REVIEW

Gene therapy in head and neck cancer: a review

E Chisholm, U Bapat, C Chisholm, G Alusi, G Vassaux

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Gene therapy for cancer is a rapidly evolving field with head and neck squamous cell cancer being one of the more frequently targeted cancer types. The number of clinical trials in the UK is growing and there is already a commercially available agent in China. Various gene therapy strategies along with delivery mechanisms for targeting head and neck cancer are reviewed.

Head and neck squamous cell cancer (HNSCC) refers to malignant tumours of squamous cell origin arising from the mucosal surfaces of the upper aerodigestive tract, salivary glands, paranasal sinuses, and skin of the head and neck.[1] In England approximately 400 persons per 100 000 are newly diagnosed as having head and neck cancer each year.[2] It is the sixth most common type of cancer in the world and most sufferers die from their disease. Untreated it has a 50% mortality at 4 months.[3]

The mainstay of treatment for HNSCC is surgery or radiotherapy, either alone or in combination with chemotherapy.[1] This results in a 60% 5 year survival for patients with newly diagnosed HNSCC.[4] Unfortunately this figure has remained largely unchanged for 30 years.[5] The search for new treatment strategies is of great clinical interest. One such approach is gene therapy.

As defined in the government's genetics white paper in 2003, gene therapy is ''the deliberate introduction of genetic material into patient's cells in order to treat or prevent a disease''.[6] Early gene therapy research was directed at disorders with a single, identifiable genetic defect such as severe combined immunodeficiency disease (SCID), cystic fibrosis and haemophilia.

Recently, attention has moved towards cancer with the realisation that it too is a genetic disease. The loco-regional nature of HNSCC makes it is accessible for both intratumoral injection and tissue biopsy. For this reason it is one of the cancers most frequently targeted by gene therapy.[7] We will discuss the genetic changes in cancer followed by the different strategies of gene therapy. We will then describe monitoring of gene therapy, gene delivery and ways of enhancing cancer cell specificity.

GENETIC CHANGES IN CANCER

In order for a cell to become ''cancerous'' or have malignant potential it must undergo mutations or epigenetic changes. These are usually somatic, brought about by chance or environmental factors, with only a small proportion being caused by inherited factors. The normal cell cycle is regulated by numerous genes, including proto-oncogenes and tumour suppressor genes, held in equilibrium. An upset to this balance by increased (proto-) oncogene expression or a reduction in tumour suppressor gene expression leads to aberrant proliferation and hence ''cancer''. On a cellular level the hallmark changes of a cancerous cell are: self sufficiency in growth signals; insensitivity to anti-growth signals; evading apoptosis; sustained angiogenesis; limitless replicative potential; tissue invasion and metastases[8] (box 1).

Cancer gene therapy is based on the insertion of a gene (transfection) into a cell. This new DNA is then ''transcribed'' to make mRNA which encodes a specific protein that is made through the process of translation (fig 1).

As the whole of the human genome has been mapped there are an enormous number of genes from which to choose. The type of gene therapy can be characterised as ''corrective'', ''cytoreductive'' or ''immunomodulatory''.[9]

CORRECTIVE GENE THERAPY

When a gene is causally implicated in oncogenesis it is termed a ''cancer gene'' (either an oncogene or tumour suppressor gene). So far, 291 oncogenes have been reported, more than 1% of all the genes in the human genome.[10] Ninety per cent of cancer genes show somatic mutations in cancer, 20% show germline mutations and 10% show both.[11] Corrective gene therapy attempts to block oncogenes or replace tumour suppressor genes. In the case of tumour suppressor genes, the aim is to express a gene under the control of a suitable promoter so there is an increase in the production of that therapeutic gene product. The archetypal tumour suppressor gene in HNSCC and most other forms of cancer is p53, which is a built-in safety mechanism within each cell. If there is damage to the genetic material within the cell, which may cause it to behave in an abnormal way, the protein encoded by the p53 gene stops the cell cycle by binding to DNA (it is a transcription factor). If the damage is not repairable it triggers cell death (apoptosis).[12] Alteration to p53 results in continued propagation of the damaged cell line. Replacement of p53 results in reduced HNSCC growth and increased radiochemo-sensitivity.[7]

A US trial in advanced recurrent HNSCC showed a positive response in 50% of cases.[13] In China there is a commercially available gene therapy agent for HNSCC based on p53. This is in the form of Gendicine from Schenzhen SiBono GenTech. In phase I trials in 12 patients with advanced laryngeal cancer there was a claimed response, without relapse 5 years later, in 11 of the 12 patients.[14 15] In phase II and III clinical trials it was

See end of article for authors' affiliations

Correspondence to: Dr E Chisholm, 44 Eastbury Grove, London W4 2JY, UK; edwardchisholm@doctors.org.uk

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Box 1: The features of a ''cancer'' cell

- Insensitive to anti-growth signals
- Self sufficient in growth signals
- Evade apoptosis (programmed cell death)
- Sustained angiogenesis
- Tissue invasion and metastasis
- Limitless replicative potential

shown to have synergistic effects with radiotherapy and chemotherapy. One hundred and thirty-five HNSCC patients (77% stage III or IV) were randomised to receive radiotherapy alone or in combination with Gendicine. Those receiving the gene therapy in addition to radiotherapy had a 93% response rate with complete remission in 64% as compared to 79% and 19%, respectively, in the radiotherapy group.[15–17] To date it has been given by various routes to more than 2500 patients with varying types of cancer.[15]

