GENE THERAPY, AN AID TO CURING CANCER?

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ABSTRACT

Gene therapy is the use of gene silencing and gene manipulation to overcome genetic diseases. In this paper I am going to look at ways in which gene silencing could be used to solve diseases such as cancer. The background to my work is an interest in this area of biology and therefore further research into it. This has meant that my central idea has formed around gene silencing, the methods and problems which could be solved in the future using this method, following the process of genes to proteins, DNA transcription and translation to protein and the problems this causes for a disease sufferer.

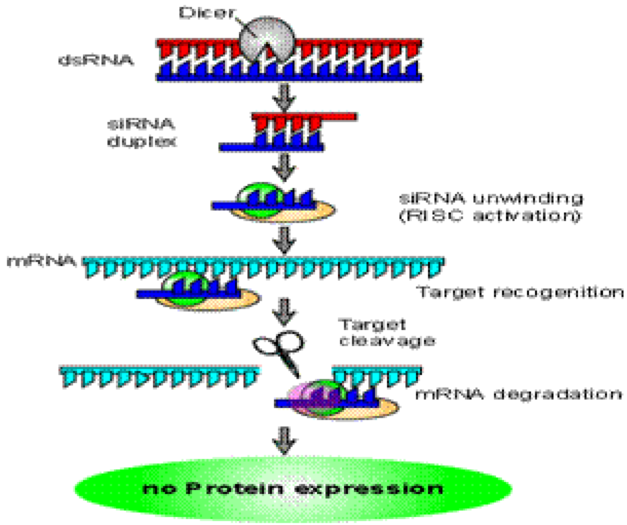
INTRODUCTION

The long double strand of DNA of a chromosome carries a linear sequence of many genes, the units of inheritance. DNA stands for Deoxyribonucleic acid. This is a polymer of nucleotide molecules that form the instructions for the synthesis of proteins found within organisms. These nucleotides are the monomers of all nucleic acids. Each nucleotide is formed by binding together a phosphate group, a sugar molecule and a nitrogenous base. Two of these strands then bond together using the base pairing rules and hydrogen bonds to form a double strand of DNA and further the double helix model. The base pairing rule means that complementary bases pair together, pyrimidines to purines. Pyrimidines are the three bases Thymine, Cytosine and Uracil (cytosine in DNA where as it is Uracil in RNA). Purines are the bases Adenine and Guanine these remain the same in DNA and RNA. In DNA Adenine (purine) always bonds to Thymine (pyrimidine) and Guanine (purine) always bonds to Cytosine (pyrimidine). RNA stands for ribonucleic acid. It is structurally different from DNA because the sugar molecule that makes up a nucleotide is ribose. The nitrogenous base Uracil is found instead of the organic base Thymine. The polynucleotide chain is usually single-stranded and three forms of RNA molecule exist. These are messenger RNA (mRNA), ribosomal RNA (rRNA) and transfer RNA (tRNA).

During the process in which DNA is used to make proteins, the DNA primarily undergoes the process transcription. This is when the DNA spirals separate and copies its instructions to mRNA (messenger RNA). It is made as one complementary strand of the DNA molecule. The next stage of the process is translation, this is when the mRNA arrives at the ribosome. The information is copied over to rRNA (ribosomal RNA) from here the ribosome can use the information to construct the new protein. To stop this process and therefore stop diseases like cancer and foot and mouth translation could be stopped at the ribosome if the mRNA never arrives. This would mean any gene could be switched off at any time giving us a cure for any virus.

RNAi (RNA interference) is responsible for turning off genes as it is a process of gene silencing that plays an important role in the development and maintenance of the genome which is the genetic material of a living organism. The RNAi pathway is complex and is initiated by an enzyme called 'dicer'. This enzyme splits up the strand of RNA into smaller fragments. A new RNA-induced silencing complex is then formed by base pairing between complementary mRNA and one of the fragments. The formation of this complex is then followed by the degradation of the complementary mRNA which is enduced by a catalytic component. The fragments become known as small interfering RNA. RNAi has become a valuable research tool as it allows the prevention of specific genes by introducing these small interfering RNA to the mRNA one wishes to suppress. The mechanism is shown in figure 1 (1).

