiley.co.uk/genetherapy/clinical/).

<table>
<thead>
<tr>
<th>Gene type</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunotherapy</td>
<td>179</td>
<td>29.4</td>
</tr>
<tr>
<td>Tumour suppressor</td>
<td>114</td>
<td>18.8</td>
</tr>
<tr>
<td>Antigen</td>
<td>111</td>
<td>18.3</td>
</tr>
<tr>
<td>Antisense</td>
<td>6</td>
<td>1.0</td>
</tr>
<tr>
<td>Suicide</td>
<td>68</td>
<td>11.2</td>
</tr>
<tr>
<td>Viral oncolysis</td>
<td>51</td>
<td>8.4</td>
</tr>
<tr>
<td>Drug resistance</td>
<td>13</td>
<td>2.1</td>
</tr>
<tr>
<td>Receptor</td>
<td>25</td>
<td>4.1</td>
</tr>
<tr>
<td>Oncogene regulator</td>
<td>4</td>
<td>0.7</td>
</tr>
<tr>
<td>Hormone</td>
<td>3</td>
<td>0.5</td>
</tr>
<tr>
<td>Others</td>
<td>34</td>
<td>5.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>608</td>
<td>100</td>
</tr>
</tbody>
</table>

Genetic immunotherapy

The main principle of genetic immunotherapy is to improve the host’s immune response to a particular tumour. Passive immunopotentiation involves boosting the natural immune response to make it more effective. Active immunopotentiation requires the initiation of an immune response against a previously unrecognized tumour. Strategies of passive immunopotentiation include transfection of cytotoxic T cells, natural killer cells, macrophages, and dendritic cells with genes encoding cytokines and growth factors. The population of transfected cells is expanded and then returned to the patient. Immune cells can also be induced to recognize specific cancer cells. Active immunotherapy (tumour vaccines) is used to initiate an immune response against an unrecognized or poorly antigenic tumour. Tumour cells can be genetically modified to express a variety of factors (e.g. major histocompatibility antigens, cytokines, tumour antigens). The transfected tumour cells are then
irradiated both to minimize malignant potential and to improve immunogenicity before being reintroduced by vaccination into the patient. Nowadays, the majority of cancer gene therapy trials use the immunotherapy approach. Phase II immunotherapeutic clinical trials based on dendritic cell immunotherapy for patients with glioma and hepatocellular carcinoma patients are ongoing and a variety of melanoma tumour cell vaccines have been developed, including immunization with IL-7 and IFN-γ.

**Gene replacement**

Loss of tumour suppressor genes is an important mechanism in the carcinogenesis by which cancer cells with DNA mutations can continue to proliferate and avoid apoptosis. The potential to correct these losses in a tumour genome seems an attractive approach for cancer gene therapy to inhibit cell growth and induce apoptosis. Phase I and II clinical trials with tumour suppressor gene replacement for non-small cell lung cancer have demonstrated that direct intratumoural injection can cause tumour regression or prolonged stabilization of local disease. In addition, this gene transfer was associated with low toxicity and therefore it has been hypothesized that tumour suppressor gene replacement can be readily combined with existing and future treatments. More recent data from laboratory studies demonstrate that these genes may even be useful in early intervention, diagnosis, and even prevention of cancer.

Another form of this type of cancer gene therapy is inactivation of oncogenes. The propagation of cancer cells by activation and amplification of oncogenes can be inhibited using the following strategies: 1) Inhibition of oncogene transcription into mRNA by using DNA oligonucleotides (i.e. short single-stranded DNA sequences) that are designed to bind to specific oncogene promoter regions. These oligonucleotides form triple helix structures that prevent oncogene transcription. 2) Reduction of mRNA translation into protein by using antisense RNA oligonucleotides that bind in a complementary manner to the mRNA. The resulting double-stranded RNA complexes are subsequently destroyed by intracellular enzymes. Antisense oligonucleotides have been used to block bcl-2 oncogene translation in prostate and breast cancer cells. 3) After mRNA translation, transportation of the oncoprotein to its target can be blocked by intracellular expression of single chain antibodies directed against the oncoprotein, called intrabodies. Although there are only a few clinical studies with this approach, intrabodies have been developed against p53, erb2, and cyclin E proteins expressed in ovarian and breast cancers with good *in vitro* results.
Suicide gene therapy
One of the most promising strategies of gene therapy is the transfection of cancer cells with suicide genes that encode enzymes able to transform a non-toxic 'prodrug' into a toxic product. The advantage of this approach is the so-called bystander effect of the toxic drug that can induce cell death of neighbouring tumour cells without the need of viral transduction. There are two commonly used approaches: 1) The use of Herpes Simplex Virus (HSV)-thymidine kinase (tk), followed by treatment with nucleoside analogues such as ganciclovir. Viral tk converts the monophosphate ganciclovir into the triphosphate form by cytoplasmic enzymes. This triphosphate ganciclovir is incorporated into replicating DNA and stops chain elongation resulting in cell death. 2) Transfection of cancer cells with the Escherichia coli gene coding for Cytosine Deaminase (CD) followed by systemic use of 5-fluorocytosine (5-FC). CD transforms the non-toxic pro-drug 5-FC into the cytotoxic drug 5-fluorouracil (5-FU). Sixty-eight clinical trials for various carcinomas (e.g. brain, prostate, bladder, kidney) using the suicide gene therapy strategies are currently being performed, most of which are still in a phase I stage.