This strategy depends on the aberrant suppressor gene(s) being consistent in all HNSCCs. Although p53 anomalies are common, they are only evident in 40–60% of cases.[18]

In contrast to the under expression of tumour suppressor genes, oncogenes (for example, ras, myc, bcl-2, erbB2) may be over expressed or amplified in cancer. This often results in the cell avoiding nature's control mechanism, apoptosis. The effects of oncogene abnormalities can be overcome by blocking the faulty gene. This may be by inserting DNA into the cell which binds to, and hence blocks, the oncogene expression (for example, transfecting antisense cDNA or oligonucleotides), inhibiting the oncogenes' DNA from making RNA (transcription) and/or the RNA from making protein (translation). These agents are entering phase I clinical trials. There are no examples to date for HNSCC.

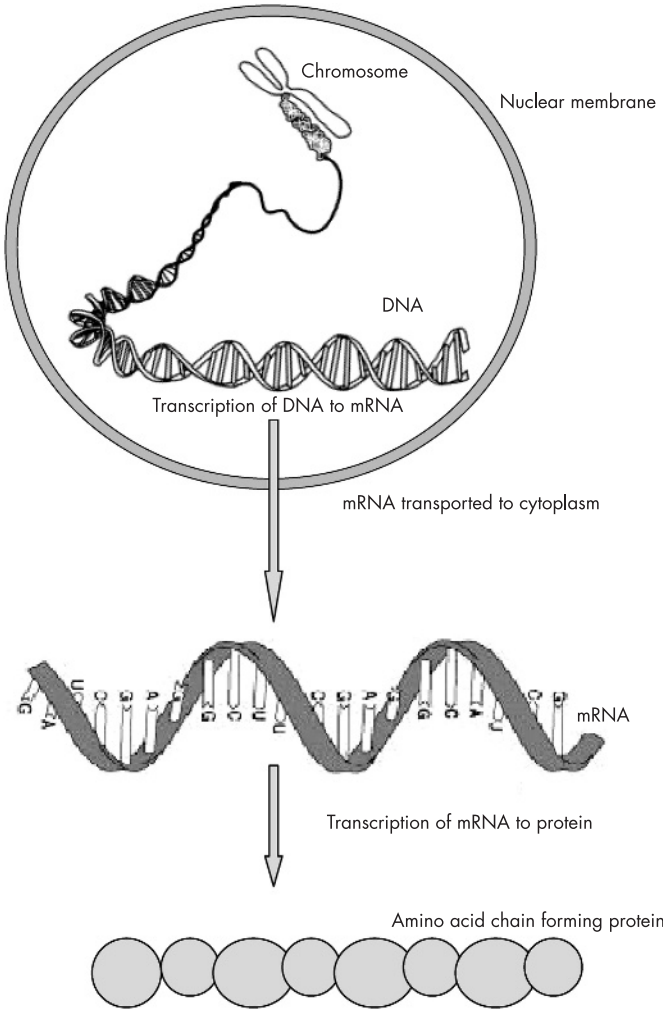

Figure 1 Transcription and translation of DNA to protein.

CYTOREDUCTIVE GENE THERAPY

Cytoreductive gene therapy aims to directly or indirectly kill the cancerous cell rather than correct the underlying genetic defect. As there may be many genetic defects by the time the cancer becomes clinically apparent this would seem a logical approach. This can be done by augmenting the effects of other anti-cancer therapies such as chemotherapy, concentrating cytotoxic agents in cancerous cells, interfering with the tumour's blood supply or inducing apoptosis.

Augmentation of chemotherapy

Gene therapy can be used to augment chemotherapy by either a drug sensitisation or resistance approach. With the sensitisation approach, a gene is transfected to convert a pro-drug into its active metabolite. This allows for drug conversion and a high level of active drug only in the tumour bed. One example is the herpes simplex virus thymidine kinase (TK) gene, which converts gancyclovir into its cytotoxic triphosphate.

Phase I trials of a nitroreductase gene inserted into HNSCC have been completed. The cancer cells express this gene and manufacture nitroreductase. The nitroreductase then activates an inactive pro-drug CB1954 into a potent chemotherapeutic agent.[19]

Another way to augment chemotherapy is to use a drug resistance approach. A drug resistant gene is added into normal cells that are sensitive to chemotherapy, such as haematopoietic stem cells, so that they can resist chemotherapy. This allows higher doses of chemotherapy to be used.[20]

Concentrating radionucleotides

A successful form of treatment of thyroid cancer is the administration of iodine^{131}. The gene encoding the membrane protein responsible for the uptake of iodide is the sodium iodide symporter. This gene can be inserted into other cancer cells to cause them to concentrate radioisotopes of iodine.[21] This can be used for imaging and to administer a concentrated local dose of radiotherapy.