Figure 1.



This was demonstrated in a paper contributed by Richard O. Hynes. (Published in 2008) which stated 'RNAi has proven to be a valuable research tool for investigating biological processes (2). Various groups have devoted significant efforts toward developing genome-scale, vector-based RNAi libraries...' he continued to write 'These efforts are beginning to offer insights into the complex network of interactions that potentiate the development of differentiated cell types or drive the transformed phenotypes of cancer cells.' The development in RNAi research and knowledge is widely useful in veterinary medicine and in some cases relates directly to it. As the knowledge of animal genetics is extremely important in the application of biotechnology to manage genetic disorders and improve animal breeding. These technologies are being used to benefit many areas, the transgenic technologies have been used widely to increase efficiency for the food production industry, for example it has been used for improving the milk production and the meat in farm animals as well as for creating models of human diseases. We can now see that biotechnology has potential applications in the management of several animal diseases such as foot and mouth disease, swine flu and avian flu. Most prominently in the discovery of vaccinations against these. Another developing area is gene therapy for diseases in pet animals. This is an option because many of the technologies for humans were developed in animals and due to the close relation in the diseases cats and dogs get compared to humans. This provides me with enough information to look at how gene silencing could be used as a cure for many diseases.

DISCUSSION

RNA- mediated silencing is a naturally occurring process for controlling the processes undergone by DNA to make proteins seen in every organism ranging from plant to man. The mechanism is referred to as RNA interference and is a powerful tool in many medical processes in use today. Scientists and researchers can use it in drug target screening. This method takes into account that RNA can fold into complex structures and interact specifically with different proteins. This feature of interactions is what makes it so essential for various biological functions within living organisms. In order to discover small molecules which can affect these protein complexes, which in these cases are drugs, a thorough analysis of the RNA-protein binding is required. We also use RNA interference in the development of therapeutic drugs and in particular anti-viral drugs. Anti-viral drugs are medicines that are not curative but they can control virus infections if used in the early development of the infection (3). They are able to do this because of their mechanism. Their mechanism typically inactivates the enzymes needed for the viral replication. This therefore reduces the rate of viral growth but cannot inactivate the virus already present. The antiviral therapy must be started within 48 hours of the onset of an infection to provide any benefit. Examples of this in use in the world are as follows, drugs used for influenza may be used throughout the influenza season in high risk patients or within 48 hours of exposure to a known carrier. Other antiviral drugs like these are advised to be used at first signs of symptoms and after initial response this is to prevent reinfection and make them as effective as possible.

Gene therapy is a technique for correcting defective genes responsible for disease development. In lung cancer, scientists have identified a genetic mutation that makes lung cancer more aggressive and increases the likelihood of it spreading. The LKB1 gene is a "master gene" which has been shown to play a role in preventing cells becoming cancerous. Professor Kwok-Kin Wong, of the Dana-Farber Cancer Institute, in Boston, Massachusetts, found that mice genetically engineered to have lung cancer and a defective form of LKB1 developed tumors which spread more quickly than mice with an effective LKB1 gene. Professor Wong and colleagues examined 140 samples of human lung tumors and discovered defective LKB1 genes were also more likely to develop more aggressive forms of lung cancer that spread more quickly. Patients who undergo a test for defective LKB1 could be given varying doses of chemotherapy drugs depending on which form of the gene they had. The mutation occurs spontaneously in around 30 per cent of lung cancer cases. Professor Wong (4) said: "As we make more and more discoveries about the relationships between different genetic variations and disease, we will be able to tailor more specific and effective therapies." As these discoveries Professor Wong talks about are found out about, we could use gene therapy in many cases to effectively eliminate the disease. A 'normal' gene could be inserted into the genome to replace an 'abnormal' disease causing gene. A carrier molecule could be used to deliver the therapeutic gene to the patient's cell containing the 'abnormal' gene. At the moment we can use a genetically modified virus to carry normal human DNA. Viruses are able to carry out this function due to their evolved way of enclosing and delivering their own genes to human cells in a pathogenic manner. Scientists have been able to take advantage of this capability and manipulate the virus genome to remove disease causing DNA and insert therapeutic genes. Other methods of gene delivery are direct introduction of therapeutic DNA into target cells. This approach is limited because it can only be used with certain tissues and requires large amounts of DNA. The advantages of this method would be that it is non viral.