Drug resistance gene therapy
One major obstacle to successful treatment with high-dose chemotherapy is myelosuppression. The transfer of drug-resistance genes into haematopoietic stem and progenitor cells has been explored as a method for overcoming this problem. Overexpression of the human multidrug resistance 1 (MDR1) drug-efflux P-glycoprotein in cells confers resistance to a variety of chemotherapeutic agents including paclitaxel and etoposide. Retroviral transfer of the MDR1 gene into human stem and progenitor cells prior to autologous transplantation has been used with variable results in the treatment of human germ cell tumours, metastatic breast cancer, and other advanced cancers not involving bone marrow.

Viral oncolysis
Most strategies for cancer gene therapy as described above use non-replicative viral vectors encoding for a target gene. A relatively new class of therapeutic vectors are replicative viruses which induce destruction of tumour cells as a result of viral replication with the advantage of subsequent multiplication and spread (Figure 1). Viruses that have been examined for their oncolytic potential include the adenovirus, herpes simplex virus and vaccinia virus. The adenovirus has the most favourable characteristics since the natural adenoviral replication cycle is lytic and results in killing of the infected cell. For safety and effectiveness reasons,
FIGURE 1
The concept of adenoviral oncolysis: conditionally replicating adenoviral vectors (CRAds) infect tumour cells, replicate their genome, assemble new viral particles and kill the host tumour cell by lysis. This results in the release of progeny viruses, which are able to transfect neighbouring cells. In normal cells the viral replication is attenuated.
it is of crucial importance that these constructs have tumour specific characteristics. In the adenoviral context this has led to the development of conditionally replicative adenoviruses (CRAds). Two general mechanisms have been used to achieve tumour-selective viral replication: 1) The deletion of viral genes that are dispensable on infection of neoplastic cells but are critical for viral replication in non-neoplastic cells. An example is the ONYX-015 virus, in which tumour specificity is obtained by a deletion in the viral E1B region that normally encodes a 55-kDa protein. When an adenovirus infects a normal cell, p53 levels are upregulated and the cell undergoes cell-cycle arrest or apoptosis, thereby preventing viral replication. Wild-type adenoviruses escape this ‘cellular suicide’ by expressing the E1B 55-kDa protein which binds to and inactivates p53, allowing viral replication to proceed. ONYX-015 lacks this gene, restricting viral replication to cells with a defective p53 pathway. ONYX-015 has been analysed in clinical trials for the treatment of several p53-deficient malignancies, including head and neck cancer and ovarian malignancies. Phase I studies demonstrated the safety of ONYX-015 administered intratumourally and intravenously, but most phase II studies did not induce significant tumour regression. 2) Replacing the replication controlling viral EA1 promoter by a tumour specific promoter. Such a selective promoter can be derived from genes that are upregulated specifically in tumour tissue. An example is the oncolytic adenovirus under transcriptional control of an alfa fetoprotein (AFP) promoter that replicates selectively in AFP-producing cell lines (e.g. HCC cell lines).

GENE THERAPY FOR OESOPHAGEAL CANCER

Potential applications
There are at least three potential strategies for application of gene therapy in the treatment of oesophageal carcinoma. The first scenario is the patient with locally irresectable or metastatic disease who is not a candidate for surgical resection. For these patients, gene therapy could be used to reduce tumour load as a palliative regimen by either systemic or local endoscopic administration. In another scenario gene therapy could be used as adjuvant therapy either to treat local residual disease after a non-radical resection or to treat (micro-) metastases. It has been demonstrated that even after a presumed radical oesophagectomy, undetected malignant cells may still be present in bone marrow or lymph nodes. With a specific vector one could eradicate these metastases by systemic delivery.
The third scenario is for the patients with high-grade dysplasia in a Barrett's oesophagus, for whom there is very high risk of the presence or soon development of cancer, or patients with an intramucosal lesion without lymph node metastases. So far, these patients are advised to undergo an oesophagectomy which is associated with a significant morbidity and substantial mortality. Currently, various endoscopic techniques (e.g. endoscopic mucosal resection and photodynamic therapy) are being explored as local treatment strategies for these lesions. In the context of gene therapy, a specific gene delivering vector eliminating dysplastic and neoplastic cells could potentially be used for these early lesions endoscopically.