Anti-angiogenic

After growing to more than 1–2 mm in diameter a tumour needs its own blood supply to survive.[8] Targeting new blood vessel formation either by up regulating anti-angiogenic or down regulating pro-angiogenic factors may prove to be useful in controlling tumour growth.

Pro-apoptotic

Normal cells are programmed to kill themselves if they accumulate potentially harmful levels of genetic damage or suffer cytotoxic insults. Like angiogenesis, this is under numerous controls such as tumour necrosis factor (TNF). These control mechanisms can be targeted by gene therapy to induce cancer cells to undergo apoptosis. This method is yet to reach any clinical trials.

MODIFICATION OF THE IMMUNE RESPONSE TO CANCER

The body's own immune system can be used to help clear HNSCC cells by introducing a gene into cancer cells, but not

Box 2: Effective gene therapy requires the following key points or steps

- The disorder has a known genetic defect
- The gene can be carried to the cell (by a vector)
- The new genetic material can be expressed by the cell (transduction)
- The transduction must be in the desired cell type in a targeted manner (usually required)
- It can be carried out in vivo
- The gene product can be produced for a long period of time and in a regulated manner (usually required)
- It must be safe

normal cells, which produces a foreign protein on the cell's surface. This tumour specific antigen allows the cell to be seen and destroyed by the immune system. Cytokines or immune regulatory proteins can be introduced into the HNSCC cells to upregulate the body's own immune response to the tumour cells. Cytokine gene transfer can be performed in vivo where tumour cells or immune cells are transfected in the body, or ex vivo where the cells are removed from the body for transfection and replaced back into the body. Immunotherapy is moving towards acceptance in melanoma, lymphoma and some virus induced malignancies.[22]

Gene therapy can also be used to vaccinate an individual against the antigens expressed by HNSCC cells. A specific antigen gene is injected into cancer cells, helping the body recognise them and mount an immune response against the tumour cells. A problem is the lack of reliable tumour specific antigens. Another vaccination approach is to add a gene that can produce a co-stimulatory molecule. This co-stimulatory molecule is essential for the tumour cell and produces an immune response.

MONITORING OF GENE THERAPY

The easiest way to evaluate these techniques is indirectly through cross-sectional imaging to assess the change in size of the tumour bulk. Otherwise the tissue in question has to be excised and examined by immunohistochemical methods to see if it was expressing the newly engineered gene. The need to assess response has led to the evolution of the field of molecular imaging to monitor gene therapy. By introducing a ''reporter gene'' the gene expression can be monitored. Techniques are based upon the premise that the cells with the transfected gene concentrate or activate a marker.

A promising avenue is the sodium iodide symporter, the membrane protein responsible for the thyroid gland taking up iodide. This can be inserted into cancer cells and the patient given tracer doses of iodide. Positron emission tomography (PET) can then be used as a non-invasive imaging technique to monitor the tumour cells.[23 24] The quantitative PET images obtained may guide the injection of a relevant, therapeutic dose of ^{131}iodide.

GENE DELIVERY (VECTORS)

Once selected, the manufacture or isolation of the gene segment is a relatively straightforward procedure. The main limiting factor to gene therapy is the accuracy and efficacy of delivery of the gene by the gene delivery vector.[25] The route of delivery for gene therapies in HNSCC is almost always direct tumour injection. Systemic injection results in the damage or destruction of the gene and vector before it reaches the tumour cells or sequestration by the liver and spleen.[24] If nucleic acids are injected systemically they are rapidly degraded by serum nucleases. DNA and RNA are negatively charged macromolecules and hence do not readily cross the cell or nuclear membranes so a carrier mechanism is needed. An ideal vector would be highly specific (targeting only the tumour cells and not the host's normal cells), highly efficient (all targeted cells become transfected) and safe. Unfortunately, so far this ideal does not exist.

Numerous types of vector have been evaluated. These can be characterised as viral and non-viral.

Non-viral vectors

Non-viral vectors tend to be less immunogenic and hence may be given repeatedly, an advantage over viral vectors. Typically they can carry more DNA and are cheaper to produce.[26] Non-viral vectors include physically forcing DNA into the cell by direct injection into the tumour.[27] Described methods to improve the efficiency of this physical transfer of DNA/RNA into cells include:

- *Electroportation*—an electric current increases cell permeability
- *Bio-ballistics (gene gun)*—gold particles coated with DNA are ''shot'' into superficial tissue; ultrasound increases the permeability of the cell membrane
- *High pressure hydrodynamics*—a rapid, high volume naked DNA solution injection.[28]

Gene delivery is also possible with chemical carriers such as liposomes carrying cationic lipids and polymers.[29] The gene expression from this can be further enhanced by anionic pH sensitive peptides.[30] The result is a small container or ''nanoparticle'' in which the genetic material is protected until within the cell.

