Gene therapy and cancer

Science, Genetics

Abstract:

Gene therapy is the introduction of normal genes into cells in place of missing or defective ones in which lead to disease development. There are two overarching forms of gene therapy; Germ Line which results in permanent change and elimination of disease inheritance and Somatic gene therapy which is more targeted. In this EPQ the therapeutic avenue investigated will be Somatic hematopoietic stem cell (HSC) gene therapy, in particular Ex Vivo therapy where the cells are modified outside the body then inserted. The most common type of gene therapy is replacing a mutated/ abnormal gene that causes disease with a healthy copy of the gene, other approaches include inactivating a mutated gene that isn't functioning properly or introducing a new gene into the body to fight disease. In a world where the role of genetics in the most debilitating conditions is being further clarified, gene therapy is being hailed as a cure for many previously incurable genetic defects.

Process of gene therapy:

The process of Somatic, Ex Vivo gene therapy begins with isolating which is the abnormal/ defective gene that needs to be corrected. A plasmid is then created with the correct form of the gene within it and its promoter. A promotor is a region of DNA that initiates the transcription of a particular gene. Promoters are located near the transcription start sites of genes, on the same strand and upstream on the DNA. On a plasmid they are a sequence that interacts with transcription machinery in order to ' trigger' the expression of the chosen gene. This plasmid is taken up by a virus (usually a retroviral which are a group of RNA viruses which insert a DNA copy of their

genome into the nucleus of a host cell in order to replicate, such as an inactivated form of Human Immunodeficiency Virus/ HIV). The most effective retroviral vectors are lentivirals which have a long incubation period and able to deliver large amounts of genetic information into the DNA of their host cell. Examples of lentiviruses include HIV, Simian Immunodeficiency Virus, and Feline Immunodeficiency Virus. (1) The altered virus is mixed with the patient's cells (in this case stem cells) and the stem cells become genetically altered as they are transfected by the virus. The stem cells are returned to the patient and begin to replicate- these replications contain the corrected gene.

Previous successes:

The above method of Somatic ex vivo gene therapy has been successful in treating a form of immuno- deficiency disease called Severe Combined Immuno- Deficiency (SCID). SCID is caused by inherited genetic abnormalities resulting in reduced or malfunctioning T- and B-lymphocytes, the specialized white blood cells which fight infection. This debilitates the affected patient's immune system making them highly susceptible to disease and severe long-lasting infection. Gene therapy routes are becoming mainstream as a way of treating a particular form of SCID, adenosine deaminase or ADA SCID. ADA SCID is caused by mutations in the ADA gene resulting in a deficiency of the enzyme adenosine deaminase which is vital to the functioning of lymphocytes. (4) Adenosine deaminase removes a molecule generated when DNA is broken down called deoxyadenosine. Deoxyadenosine is toxic to lymphocytes in high levels so adenosine deaminase breaks it down to form another molecule called deoxyinosine that

isn’t harmful. Mutations in the ADA gene stop or impair activity of adenosine deaminase, resulting in the build-up of deoxyadenosine to levels that are toxic to lymphocytes. In this case, the lymphoces- both b cells and t cells, are then destroyed and therefore the body is unable to fight infection other than through phagocytes. Gene therapy is used to insert a working form of the ADA gene thereby preventing the destruction of lymphocytes. This is done by introducing the correct form of the ADA gene via a plasmid into a viral vector. (5) The patient undergoes chemotherapy to kill any of the previous immune system and their own stem cells are collected before being transfected by the virus containing the correct ADA gene.

The stem cells are replaced and begin to undergo the differentiation process; lymphocytes (both t and b cells) are created at the correct levels and work at the needed activity meaning that the immune system is now fully functional. (6) (7)Use for leukaemia through CAR t-cell modification and Immunotherapy: Gene therapy is already being used as a therapeutic avenue for cancer through CAR t-cell modification.