**In vitro studies**

Most studies on gene therapy for oesophageal cancer make use of *in vitro* models in which the various strategies of genetic immunotherapy, gene replacement therapy and suicide gene therapy are analysed in human oesophageal cancer cell lines. For genetic immunotherapy it was demonstrated that a replication-deficient adenoviral vector encoding TNF-α under control of the early growth response-1 gene (EGR-1) promoter, which is sensitive for radiation, resulted in TNF-α secretion and cytotoxicity upon radiation of transduced oesophageal adenocarcinoma cells. In another study, retrovirally expressed interleukin-2, interleukin-6 and granulocyte macrophage-colony stimulating factor inhibited the metabolism of transduced oesophageal squamous carcinoma cells after irradiation. Gene replacement therapy with the tumour suppressor genes p53 and p21 transduced in several oesophageal squamous cell carcinoma cell lines using adenoviral vectors, resulted in efficient apoptosis and a significant reduction in cell growth. Antisense therapy has also been used in oesophageal cancer cell lines. Proliferating cell nuclear antigen (PCNA) has been shown to stimulate DNA synthesis by DNA polymerase delta, and it is expressed in the majority of oesophageal cancers. Antisense oligonucleotides specific for PCNA mRNA were demonstrated to inhibit the growth of oesophageal carcinoma cell lines, whereas random sequence oligonucleotides had no effect. For suicide gene therapy, the HSV-tk system was tested on oesophageal squamous carcinoma cell lines. It was shown that the HSV-tk system resulted in a cytotoxic effect of transduced cells, accompanied by a cytotoxic bystander effect of the surrounding non-transduced tumour cells. In this study, the effect of HSV-tk was enhanced by uracil phophoribosyltransferase, which alters the 5-FU metabolism and sensitivity.
In vivo animal studies

In vivo gene therapy studies for oesophageal cancer are more limited, since most animal models are laborious and associated with a high morbidity and mortality of the animals. Subcutaneously injected cell lines in nude mice, is the most commonly used animal model. In this model it was demonstrated that immunotherapy with TNF-α under control of the EGR-1 promoter resulted in regression of oesophageal carcinoma cell line tumours after radiation. When the HSV-tk system was used for subcutaneous tumours in nude mice, the cell lines with wild type p53 showed better response on adenoviral suicide gene therapy when compared to tumours with mutated p53. For this latter group adenoviral p53 gene replacement therapy with the recombinant adenoviral-p53 vector is suggested to be more effective since significant growth suppression in a xenograft nude mice model was demonstrated. A gene transfer study in normal rat oesophagus showed that squamous epithelium can also be transduced with the use of liposomes, although this transduction is limited. This transduction of normal squamous epithelium is an application that has successfully been used to transduce the manganese superoxide dismutase transgene which prevented radiation-induced damage.

Clinical studies

So far, two clinical phase II trials using gene therapy for oesophageal carcinoma have been initiated. Both protocols are for locally advanced oesophageal carcinoma. One multi-center study at the University of California uses preoperative immunotherapy with an adenovirus encoding TNF-α (AdGVEGR.TNF.11D) in combination with chemotherapy (http://www.wiley.co.uk/genetherapy/clinical/). The other study, performed at the University of Tokyo, Japan, introduces an adenoviral vector intratumorally encoding for the wild type p53. Although the results have to be awaited, the latter trial has reported preliminary data indicating that the feasibility of this treatment appeared fairly good in nine patients and that so far no serious adverse events were seen.

In patients with head and neck cancer remarkable clinical effect was achieved with the use of ONYX-015, the adenovirus that selectively replicates in p53-deficient cancer cells as described above, in combination with chemotherapy. Since head and neck cancers are squamous cell carcinomas with malignant characteristics comparable to oesophageal squamous cell carcinomas, ONYX-015 potentially could have a role in the management of these cancers.
OPTIMIZING GENE THERAPY FOR OESOPHAGEAL CANCER

As with all forms of cancer gene therapy, the main problem in developing this new therapeutic approach for oesophageal malignancies is to optimize gene delivery in order to maximize the proportion of successfully transduced tumour cells and increase selective transduction to spare normal cells. Although numerous vector systems for gene transfer have been developed, a perfect vector system has not yet been constructed. So far the adenovirus seems the most promising vector due to its relatively high transduction efficiency, but clinical studies still demonstrate difficulties of gene transfer due to resistance of human cells for viral infection. To increase infection efficiency and selectivity targeting is used. Several targeting approaches have been developed with promising in vitro results. However, it has been demonstrated that there is a significant discrepancy between the vector efficiencies observed in vitro using established cell lines and the tumour transduction rates achievable in vivo. Therefore, optimising gene therapy should focus on both developing adequate study models and improving vector systems.