I have many ideas for uses of this new science which could potentially in theory help cancer. In one approach, we could simply replace missing or faulty genes with healthy working genes. This is because many of these missing or faulty genes are the causes of cancer. By replacing these we are getting rid of the problem. For example, if we used the research of Professor Wong that I described earlier we could help eliminate lung cancer. Every embryo could be screened for the LKB1 'master gene'. If this gene is missing or defective it would be up to the mother whether she puts her baby through gene therapy in which this gene would be replaced with a working one. If this were the case and the mother decided her baby would have the treatment it could reduce the chance of lung cancer. We know this as in 30% of lung cancer cases in humans, a faulty LKB1 gene is seen.

Another use of gene therapy which could help cancer is, if it were used to stimulate a patient's immune response to cancer itself. The gene therapy would improve the body's natural ability to attack cancer cells. This method is being researched into at the moment, where researchers take a small blood sample from the patient and insert genes that will cause each cell to produce the protein called a T-cell receptor. The genes then transfer into the patient's white blood cells and replaced back into the patient. When back inside the body of the patient, the white blood cells produce these T-cell receptors which can then attach themselves to the outer surface of the white blood cells. The T-cell receptors then recognize and attach to certain molecules found on the surface of tumour cells. They then can activate the white blood cells to attack and kill the tumour cells. The white blood cells are specifically called T-lymphocytes.

Another method could be to insert genes into the actual cancer cells which would make them more sensitive to chemotherapy, radiation therapy and other treatments. This would mean they would respond quicker to treatments making it a quicker and cheaper experience for the patient. Similarly you could remove healthy blood forming stem cells from the body and insert the genes that make the cells more resistant to the side effects of high doses of anticancer drugs and then inject the cells back into the patient. Genes could also be inserted called 'suicide genes' which would cause the destruction of the cancer cells.

The first success story using gene therapy in a human subject was a 4 year old girl with a severe immune deficiency disease (5). This disease is caused by a faulty gene that fails to produce a vital enzyme. In the therapy procedure, they extracted some of the girl's white blood cells. Then, they exposed them to a genetically engineered virus that had lost its virulence but still carried normal versions of the gene that was not functioning correctly in the girl. The virus invaded the white blood cells, and then these cells were transfused back into the girl. Once back inside the girl's bloodstream, the cells began producing the proper enzyme. Although the girl still needs follow-up treatments, she now leads a relatively normal life following the gene therapy.

There are many potential problems with using gene therapy for treatments in such serious diseases as cancer. There are many considerations to be made such as the short lived nature of gene therapy. This is because before gene therapy can become a permanent cure for any condition, the therapeutic DNA introduced into target cells must remain functional and the cells containing the therapeutic DNA must be long lived and stable. The cell is also a problem due to the rapidly dividing nature which prevents gene therapy from achieving any long term benefits for the organism, especially in bone-marrow stem cells. These cells divide infrequently, and the viruses used in gene therapy only insert genes in to cells that are dividing. Patients would have to undergo multiple rounds of it which is time consuming and expensive. There is also the risk of stimulating the immune system due to the foreign object being introduced into human tissues, because the immune system is designed to attack and destroy invaders. If the immune system was to be stimulated it could reduce the effectiveness of the gene therapy. This then spirals because of the immune system being able to respond quicker to repeat invaders. Another problem is using the virus as a vector to transport the new DNA to the target cell. This is because the actual carrier could present problems to the patient. These would include toxicity (the degree to which a substance can harm humans and animals), immune and inflammatory responses (responses with classical signs and symptoms). There is also the fear that the viral vector, while inside the patient, may recover its' ability to cause disease. This would be especially trouble causing in the patients who are already ill and experiencing the effects of their disease. One of the biggest problems would be that the most commonly occurring disorders such as heart disease, high blood pressure, Alzheimer's disease, arthritis and diabetes are all caused by the combined effects of variations in many genes not mutations in single genes. Theses multi gene disorders would be especially difficult to treat effectively using gene therapy. The viruses may also target the wrong cells, meaning they insert themselves into the wrong place in the DNA, which could easily lead to abnormal, uncontrollable growth. These would develop into tumours. The new gene could also fail to express itself. For example, a virus used in cystic fibrosis produced an inflammatory response. The trials with that virus had to then be halted and other virus vectors had to be developed.

