

Gene Silencing~
Use in Cancer Treatment
And Transportation of siRNA.

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PASS WITH MERIT

Research Paper
Based on
Pathology Lectures
At Vet-Medlink 2009

Abstract:

Many people and animals suffer from Cancer every year, after listening to lectures at Vet-Medlink in 2009; it seems a cure for this horrible disease may finally have been discovered. RNA interference, otherwise known as RNAi, is a gene-silencer which could stop the transcription process in which the gene for cell mutation is translated. Scientists are very interested in this new discovery and have explored every possible case in which it could be used. From a veterinary and economic point of view it could also be used in new viral outbreaks to suppress diseases quickly without there being the need for culling, such as in the case of Foot and Mouth disease.

Introduction:

DNA (Deoxyoxyribosenucleic Acid) is the blueprint for the structure of the organism it belongs to; it defines the features and how the organism is to look. However, it can be wrong. In these circumstances, the consequences can be very severe. The single gene (section of DNA, that encodes for a certain protein to be made and therefore allowing and controlling thousands of chemical reactions to take place in the cell) that encodes for this 'Misprint' could lead to the death of the organism.

To enable the specific protein to be made, there is a process in which the gene is translated. A process called transcription allows the DNA spirals to separate in a process called RNA polymerase, which then copies base instructions to mRNA (messenger RNA). The mRNA takes the base instruction to the ribosomes where the gene is translated and built, in a process called proteinsynthesis (translation). If the mRNA never arrives, translation will not take place, meaning the harmful gene is stopped and is incapable of causing damage to the organism.

RNAi happens naturally in plants and invertebrates as an immune defence against viral infection and was discovered in petunias, which when extra coloured genes were inserted, the result was not a darker purple but co-suppression. Co-suppression is where the extra genes affect the other genes inside the nucleus.

Viruses contain RNA or dsRNA (double-stranded RNA) as this invades a plant or invertebrate cell; it is identified and dissected by an enzyme dicer. After this process a second enzyme destroys the RNA and is known as RISC (RNA-Induced-Silencing-Complex). RISC also copies the DNA fingerprint of the intruding RNA allowing matching RNA to be destroyed.

Humans and higher mammals do not produce this response; instead long strands of dsRNA cause protein production to be shut down, known as the interferon response. This can be overturned by introducing siRNA (short-interfering-RNA) which triggers the interferon response and can then allow any matching RNA (including the mammals own mRNA to be destroyed).

At the forefront of RNAi development in cancer, is the research involving carcinogenic molecular pathways and how the carcinogen develops into a cancerous cell. As well as solving the major problems involving RNAi, such as; how to get siRNA to a cell, and how to keep a prolonged supply of siRNA there.

Discussion:

There are many cases in which humans and animals can benefit from the development of RNAi and its use in medicine. At the moment the advances in technology and medicine mean that genetic diseases (For example; some types of cancer or Down's syndrome) can only be removed by IVF (In vitro fertilisation), however this may not be necessary if RNAi will be able to prevent the translation of the gene responsible.

There are many genetic disorders in the world and with RNAi, it could be possible to detect, treat, cure and possibly eliminate these disorders as well as destroying many viral diseases. Some of the diseases it is possible to treat are; cancer, Down's syndrome, Autism, Cystic Fibrosis, Parkinson's and so many more which affect people's lives in such a drastic way.

Although scientific work into the way RNAi works is supported on many levels, not only by the medical community, but by many who see this as a great advantage to modern day life and who are happy to see the development of a cure for some of the world's greatest diseases, for example HIV/AIDS which in 2008 caused the deaths of an estimated 2 million people worldwide, or Cancer which caused the deaths of 155,484 people in the UK in 2007. Although these statistics support the movement of medical advancements in RNAi, ethical issues are major problems to the speed at which RNAi is developed into a useable state. To be able to clinically use RNAi, it first has to go through extensive testing.

It is required that any new treatment must be tested thoroughly before it used in practice. This can majorly increase the amount of time before RNAi is allowed to be used in any type of mammals, as well as a build up of opposition on ethical grounds. This could be because of necessary use of animals in their testing facilities or because of religious reasons, such as Christians believing that it is wrong to 'play God' and change someone's genetics. Therefore we can safely assume that there will be opposition to the development of this treatment by animal rights activists or religious people.

Cancer is a major problem in all mammals, as a case study, I would like to propose how RNAi could be used to protect one of the world's endangered animals. The Tasmanian devil has been recovering from an almost total wipe-out after hunting it was finally made illegal. However, recently the numbers of the Tasmanian devil have dropped massively by over 70%. This is due to a new disease, known as the Tasmanian devil Facial Tumour Disease (DFTD) which is a type of cancer, see figure 1 below; it has occurred due to a large amount of inbreeding in Tasmanian devil's, this has made their genetic make-up so similar that their immune systems do not recognise other Tasmanian devils cells as being foreign.

When a Tasmanian devil contracts Facial cancer, a large open tumour occurs on the side of its face. When two Tasmanian devils fight over food or a mate, they are likely to bite the other devil on this open tumour. When the infected cells enter the attacking devils system they are not recognised by the immune system and therefore are not destroyed by Phagocytes. The infected cells can then infect an open cut in the mouth and hence cause a tumour on the Devil's face.

This has caused major problems in the recovery of the Tasmanian devil. The two things it needs to survive have started to destroy the population. DFTD is weird as it is one of three types of cancers that can spread like a contagious disease and since its discovery in 1996 it has spread at a rate of between 7 and 20km per year and reduced sightings by up to 95%. T

RNAi could be very important here as it could help keep a species from extinction. If enough Devils were protected by siRNA causing an interference response for long enough the disease could be isolated and destroyed.



