



Gene Therapy

This Factsheet will describe in simple terms the process of gene therapy and review some of the most serious conditions which may be treated by this method in future. Gene therapy can also involve treatment which leads to the body correcting its own gene mutation.

- Genes are long sequences of nucleotide bases on DNA
- They are the instructions which a cell needs to make proteins
- Proteins may be structural or they may be enzymes
- In some people, due to mutation, the genes are missing or don't work properly
- Hence these people suffer disease or impaired health

Definition of gene therapy

Introduction of genes into an individual with abnormal genes; so allowing the individual to produce proteins which otherwise they would not be able to produce. The principle of the process is very simple:

1. Take some cells from an individual
2. Add genes to the cells
3. Replace the cells (i.e. put the cells back into the individual)
4. These cells can now produce the proteins the individual needs

Cells contain all the genetic information – in the form of genes on chromosomes – of an individual. Genes work by coding for protein production – they are essentially the instructions for making proteins.

Non-reproductive cells are used, i.e. not the sperm or eggs, since it is essential that the altered cells cannot be inherited/passed to the next generation. For this reason, blood cells, liver cells, skin cells or bone marrow cells are used.

In practice, things are a bit more complicated.

1. Extracting the cells from the individual

This is relatively easy using a biopsy syringe under local anaesthetic.

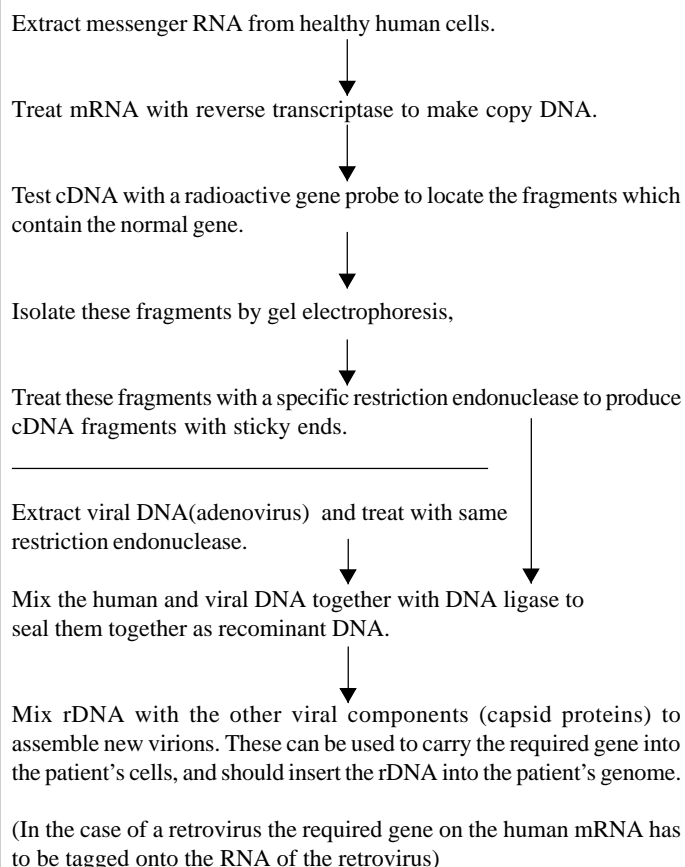
2. Getting the gene into the patient's cells

The genes are carried into the cells using a vector. The vector is often a **retrovirus**, which contains RNA. Before the virus is allowed to enter the cells, it is made harmless – this may involve cutting out some of the genes in the virus. Once inside the patient's cells, the retrovirus's RNA is used as a template to make DNA. This viral DNA then becomes inserted into the patient's DNA.

In making the vector retrovirus safe, the virus is unable to enter the human cells by itself. Thus the vector virus is mixed with normal (harmless) retroviruses, called helper viruses. These are able to penetrate the membrane of the host cell, allowing the vector virus to enter too. The helper virus then supplies genes for the replication of the vector virus. Millions of vector viruses and helper viruses are now replicated in the host cells. Fig 1 illustrates the way in which a normal gene is inserted into the viral vector.