Study models

With the currently available study models, human cancer cell lines are most often used to analyse vector efficiencies. However, established cell lines are transformed due to multiple passages and consist of only one cell type, which makes the similarity to the original heterogeneous tumour cells limited. Therefore, it is important to create a cell culture system with human tumour cells that is more comparable to the in vivo situation. Primary cell cultures from human biopsies or resection material have been established in which different (heterogeneous) cell types could be studied, but this is still an artificial monolayer test-system in which the original tumour architecture is lost. To perform experiments in complex three-dimensional heterogeneous tumours, xenografts can be used. For survival of these xenografts, the primary human specimens are often implanted in a nude mouse. Although this is an attractive model comparable to the in vivo situation, the results should be interpreted with caution because of the lack of immune-response on adenoviral administration in nude mice, possibly resulting in false-positive results. Another option to analyse vector efficiencies for gene therapy is the use of animal models with spontaneously developed tumours. Several studies have demonstrated that the carcinogen 2,6-dimethylnitrosomorpholine (DMNM) induces squamous cell carcinomas and adenocarcinomas in rats. Other reports also showed the development of a Barrett's segment and
adenocarcinoma after gastro-duodenal-oesophageal reflux without carcinogens, but so far the true nature of these lesions remains to be elucidated. In addition, one also has to realize that as a general rule human adenoviral vectors do not replicate in most animal cells and that animal models most frequently used to study cancer gene therapy (e.g. rat and mouse) are therefore not suitable to assess the selectivity of CRAds.

Targeting
Several targeting approaches to increase the selectivity and/or efficiency of gene delivering vectors for oesophageal cancer were analysed. To increase tumour selective transduction, peptides that bind a specific tumour antigen can be cloned into the viral vector allowing specific tumoural infection. For oesophageal carcinoma a marked difference in expression of the human epithelial cell adhesion molecule (EpCAM) between normal and (pre)malignant lesions of the oesophagus was demonstrated. Based on these findings, the feasibility of a bispecific antibody against the adenovirus fiber-knob protein and EpCAM was explored for selective gene transfer.

Adenoviral vectors redirected to EpCAM using bispecific antibodies specifically infected gastric and oesophageal cancer cell lines. Using primary human cells, an improved ratio of tumour transduction over normal epithelium transduction was accomplished by these EpCAM-targeted vectors. Other targets like the epidermal growth factor receptor, c-met and HER2/neu have also been suggested as potential epitopes to which a gene delivering vector could be targeted to for specific transduction.

To increase transduction efficiency, genetic targeting can be used. From literature it is known that adenoviral cell entry via the Coxsackie and adenoviral receptor (CAR) is the rate-limiting step of gene delivery. An important problem in vivo is the decreased expression of CAR in tumour cells. By inserting an arginine-glycine-aspartate (RGD) tri-peptide in the fiber knob, the adenoviral vector can be targeted to integrins. Studies have demonstrated that this RGD-retargeted adenovirus infects pancreatic and ovarian carcinoma cells with enhanced efficiency. Since oesophageal tumour cells also express various integrins abundantly, genetic targeting might be an attractive approach for this malignancy.

The use of tumour specific promoters to develop selective CRAds has also been proposed as an attractive targeting strategy for oesophageal cancer. All genes upregulated specifically in dysplastic or tumour tissue can potentially be used. Examples are the Cyclooxygenase-2 (COX-2) and midkine enzyme which are frequently upregulated in oesophageal cancer while undetectable in normal squamous cell epithelium.
CONCLUSIONS

Oesophageal cancer is an aggressive disease for which current treatment modalities are limited. For the last two decades there have been significant developments in the field of surgery and adjuvant regimens, but even despite these advances in multimodal therapy, the prognosis for patients with invasive oesophageal cancer remains poor. Therefore, new and innovative treatment modalities are needed to improve the management of these patients. The field of cancer gene therapy has evolved tremendously with many potential applications. Especially in the field of targeting and new therapeutic genes there have been promising developments in recent years. Most of this research is still in a preclinical phase and so far clinical studies have mainly focused on safety issues. The results of the first clinical efficacy trials are anxiously awaited, but obviously a lot of research is still needed before gene therapy can play a significant role in the management of oesophageal cancer. The focus of this research should be on developing new experimental models as well as on improving gene delivering vectors or novel therapeutic genes that can have a synergistic effect with current adjuvant therapies. Although it is unlikely that cancer gene therapy will replace the conventional methods of treatment, selective and efficient vectors might be used as monotherapy for a specific subset of patients or it might be used as (neo-)adjuvant therapy improving the therapeutic options for patients with oesophageal cancer in the future.
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