A problem with non-viral vectors has been the low transfection rates, but recent studies have demonstrated encouraging progress.[29]

Viral vectors

Viral vectors are based on the fact that viruses rely on the transcriptional apparatus of the eukaryotic cell for their own replication. They have evolved over millions of years to be highly effective at infecting cells and transferring their genetic material to the nucleus, where it is expressed. For this reason they have logically become natural candidates to carry synthesised genes into HNSCC cells. They are more efficient than non-viral vectors but do cause a ''viral type illness'' and have the potential for acute toxicity. Ideally the pathogenic elements of the viral genome are removed and replaced by exogenous genes with or without added specificity for infection of cancer cells. The virus itself can also exert an anti-cancer effect—''oncolytic viruses''. The oncolytic effect of viruses was noticed at the beginning of the 20th century when a patient's leukaemia dramatically improved following a flu-like illness.[31]

The five main types of viral vector can be classified according to whether their genome integrates into the host cell DNA (retroviruses and lentiviruses) or persists in the cell nucleus as episomes (adenovirus, adeno-associated viruses (AAVs, herpes virus)). They can also be divided into two groups by virtue of their ability to replicate (oncolytic) or if they are replication deficient. Viruses are currently the most commonly used vectors in research.[32]

Retroviruses

Retroviruses have been mainly used ex vivo. Target cells are removed from the patient, genetically modified and then reimplanted into the same recipient. For safety reasons they

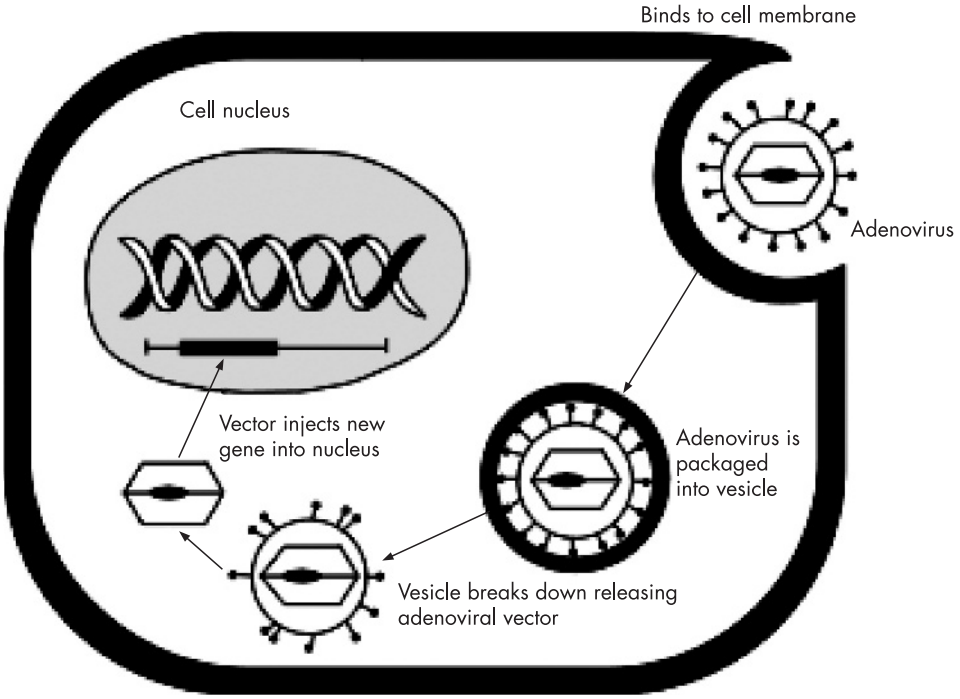

Figure 2 The process by which an adenoviral vector inserts DNA into the target cell.

are incompetent for replication. The retrovirus integrates its genome with the host DNA in the cell. They have a natural tendency to transduce dividing cells as it is only during division that they can enter the nucleus. Apart from the risk of retroviral infection they may also disrupt the host genome (insertional mutagenesis) as was unfortunately shown in two children being treated for X linked SCID who developed a leukaemia-type illness.[33] Lentiviruses are members of the retrovirus family but have the ability to infect non-dividing cells.

Adenoviruses

Adenoviral vectors are strongly immunogenic and early examples were cleared by a vigorous T cell response. This has been reduced by removing the immunogenic genes which are first expressed upon cell infection. A schematic view of the adenovirus vector is shown in fig 2. Adenoviruses can be either replication defective or replication competent. Replication defective adenoviruses can be produced in large amounts in producer cell lines and have the ability to infect non-dividing cells. As they are not inserted into the host genome there is minimal risk of insertional mutagenesis. An adenoviral vector is used in Gendicine, the p53 based gene therapy agent commercially available in China. Recombinant adenovirus vectors carrying p53 are also being used in phase III clinical trials for HNSCC in the USA in the form of Advexin by Introgen Therapeutics (Austin, Texas).

Herpes simplex viruses

Non-replicating herpes simplex virus (HSV-1) was originally used as it has the ability to persist after the initial infection in a latent state in neuronal cells for the lifespan of the cell. They have a large cloning capacity that allows for simultaneous delivery of several genes. Unfortunately this has not conferred any benefit in HNSCC therapy so far.