Modified viruses, or cells containing the modified viruses, or liposomes (microscopic fat droplets) containing the modified viruses, can be introduced into the patient via a nasal spray or by injection.

Fig 1. Insertion of the normal gene into a viral vector



Problems

1. Insertion of new gene may disrupt another normal host gene.
2. The viruses may cause toxic side effects in the patient.
In September 1999 the first patient died as a result of gene therapy. The patient had a disorder that made his liver unable to break down ammonia. Huge doses of adenoviruses were used to deliver the necessary gene, but the patient suffered liver failure.

Exam Hint - Candidates may be asked to apply their knowledge of recombinant DNA techniques in the treatment methods for cystic fibrosis and α -1 antitrypsin deficiency, and also the use of transgenic organisms in the treatment of these diseases.

Alpha-1 antitrypsin deficiency

In this condition the gene coding for the enzyme alpha-1 antitrypsin mutates so that the liver cells cannot make the enzyme. The function of the enzyme is to destroy any unwanted protease enzymes in the tissues. If the enzyme is missing then body tissues are damaged by protease activity. This particularly affects lung tissue, leading to emphysema, and liver tissue, leading to cirrhosis. The disease can be treated in two ways:

1. By dosage with alpha-1 antitrypsin using a nasal spray. The enzyme is produced by transgenic sheep by the technique outlined in Fig 2.
2. By gene therapy. In this case the vectors used are liposomes or adenoviruses (common cold), both of which can be sprayed into the lungs via nasal sprays, or retroviruses which are grown in cultures of the patient's liver cells and then implanted back into the liver.

Advantages and problems

- Implanting the normal gene into a transgenic sheep which then produces enough alpha-1 antitrypsin to be able to directly treat patients means that patients do not have to suffer invasive techniques of viral or liposome delivery or give tissue samples for culture.
- Adenoviruses tend to cause toxic side effects but are very efficient at delivering the gene into the host genome.
- Liposomes have very low toxicity and are easily produced, but are not very efficient in delivering the gene into the host cells.
- Retroviruses need actively dividing cells to infect. Liver cells and lung cells tend to be mature or 'terminally differentiated'. They can only be infected with retroviruses if the cells have been induced to divide in tissue culture.

Cystic Fibrosis

Cystic fibrosis sufferers lack the gene which produces a transport protein in cell membranes. The transport protein is responsible for transporting Cl^- ions out of cells. Cystic fibrosis sufferers cannot produce this transport protein, so Cl^- ions accumulate in their cells. These ions draw water into the cells and leave behind a sticky mucus in the body's passageways, especially the airways. These areas of mucus build-up become infected. Cystic fibrosis sufferers die from respiratory tract and lung infections. There are two commonly used techniques for getting the normal genes into the patient's cells.

- (a) Normal genes are placed in liposomes which are sprayed through the nose into the lungs and taken up by the lung cells.