Because gene therapy involves making changes to the body's set of basic instructions, it raises many unique ethical concerns. Many people would ask how can 'good' and 'bad' uses of gene therapy be distinguished, as what seems viable and fair to one individual may not to another. I think that gene therapy being used to change someone's genes so they are not so susceptible to a life threatening, carcinogenic disease in the future seems a good use of the new medical tools we have, where as others in this situation may add that it would be interfering with the body and the science could easily be manipulated for uses not so viable, for example enhancing human traits such as height, weight, intelligence and athletic ability. It could become a slippery slope once we have started saying 'yes' to some uses of the new science but where do we say 'no' to the same method being used for different reasons. There is also the argument about who decides which traits are normal and which traits constitute as a disability or a disorder. I think that in the context I am discussing, which is using the genes to replace faulty or missing ones to benefit diseases which are life threatening is reasonable and suitable. Others may take offence to be classified and grouped in such ways that label them as having disabilities. This grouping could lead to society becoming less accepting towards people who are different. A query about who the therapy will be available to, due to cost is also a worry for many people. This would be due to the high costs of the treatment. Current gene therapy research has focused on treating individuals by targeting the therapy to body cells such as bone marrow or blood cells. This type of gene therapy cannot be passed on to a person's children. Gene therapy could be targeted to egg and sperm cells (germ cells), however, which would allow the inserted gene to be passed on to future generations. This approach is known as germ line gene therapy. The idea of germ line gene therapy is controversial. While it could spare future generations in a family from having a particular genetic disorder, it might affect the development of a fetus in unexpected ways or have long-term side effects that are not yet known. Because people who would be affected by germ line gene therapy are not yet born, they can't choose whether or not to have the treatment. These questions only consider the questions behind the science while it is actually happening. We also need to consider ethical concerns with the research into gene therapy and similar treatments. Does searching for a cure demean the lives of individuals presently affected by disabilities or does it present hope for future generations.

CONCLUSION

In this paper and in my research investigating both gene therapy and gene silencing I have been able to identify many existing and potential future applications for its use. Gene modification is already being used extensively in the field of agriculture to improve crop resistance to disease and to provide improvements in yield. In medicine, both human and animal, I have seen how gene therapy and gene silencing could be used to enormous benefit. The biotechnology industry continues to push the boundaries in these fields, given the enormous potential benefits and financial gain potentially available through gene modification of both plants and animals. However, there are potential inherent dangers raising questions of ethical, environmental and sometimes legal origin. I have tried to demonstrate in this paper that the potential advantages of gene therapy out weigh the disadvantages in that being able to give someone that is born with a genetic disease or someone who develops cancer the opportunity of overcoming the disease, outweigh the ethical arguments against it. The future of gene therapy and gene silencing seems promising, exciting and challenging with ongoing trials and research taking place worldwide to help improve knowledge of how it could be used in all fields of science. That there is a future for gene therapy and gene silencing is not in doubt, however, it will continue to face constant scrutiny as it deals with the very thread of life itself. I hope that in time the ethical, environmental and financial negatives currently associated with the research may be overcome to allow the controlled growth and acceptance of this science.

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