Replicating viral vectors

If the new genetic material is successfully transfected into the HNSCC cell and the cell is destroyed, so is the new genetic material it contains. In effect the gene therapy is responsible for its own suicide while the surrounding cancer cells are untouched. In order to be successful the effect of the gene therapy must be able to spread to surrounding cells. This can be done by a replication competent (oncolytic) vector. Oncolytic viral vectors only require a limited initial transduction of target cells. They can then replicate and release a second wave of viral vectors and so on. Another way to circumvent the self destruction of the vector is for it to exert a secondary effect on neighbouring cells—the so called ''bystander effect''.[34] This can be either local, where toxic metabolites are transferred to neighbouring cells, or immune mediated.[25]

Replicating adenoviruses

Replicating adenoviruses are the most commonly studied oncolytic viral vectors.[35] One such is ONYX-015, a conditionally replicating adenovirus. ONYX-015 has the gene responsible for binding to and inactivating p53 removed. The result is a virus unable to replicate in normal cells but capable of replicating in p53 negative cells. As a lack of p53 is a hallmark of most cancers, ONYX-015 is considered an oncolytic virus. This has been used in phase I and II clinical trials in advanced recurrent HNSCC.[36 37] In the phase II trials of 40 patients, there was no viral replication or toxic effects in normal tissue. There was tumour regression in 10%, tumour growth stabilisation in 62% and disease progression in 29%.[37 38] The dose was increased fourfold with no change in response, but an increase in the side effects of fever and injection site pain.[38] It was felt that this justified further assessment in earlier stage HNSCC in conjunction with cisplatin and 5-fluorouracil (5-FU). There was no increase in the chemotherapy toxicity with the addition of ONYX-015. Dramatically, a response rate of 63% versus the expected 35% was observed.[39]

Replicating herpes simplex viruses

Unlike their non-replicating herpes viruses (HSV) vector counterparts, replicating or oncolytic HSVs have shown some success in clinical trials. With the deletion of genes from the HSV which control virulence (for example, ICP6 and/or ICP34.5) the virus becomes dependent on dividing host cells in order to replicate.[35 40] This results in cancer cell selectivity. An oncolytic HSV with both these deletions and added granulocyte–macrophage colony stimulating factor (GM-CSF), named Oncovex, is available. The GM-CSF enhances the anti-tumour

Table 2 Gene Therapy Advisory Committee approved clinical trials of gene therapy agents for head and neck squamous cell carcinoma in the UK

Phase	Title	Gene	Gene type	Vector	Date/status
I	Gene directed enzyme prodrug therapy for the treatment of head and neck cancer	Nitroreductase	Tumour suppressor	Adenovirus	1999 open
I	A multiple ascending dose study evaluating the safety and gene transduction into malignant cells after administration of E1A–lipid complex by intratumoral injection with unresectable or metastatic head and neck tumours	E1A	Tumour suppressor	Lipofection	Withdrawn
II	Intravenous cisplatin, 5-FU and intratumoral injection with ONYX-015 into recurrent, chemotherapy naive squamous cell tumours of the head and neck	E1B deleted	Tumour suppressor	Adenovirus	1996 closed
I	Phase I study in patients with recurrent metastatic squamous cell carcinoma of the head and neck using SCH 58500 (rAd/p53)	P53	Tumour suppressor	Adenovirus	Never initiated
II	Preoperative intratumoral injection with an E1B attenuated adenovirus in patients with resectable head and neck tumours	E1B deleted	Tumour suppressor	Adenovirus	1999 closed
I	Open-label, dose-escalation trial of intra-tumoral injection with an E1B attenuated adenovirus ONYX-015, into recurrent and locally advanced p53(-) squamous cell tumours of the head and neck	E1B deleted	Tumour suppressor	Adenovirus	1996 closed
II	Preoperative intratumoural injection with HSV1716 in patients with resectable squamous cell tumours of the head and neck	ICP34.5 deleted	Other	Herpes simplex virus	2001 open
I	First administration to man of an oncolytic herpes virus vector containing a transgene for granulocyte macrophage colony stimulating factor (Oncovex GM-CSF)—a study of its safety, biodistribution and biological activity	ICP34.5 deleted ICP47 deleted Granulocyte–macrophage colony stimulating factor (GM-CSF)	Other	Herpes simplex virus	2001 open
I	Gene therapy for squamous cell head and neck cancer that has spread to the skin	Granulocyte–macrophage colony stimulating factor (GM-CSF)	Cytokine	Herpes simplex virus	2002 open
I	A recombinant vaccinia Ankara (MVA) based vaccine encoding Epstein-Barr virus (EBV) target antigens: phase I dose escalation trial to determine immunogenicity and toxicity in patients with EBV + nasopharyngeal malignancy	Epstein-Barr virus epitopes (EBNA1 and LMP2A)	Other	DNA + MVA	2002 open

immune response.[41 42] Phase I trials of Oncovex, in conjunction with chemo- and radiotherapy, have been completed in HNSCC as well as breast, melanoma and certain gastrointestinal cancers. Phase II trials in HNSCC are planned.