Problems

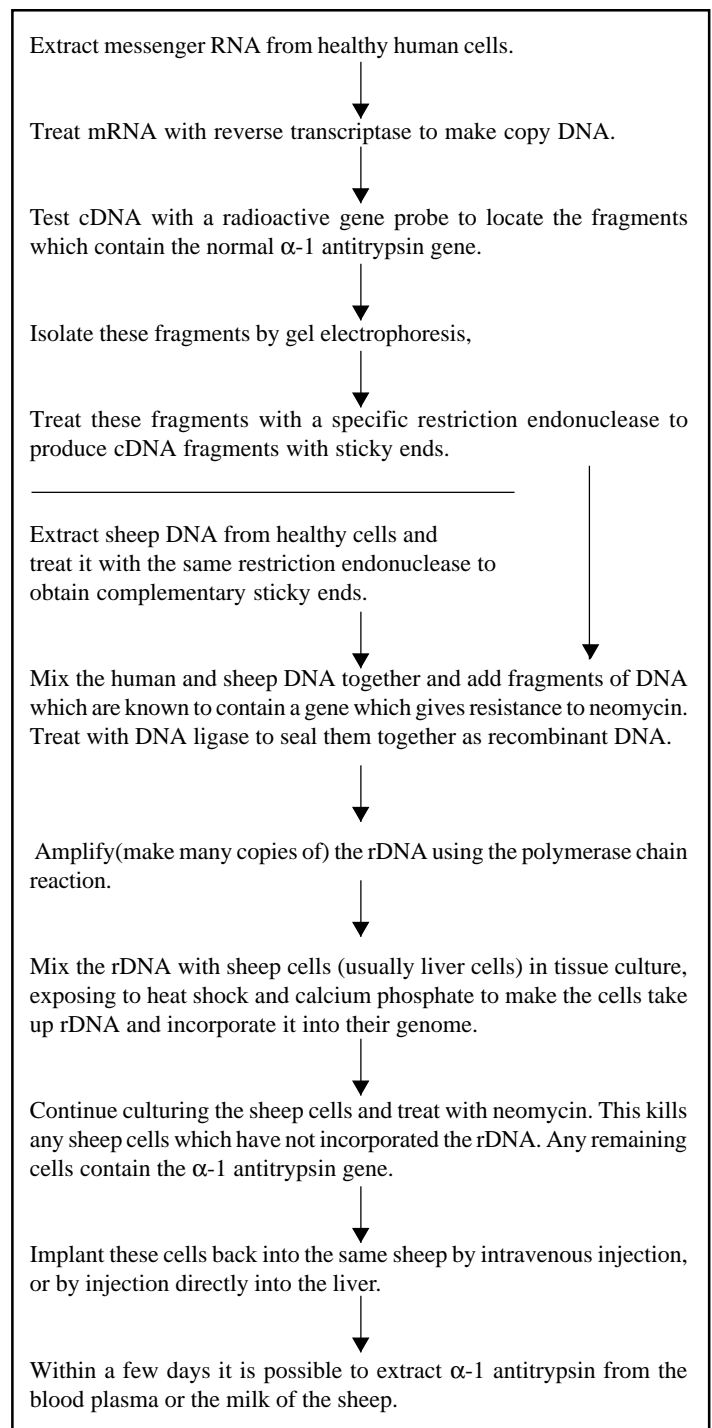
- Fusion to lung cells is not always successful.
- Beneficial effects temporary.
- Some liposomes toxic to some cells.

However, one advantage is that liposomes protect the DNA and can carry large pieces of DNA.

- (b) 1. Normal genes are inserted into adenoviruses.
 2. Adenoviruses are treated so that they cannot replicate.
 3. Adenoviruses are sprayed into nasal passages and carry gene into the lung cells.

Problem

- Adenoviruses tend to have toxic side effects.

Fig 2. Production of α -1 antitrypsin using transgenic sheep**Other examples of gene therapy****Thalassaemia**

Symptoms – inability to synthesis haemoglobin normally.

1. Normal gene for haemoglobin synthesis is inserted into a virus.
2. The virus is inserted into some of the patient's bone marrow cells.
3. Screening shows which of the marrow cells have taken up the virus.
4. Cells which have taken the normal genes up are returned to the patient.

Haemophilia

Sufferers have a mutation on a single nucleotide. A single DNA base in the gene which codes for one of the clotting proteins is altered. This means that the patient's liver cells cannot make the protein which is essential for blood clotting. Scientists have now developed a molecule, which can be injected intravenously, which travels to the liver cells and corrects the mutation.

The molecule is a fragment of genetic material known as a "chimeric RNA/DNA oligonucleotide". The technique is known as chimeroplasty, but the advantages of the technique are that the effects are permanent and cannot be inherited.