An expanding area of cancer research is into Immunotherapy; using and enhancing the body’s own immune system to eradicate the cancer. Cancers have methods of circumventing the immune response, however, with recent developments in gene therapy this can be rectified. This has seen the rise of a potentially revolutionary therapy for leukaemia; CAR t-cell modification which is where the gene therapy process of transfection is utilised to implant the patient’s own t-cells produce specialised receptor on their surface called a Chimeric Antigen Receptor or ‘ CAR’. These receptors are complementary

to a unique antibody expressed on the surface of tumour cells called CD19 antibodies. In this way the t-cell is better able to recognise and attach to the previously undetected tumour cells allowing the immune system to kill the tumour cells either through the t-cells themselves or through the other available immune responses. The patients must initially give blood so their own lymphocytes can be modified (to reduce the possibility of the modified cells being rejected by the body's immune system). The CAR receptors are added to the t-cells but before being administered to the patient the patient must be Lymphodepleted allowing the engineered cells to multiply inside the body. This technique is used in the patients of ALL (Acute Lymphoblastic Leukaemia) which has gone into remission which is the leading cause of childhood death from cancer. In a recent trial, 27/30 trial patients showed a complete response with many of them continuing to show no signs of cancer after the treatment. (10)

Apoptosis and cancer:

The definition of apoptosis is ' the death of cells which occurs as a normal and controlled part of an organism's growth or development'. Apoptosis occurs as a way of removing unnecessary cells and to regulate cell numbers. (11) The other method by which cells die occurs when a cell dies due to injury. In this event the cell swells and bursts, endangering other healthy cells by invoking a potentially damaging inflammatory response. (12) By contrast when a cell dies by apoptosis, its organelles are neatly internally digested/ disassembled and none of the harmful contents damages the surrounding cells. Most importantly, the membrane is altered during this process allowing the cell to be phagocytosed either by a neighbouring cell or

a macrophage (type of specialised phagocytic cell) as part of the immune response. This not only prevents the harmful inflammatory response caused by necrosis but also allows its organelles to be recycled by the surrounding cells. In adult tissues, cell death exactly balances cell division- or else the tissue would grow or shrink. This is the basic stimulus for the idea of inducing apoptosis in cancerous cells in order to eradicate the cancer. The process of apoptosis is highly complex; it begins with the gene BAX. The intrinsic pathway of apoptosis works on the basis of permeabilization of the outer mitochondrial membrane by proapoptotic proteins such as Bax. This happens by the BAX gene coding for the protein apoptosis regulator BAX which accelerates programmed cell death by binding to, and antagonizing the apoptosis repressor BCL2 among others, all potent apoptosis inhibitors. Bcl-2 family proteins regulate a critical step in apoptosis by controlling mitochondrial permeability and function. (13) (18) They do this through inhibition of cytochromec release while Bax directly promoted cytochromec release from isolated mitochondria.

The release of Cytochrome c, (a large transmembrane protein complex found in the mitochondrion of eukaryotes), is a vital step in apoptosis. By releasing from mitochondria to the cytosol, it activates a caspase cascade. Caspases are a family of protease enzymes playing essential roles in programmed cell death (including apoptosis, pyroptosis and necroptosis) and inflammation. Lysosomes also become permeabilised releasing their digestive enzymes- essentially phagocytosing the cell. (14) This is the point at which the cell is committed to the death process. It is thought that that the release of cytochrome c is caused by a swelling of the mitochondrial matrix triggered

by the apoptotic stimuli. Therefore, as Bcl-2 proteins inhibit this release of cytochrome c, they inhibit apoptosis and as Bax promotes release of cytochrome c, it encourages apoptosis. (16) Bcl-2 proteins in their viral form, (coded for by several DNA viruses like herpesviruses, adenoviruses, and others as opposed to being coded for by genes), can be used by diseases- most relevantly cancers- to evade apoptosis. In this way, Bcl-2 proteins are a key way in which the unnatural proliferation of cells can occur unchecked by the usual system which should end in apoptosis. (17) By escaping cellular regulatory mechanisms, viruses can ensure replication and propagation in the infected host and cancers and other disorders are associated with viruses that encode Bcl-2 homologs.