Other replicating viral vectors

Some viruses have an innate tendency to replicate in cancer cells. Newcastle virus, with infectivity usually restricted to fowl, is such an example. It replicates in cells with defects in the interferon signalling pathways.[43] An oncolytic strain termed P701, administered intravenously, has undergone phase I trials in 79 patients. Four of these had HNSCC and two had oesophageal cancer; 22% of patients' tumours stopped growing, and in the case of one man with tonsillar squamous cell carcinoma there was complete remission.[44]

Vaccinia virus was originally used to immunise the population against smallpox. The family of viruses is now being used in the fight against cancer. By deleting the thymidine kinase (TK) gene they can only replicate at certain phases of the cell cycle and in cancer cells which have a constitutively high TK activity.[45] As well as this selective oncolytic mechanism, vaccinia have the ability to carry large quantities of DNA, therefore multiple genes. The combination of vaccinia virus with immune effector cells has shown promising early results.[46] There is a long history of their safety in clinical use. This is further enhanced by their ability to replicate in the cytoplasm without affecting normal host DNA.

CANCER CELL SPECIFICITY

It would seem prudent to restrict any genetic alteration to the cancerous cells. This regulation can be by physically targeting the tissue. Intratumoral injections and other mechanisms of direct gene insertion are well suited to head and neck cancers where the tissue is accessible under ultrasound guidance. Unfortunately, these direct methods are more interventional and fail to treat metastatic disease. Agents can be made to target tissue on recognition of specific surface proteins/receptors. Well differentiated tissue displays these markers, but as cancerous cells have a natural tendency to become less differentiated they may lose these distinguishing features. The specificity can also be in the DNA itself by exploiting tissue or tumour selective gene promoters/enhancers. The regulation of protein manufacture (translation) depends on sequences of DNA which oversee gene expression. The promoters can be universal to all cells (ubiquitous) or show specificity to certain tissue types (for example, PSA in prostate cells). A useful promoter sequence would be cancer cell specific, limiting the expression of the new or altered gene to cancerous tissue. Telomerase promoter is a promising avenue.[47 48] This enzyme renews the DNA repeat sequence on the end of the chromosomes. Normal adult cells do not express it and hence the DNA is destroyed after too many cell divisions to limit the chance of mutations. HNSCC are associated with telomerase expression.[49]

HNSCC GENE THERAPY TRIALS IN THE UK

There are over 1000 clinical trials of gene therapy in progress worldwide. More than 700 of these are for cancer, of which 54 are for HNSCC.[50] In the UK the Gene Therapy Advisory Committee (GTAC) has given approval for 10 clinical trials for gene therapy in HNSCC (table 2).[51]

SUMMARY

Gene therapy in HNSCC remains confined to trials but seems likely to progress to clinical use in combination with conventional modalities. Cancer of a single cell type in a single organism is heterogeneous at the molecular level. The subtyping of these malignancies is still in its infancy. When more is

Learning points

- Head and neck squamous cell cancer (HNSCC) is one of the most frequently targeted cancers for gene therapy work.
- Safety of the gene therapy agent has to be of paramount importance.
- Viral vectors can either deliver the transgene to a single cell per virus or be replication competent in which case many cells can be transfected.
- Metastatic disease has proven hard to target due to the route of administration of the gene therapy agent.
- Clinical trials have shown some very encouraging results when gene therapy is used in combination with other treatment modalities.

Key references

- Hanahan D, Weinberg R. The hallmarks of cancer. *Cell* 2000;**100**:57–70.
- Peng Z. Current status of gendicine in China: recombinant human Ad-p53 agent for treatment of cancers. *Hum Gene Ther* 2005;**16**:1016–27.
- Boelaert K, Franklyn JA. Sodium iodide symporter: a novel strategy to target breast, prostate, and other cancers? *Lancet* 2003;**361**:796–7.
- Kershaw MH, Teng MW, Smyth MJ, *et al*. Supernatural T cells: genetic modification of T cells for cancer therapy. *Nat Rev Immunol* 2005;**5**:928–40.
- Nemunaitis J, Ganly I, Khuri F, *et al*. Selective replication and oncolysis in p53 mutant tumors with ONYX-015, an E1B-55kD gene-deleted adenovirus, in patients with advanced head and neck cancer: a phase II trial. *Cancer Res* 2000;**60**:6359–66.
- Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, *et al*. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* 2000;**288**:669–72.

known, more specific gene therapies will be able to be tailored accordingly.

Administration is mainly by viral vectors injected intratumorally. If the difficulties with systemic administration could be overcome, then the possibility of treating metastatic disease would become a realistic proposition. Safety of the gene therapies is undoubtedly a primary concern and a rate limiting factor to its widespread introduction. The first ''successful'' gene therapy agent used in SCID resulted in at least two of these patients developing a leukaemic type disorder.[52 53] Trials are usually in pre-terminal cancer patients to minimise the impact of adverse effects when balanced against the potential gain.