Anti-Cancer Therapy

The inserted gene produces tumour necrosis factor (TNF). TNF is a protein produced by human white blood cells which normally wander round the body destroying tumours – they are therefore known as tumour-infiltrating lymphocytes (TILs).

Practice Questions

1 (a) Define the term gene therapy. (2 marks)

(b) State the function of the following in gene therapy:

(i) vectors. (2 marks)

(ii) adenovirus. (1 mark)

2. The two commonest hereditary lung diseases in individuals of European descent are alpha-1 antitrypsin deficiency and cystic fibrosis. Both of the human genes involved have been cloned and gene therapy is of potential use in the treatment of both diseases.

Cystic fibrosis is due to a mutation in the CFTR (cystic fibrosis transmembrane conductance regulator) gene that codes for a chloride channel protein in the cell membranes of epithelial cells. This protein regulates the secretion of chloride ions from the epithelial cells. If the secretion of chloride ions is reduced then the membrane resting potentials are raised, the surfaces are inadequately moistened with tissue fluid and mucus accumulates. This becomes infected and inflammation occurs. The symptoms affect the lungs, liver, gastrointestinal tract and pancreas. Since 90% of deaths due to cystic fibrosis are due to respiratory failure, gene therapy has focussed on correcting the genetic defect in the lungs.

(a) (i) What is meant by the underlined phrase 'the human genes involved have been cloned'? (2 marks)

(ii) List the sequence of steps involved in cloning, naming any enzymes that are used. (5 marks)

(b) In gene therapy the cloned genes must be delivered to the body organs that need them. In the case of cystic fibrosis this is the lungs. The genes must be inserted into suitable vectors for delivery.

(i) Suggest two suitable vectors that are used to transport cloned genes to lung cells. (2 marks)

(ii) Suggest two ways to introduce the vectors to the lungs. (2 marks)

(c) Alpha-1 antitrypsin is an enzyme that destroys protease enzymes in body tissues. It is manufactured by the liver cells but its most important site of operation is in the lungs. If alpha-1 antitrypsin is deficient then lung tissue is damaged by protease activity and hereditary emphysema develops.

In gene therapy of this disease which would be the target organ for the gene vectors? Give a reason for your answer. (2 marks)

2. (a) (i) the genes have been isolated and inserted into recombinant DNA;

Answers

Semicolons indicate marking points.

- 1 (a) Introduction of normal genes into an individual with abnormal/mutant genes;
so allowing the production of the missing protein;
- (b) (i) normal gene can be spliced into the vector;
by recombinant DNA technology;
carries the gene into the cells;
- (ii) acts as a vector/carries the normal gene into the patients cells;
and multiplied many times to produce identical copies;
- (ii) DNA is copied from human RNA using reverse transcriptase;
treated with restriction endonuclease to produce DNA fragments with sticky ends;
bacterial plasmid /viral DNA treated with same restriction endonuclease to produce DNA fragments with sticky ends;
DNA fragments mixed together and sealed to make recombinant DNA using DNA ligase;
many copies made by polymerase chain reaction/amplification;
- (b) (i) viruses/adenoviruses/retroviruses;
liposomes/plasmid-liposome complexes;
- (ii) using an aerosol/inhaler;
intravenous injection;
- (c) liver;
this is where the gene operates to make alpha-1 antitrypsin;

Acknowledgements;

This Factsheet was researched and written by Kevin Byrne and Martin Griffin Curriculum Press, Unit 305B, The Big Peg, 120 Vyse Street, Birmingham. B18 6NF

Bio Factsheets may be copied free of charge by teaching staff or students, provided that their school is a registered subscriber.

No part of these Factsheets may be reproduced, stored in a retrieval system, or transmitted, in any other form or by any other means, without the prior permission of the publisher.

ISSN 1351-5136