Under stress conditions and induced by environmental factors, BAX undergoes a conformation change which is achange in the shape of a macromolecule. That causes translocation to the mitochondrion membrane, leading to the release of cytochrome c into the cytosol and triggers programmed cell death through apoptosis.

BAX promotes activation of caspases: protease enzymes, and thereby apoptosis as the caspases digest the cells organelles and rupture the cell membrane, allowing the cell to be phagocytosed by a neighbouring cell or a macrophage. (19) (22)A genetic knockout approach demonstrated that Bax and Bak are downstream of BH3-only molecules in the programmed cell death cascade and essential for apoptosis in response to multiple death stimuli. Although some data support a role for certain BH3-only proteins, such as Bim or tBid, to directly activate Bax, others have led to the

conclusion that BH3-only proteins act indirectly by antagonizing the pro-survival Bcl-2 proteins, thereby allowing Bax activation to proceed. (19) (20) (21) (22) Con-current research on apoptosis induction: Gossypol is a drug derived from a natural phenol found in the cotton plant, traditionally used as contraception. Studies are currently being undertaken which are investigating Gossypol's effectiveness as a chemotherapeutic agent by inducing apoptosis in some cancer cell lines, particularly with regard to Prostate cancer. Exposure of these cell lines to Gossypol resulted in the activation of 13 proteins, 7 transcription factors, and expression of 17 genes involved in the mitochondrial pathway of apoptosis. These studies demonstrate for the first time that gossypol treatment induces apoptosis and activates p53, the gene for tumour suppression. (23)

In a recent study into Gossypol's effect on breast cancer, it was found that Gossypol provides two essential functions with regard to apoptosis and tumour suppression. It does this through the inhibition of two vital ways in which the tumour survives. Firstly, Gossypol inhibits MDM2 which is a proto-oncogene which promotes tumour formation by selectively targeting tumour suppression proteins. When inhibited, these proteins are able to survive and work to suppress further growth. Gossypol also inhibits VEGF (Vascular endothelial growth Factor) which mediates angiogenesis in cancer. Angiogenesis is the formation of vascular tissue around the tumour which allows it to gain nutrients from the blood supply and prevents the tumour from becoming hypoxic, allowing it to grow to more than two millimetres in size. (24)Through disrupting the interactions between MDM2 proteins and VEGF mRNA gossypol proved to have anti-apoptotic and anti-angiogenesis

functions. However, though effective Gossypol remains a chemotherapeutic agent which has the effect of poisoning the whole body rather than just the cancer. It also does not eliminate the possibility of a remission/ relapse. PH+ acute lymphoblastic Leukaemia (PH+ ALL): Acute lymphoblastic leukaemia (ALL) is a type of blood cancer that starts from young white blood cells called lymphocytes in the bone marrow. In ALL, large numbers of immature white blood cells are released before they are ready known as blast cells.

As the number of blast cells increases, the number of red blood cells and platelet cells decreases resulting in the symptoms of anaemia like tiredness, breathlessness and an increased risk of excessive bleeding. Vulnerability to infection also increases as the underdeveloped blast cells are less effective at fighting off infection than mature lymphocytes. Adults and children can get it but it is most often diagnosed in younger people. It is aggressive and progresses rapidly requiring immediate treatment. Chemotherapy and a marrow transplant are the most effective treatments currently. Philadelphia positive acute lymphoblastic leukaemia is a type of acute lymphoblastic leukaemia where the Philadelphia chromosome is present making it much harder to treat with chemotherapy and the treatment often results in remission anyway. (25) It occurs in adult leukaemia patients with ALL, approximately 20% to 30% of patients but only in about 5% of children with ALL. The incidence of PH+ rises with age and it occurs roughly 50% of times in patients 50 years or older. (26) It is caused by a mutation in which the ABL1 gene of chromosome 9 and the BCR gene of chromosome 22 become fused during cell division to form a new chromosome called the Philadelphia chromosome. This swapping of genetic material is called chromosomal