MULTIPLE CHOICE QUESTIONS (TRUE (T)/FALSE (F); ANSWERS AFTER THE REFERENCES)

(1) The main limiting factor for cancer gene therapy is selecting the correct gene

(2) There are no commercially available gene therapy agents for head and neck squamous cell carcinoma

(3) Most gene therapy agents are based around an adenoviral vector

(4) Most gene therapy agents are administered intravenously

(5) p53 repairs damaged genetic material

Authors' affiliations
E Chisholm*, U Bapat*, G Vassaux, Molecular Oncology, Cancer Research UK, Barts and The London NHS Trust, London, UK
G Alusi, Department ENT, Barts and The London NHS Trust, London, UK
C Chisholm, Department of Clinical Oncology, Charing Cross Hospital, Hammersmith, London, UK

*Also Department ENT, Barts and The London NHS Trust, London, UK

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REFERENCES

1 **British Association of Otorhinolaryngologists and Head and Neck Surgeons**. *Effective head and neck cancer management 3rd consensus document*. London: BAOHNS, 2003.
2 **Office for National Statistics**. *Cancer statistics registrations, Registrations of cancer diagnosed in 2002, England*. London: HMSO.
3 **Kowalski LP**, Carvalho AL. Natural history of untreated head and neck cancer. *J Cancer* 2000;**36**:1032–7.
4 **Coleman MP**, Rachet B, Woods LM, *et al*. Trends and socioeconomic inequalities in cancer survival in England and Wales up to 2001. *Br J Cancer* 2004;**90**:1367–73.
5 **Ries L**, Eisner M, Kosary C, *et al. SEER cancer statistics review, 1975–2000*. SEER, 2003.
6 **Department of Health**. Our inheritance, our future – realising the potential of genetics in the NHS. Genetics White Paper. London: DoH, 2003: chapter 1.25.
7 **Harrington KJ**, Nutting CM, Pandha HS. Gene therapy for head and neck cancer. *Cancer Metastasis Rev* 2005;**24**:147–64.
8 **Hanahan D**, Weinberg R. The hallmarks of cancer. *Cell* 2000;**100**:57–70.
9 **Harrington K**, Bateman A, Melcher A, *et al*. Cancer gene therapy: Part 2. Candidate transgenes and their clinical development. *Clin Oncol (R Coll Radiol)* 2002;**14**:148–69.
10 **Kato K**, Yamashita R, Matoba R, *et al*. Cancer gene expression database (CGED): a database for gene expression profiling with accompanying clinical information of human cancer tissues. *Nucleic Acids Res* 2005;**33**:D533–6.
11 **Futreal A**, Coin L, Marshall M, *et al*. A census of human cancer genes. *Nat Rev Cancer* 2004;**4**:177–83.
12 **Schwartz D**, Rotter V. p53- dependent cell cycle control: response to genotoxic stress. *Semin Cancer Biol* 1998;**8**:325–36.
13 **Clayman GL**, el-Naggar AK, Lippman SM, *et al*. Adenovirus-mediated p53 gene transfer in patients with advanced recurrent head and neck squamous cell carcinoma. *J Clin Oncol* 1998;**16**:2221–32.
14 **Han DM**, Huang ZG, Zhang W, *et al*. [Effectiveness of recombinant adenovirus p53 injection on laryngeal cancer: phase I clinical trial and follow up]. *Zhonghua Yi Xue Za Zhi* 2003;**83**:2029–32.
15 **Peng Z**. Current status of gendicine in China: recombinant human Ad-p53 agent for treatment of cancers. *Hum Gene Ther* 2005;**16**:1016–27.
16 **Zhang SW**, Xiao SW, Liu CQ, *et al*. [Treatment of head and neck squamous cell carcinoma by recombinant adenovirus-p53 combined with radiotherapy: a phase II clinical trial of 42 cases]. *Zhonghua Yi Xue Za Zhi* 2003;**83**:2023–8.
17 **Chen CB**, Pan JJ, Xu LY. [Recombinant adenovirus p53 agent injection combined with radiotherapy in treatment of nasopharyngeal carcinoma: a phase II clinical trial]. *Zhonghua Yi Xue Za Zhi* 2003;**83**:2033–5.
18 **McWilliams JE**, Evans AJ, Beer TM, *et al*. Genetic polymorphisms in head and neck cancer risk. *Head Neck* 2000;**22**:609–17.
19 **Searle PF**, Chen MJ, Hu L, *et al*. Nitroreductase: a prodrug-activating enzyme for cancer gene therapy. *Clin Exp Pharmacol Physiol* 2004;**31**:811–6.
20 **Zaboikin M**, Srinivasakumar N, Schuening F. Gene therapy with drug resistance genes. *Cancer Gene Ther* 2006;**13**:335–45.
21 **Boelaert K**, Franklyn JA. Sodium iodide symporter: a novel strategy to target breast, prostate, and other cancers? *Lancet* 2003;**361**:796–7.
22 **Kershaw MH**, Teng MW, Smyth MJ, *et al*. Supernatural T cells: genetic modification of T cells for cancer therapy. *Nat Rev Immunol* 2005;**5**:928–40.
23 **Groot-Wassink T**, Aboagye EO, Glaser M, *et al*. Adenovirus biodistribution and noninvasive imaging of gene expression in vivo by positron emission tomography using human sodium/iodide symporter as reporter gene. *Human Gene Ther* 2002;**13**:1723–35.
24 **Groot-Wassink T**, Barthel H, Lemoine N, *et al*. Sodium iodide symporter: a new strategy to target cancer? *Lancet* 2003;**361**:1905–6.
25 **Vile R**, Russell S, Lemoine NR. Cancer gene therapy: hard lessons and new courses. *Gene Ther* 2000;**7**:2–8.
26 **Curiel D**, Douglas J. *Cancer gene therapy*. New Jersey: Humana Press, 2005.
27 **Nishikawa M**, Huang L. Nonviral vectors in the new millenium. *Human Gene Ther* 2001;**12**:861–70.
28 **Niidome T**, Huang L. Gene therapy progress and prospects: nonviral vectors. *Gene Ther* 2002;**9**:1647–52.

29 **Dufès C**, Nicol Keith W, Bisland A, *et al.* Synthetic anticancer medicine exploits intrinsic antitumour activity of cationic vector to cure established tumours. *Cancer Res* 2005;**65**:8079–84.
30 **Cartier R**, Reszka R. Utilization of synthetic peptides containing nuclear localization signals for nonviral gene transfer systems. *Human Gene Ther* 2002;**9**:157–67.
31 **Dock G**. Influence of complicating disease on leukaemia. *J Med Sci* 1904;**127**:563–92.
32 **Thomas C**, Ehrhardt A, Kay M. Progress and problems with the use of viral vectors for gene therapy. *Nat Rev Genet* 2003;**4**:346–58.
33 **Hacein-Bey-Abina S**, Von Kalle C, Schmidt M, *et al.* LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 2003;**302**:415–9.
34 **Freeman SM**, Abboud CN, Whartenby KA, *et al.* The ''bystander effect'': tumor regression when a fraction of the tumor mass is genetically modified. *Cancer Res* 1993;**53**:5274–83.
35 **Lin E**, Nemunaitis J. Oncolytic viral therapies. *Cancer Gene Ther* 2004;**11**:643–64.
36 **Ganly I**, Kirn D, Eckhardt G, *et al.* A phase I study of Onyx-015, an E1B attenuated adenovirus, administered intratumorally to patients with recurrent head and neck cancer. *Clin Cancer Res* 2000;**6**:798–806.
37 **Nemunaitis J**, Ganly I, Khuri F, *et al.* Selective replication and oncolysis in p53 mutant tumors with ONYX-015, an E1B-55kD gene-deleted adenovirus, in patients with advanced head and neck cancer: a phase II trial. *Cancer Res* 2000;**60**:6359–66.
38 **Nemunaitis J**, Khuri F, Ganly I, *et al.* Phase II trial of intratumoral administration of ONYX-015, a replication-selective adenovirus, in patients with refractory head and neck cancer. *J Clin Oncol* 2001;**19**:289–98.
39 **Khuri FR**, Nemunaitis J, Ganly I, *et al.* A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nat Med* 2000;**6**:879–85.
40 **Varghese S**, Rabkin SD. Oncolytic herpes simplex virus vectors for cancer virotherapy. *Cancer Gene Ther* 2002;**9**:967–78.
41 **Hu J**, McNeish I, Shorrock C, *et al.* A phase I clinical trial with OncoVEX$^{GM\text{-}CSF}$. *Mol Ther* 2003;**7**(suppl):447.
42 **Liu BL**, Robinson M, Han ZQ, *et al.* ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. *Gene Ther* 2003;**10**:292–303.
43 **Stojdl DF**, Lichty B, Knowles S, *et al.* Exploiting tumor-specific defects in the interferon pathway with a previously unknown oncolytic virus. *Nat Med* 2000;**6**:821–5.
44 **Pecora AL**, Rizvi N, Cohen GI, *et al.* Phase I trial of intravenous administration of PV701, an oncolytic virus, in patients with advanced solid cancers. *J Clin Oncol* 2002;**20**:2251–66.
45 **Hengstschlager M**, Knofler M, Mullner EW, *et al.* Different regulation of thymidine kinase during the cell cycle of normal versus DNA tumor virus-transformed cells. *J Biol Chem* 1994;**269**:13836–42.
46 **Thorne SH**, Negrin RS, Contag CH. Synergistic antitumor effects of immune cell-viral biotherapy. *Science* 2006;**311**:1780–4.
47 **Groot-Wassink T**, Aboagye E, Wang Y, *et al.* Noninvasive imaging of the transcriptional activites of human telomerase promoter fragments in mice. *Cancer Res* 2004;**64**:4906–11.
48 **Keith WN**, Bilsland A, Hardie M, *et al.* Drug insight: cancer cell immortality-telomerase as a target for novel cancer gene therapies. *Nat Clin Pract Oncol* 2004;**1**:88–96.
49 **Patel MM**, Parekh LJ, Jha FP, *et al.* Clinical usefulness of telomerase activation and telomere length in head and neck cancer. *Head Neck* 2002;**24**:1060–7.
50 **Edelstein ML**, Abedi MR, Wixon J, *et al.* Gene therapy clinical trials worldwide 1989–2004 – an overview. *J Gene Med* 2004;**6**:597–602.
51 **Department of Health**. http://www.advisorybodies.doh.gov.uk/genetics/gtac/.
52 **Cavazzana-Calvo M**, Hacein-Bey S, de Saint Basile G, *et al.* Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* 2000;**288**:669–72.
53 **Hacein-Bey-Abina S**, von Kalle C, Schmidt M, *et al.* A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med* 2003;**348**:255–6.

ANSWERS

(1) F. The limiting factor is the vector
(2) F. Gendicine in China
(3) T.
(4) F. Intra-tumorally
(5) F. It triggers cell death if the cell's genetic material is damaged