translocation and creates what is known as a chimeric chromosome. (27)Induced apoptosis through gene therapy: It is believed that a valid therapeutic treatment avenue for cancer can be found through the utilization of apoptosis. (28) Studies into this have shown positive results; in a 2003 study due to elevated expression of Plk1 in many cancer types, Plk1 was proposed as a diagnostic marker for several tumours.

The previously mentioned vector/ plasmid-based gene therapy technique was used to specifically deplete Plk1 in cancer cells. It was found that Plk1 depletion dramatically inhibited cell proliferation- essentially halting the cell cycle by preventing the separation of sister chromatids at anaphase. Plk1 depletion therefore induced apoptosis, as indicated by the presence of activated caspase 3 proteins, and the formation of fragmented nuclei which are a result of apoptosis. The data from this study therefore strongly supports the idea that apoptosis could be used as a cancer therapy. (29) As previously stated apoptosis is the natural pathway of death for a cell and can be triggered intrinsically through expression of the gene Human apoptosis BAX. One area of cancer, and in particular Leukaemia in which I think apoptosis induction would be easiest to apply and most effective is in Philadelphia chromosome positive acute lymphoblastic Leukaemia. The first stage would be reverse engineering a plasmid to contain the gene Bid which expresses to form BH3-only proteins. These proteins are needed because cancerous cells are excellent at evading apoptosis and one of the ways in which they do this is by overexpressing a type of anti-apoptic protein called BCl-2 proteins. BCl-2 inhibits the activation of the BAX gene therefore preventing the natural pathway of apoptosis to occur. BH3-only proteins

inhibit the action of Bcl2, thereby allowing BAX to be triggered and the cell to undergo apoptosis.

The plasmid therefore must have the gene Bid which expresses BH3-only proteins and upstream of that a promoter. This promoter will be triggered/ activated in and by the presence of the Philadelphia Chromosome- using the BCR-ABL as a therapeutic target (the chimeric chromosome as a result of a single point mutation which causes ALL leukaemia- see above paragraph.) (30) Therefore, the idea is that the BH3 only proteins will be synthesised only in leukemic cells as they will be the only ones able to trigger the promoter and so they will be the only ones to undergo apoptosis, removing one of the main issues of cancer which is how to kill the cancerous cells without killing all the healthy cells as well.

A benefit to this method of induced apoptosis as opposed to CAR t-cell modification is that it theoretically would prevent remission as the transfected marrow would continue to produce virally transfected stem cells throughout the patient’s life. In this way, every time the ALL went into remission (which not only is there a high chance of happening in ALL but also it is almost always fatal), the differentiating stem cells would identify and kill it before any symptoms were to show or harm come to the patient. Another possibility with merit is in the plasmid, promote a molecule that destroys the BCR-ABL proteins which are the way that the BH3 proteins are inhibited. In this way, there would not be such a reliance on the overproduction of BH3 from the plasmid to overcome the inhibition of the BCR-ABL proteins.

Experimental evaluation:

To accompany and back up the work I did on this project, I conducted an experiment where I genetically engineered a plasmid to fluoresce. Overall, it took around 30+ hours in the lab but in the end the experiment was a success; the bacteria were genetically modified to express the GFP gene and they glowed when UV light was shone upon them.

However, the process was not without fault as I suffered many setbacks; the entire experiment had to be redone after the bacteria were left to incubate for too long meaning that weeks of work had to be revised. After overcoming this obstacle and starting from the beginning again, the experiment was completed and was a success. The whole process not only aided my understanding of the intricacies of gene therapy, but it taught me much about the scientific process and how to safely and logically conduct a complicated, extended investigation. To review the methodology, see reference 28 at end of document.