Figure 1 (DFTD)

However, for this to work siRNA would need to be supplied to the protected devils for a prolonged period of time as well as solving the other problem of delivering it to certain cells. As well as this, siRNA is very specific so any mutation in the 'Genetic Fingerprint' of DFTD and the devils would no longer have immunity to DFTD.

To transport siRNA to certain cells, a way of identifying the necessary cells has to be found. Using nanobiotechnology it has been suggested that siRNA can be delivered to cells via their surface receptors and Carbon-Nanotubes (in this instance it is relating to cells infected with the HIV virus). Cell surface receptors are glycoproteins attached to the plasma membrane of a cell.

The developers of this theory Zhuang Liu, Dr. Mark Winters, Dr. Mark Holodniy and Prof. Hongjie Dai; published online in 2007, Functionalized single-walled Nanotubes (SWNT) can be used as molecular transporters to shuttle short interfering RNA (siRNA) into human T cells and primary cells and silence the expression of HIV-specific cell-surface receptors and co-receptors (see figure 2). This silencing effect, known to block HIV viral entry and reduce infection, is superior to that observed with conventional liposome-based nonviral delivery agents.

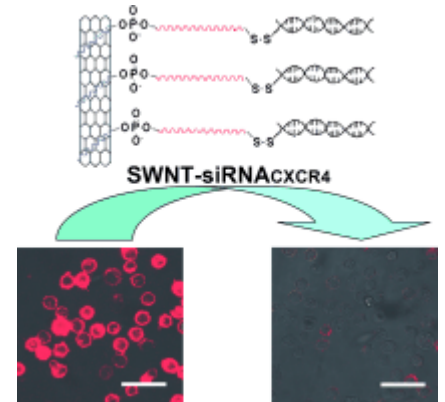


Figure 2

When a virus infects a cell they are likely to leave their cell-surface receptors on the host cell. This means that using this theory, scientists will be able to send complimentary siRNA to the infected cell via carbon Nanotubes (see figure 3). As Nanotubes are exceedingly small they will be able to travel through the blood stream without causing blockage of damage to arteries, veins or capillaries. This theory helps to solve one of the major issues of using RNAi in clinical practice.

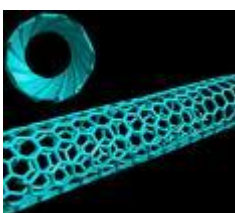


Figure 3 ~ source: biomedme.com

Cell surface receptors are also recognisable by antibodies. Using this knowledge it is possible to attach specific items to antibodies. If it is possible to attach siRNA to antibodies, then it is possible to insert the antibodies with the attached siRNA into the blood stream where they will deliver the siRNA to any cell with complimentary cell receptors to the shape of the antibody, allowing the interference process to take place. If regular injections are taken then the siRNA would be able to keep the interference process continued for a prolonged period of time, hence protecting the mammal from further harm or infection.

Figure 4 shows us how viral surface receptors are relayed to the plasma membrane of the host cell. After a virus has gain entry to a cell its DNA is synthesised and replicated before being transcribed and translated. After this the translated genes are assembled as surface receptors. If we can discover the set surface receptor and find the complimentary antibody, then we can also deliver the siRNA.

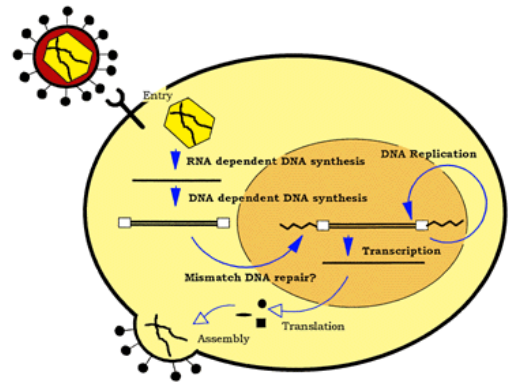


Figure 4~ source: home.ncifcrf.gov/hivdrp/Virology_scan.gif

The main problem with this solution is due to viral mutations. Viruses can mutate to survive and in doing so will no longer be complimentary to the antibody or siRNA, which being very short is very specific and will not code for mutating viral RNA.

Both Antibodies and Carbon Nanotubes are applicable for use as deliverers for siRNA. They are both small able to flow with the blood stream without causing blockages or problems. Antibodies are also a common finding in mammalian bodies as B cells, which are part of the immune system and therefore should not trigger an immune response. However without further knowledge, I am unable to comment on whether the same applies to the carbon Nanotubes.

Conclusion:

Cancer is still a much feared disease, it is known for the deaths of many. RNAi can change all that for many humans and any mammals. The use of siRNA to produce an inference reaction to cause RISC to be activated needs to be developed. One of the main problems is the transportation and delivery of siRNA to particular cells. The two ways explored above both involve the transportation of siRNA via microscopic particles.

The use of antibodies and Carbon Nanotubes are both mentionable in these circumstances. This is because of their size will enable them to fit through capillaries and if necessary in between cells and they will also be able to do so quickly and efficiently. Antibodies are not likely to cause harm to the human body and neither are carbon Nanotubes. However, further to this paper, more research would need to be done in the possible causes of harm to the patient if either process is used. Also in my research, I have defined a few uses of RNAi in genetic disorders and cancer, as well as treatment in viral disease

I have left many unanswered questions due entirely to my lack of knowledge and facilities. More research would have to be done on long-term effects of the two methods of transportation above, as well as whether they will be able to adapt to many of the conditions they might face in a mammalian body.

Referring back to the area on Tasmanian devils, there would need to be a process developed in which you were able to give a Tasmanian devil siRNA for a prolonged period of time without its capture and possible it being domesticated.

In conclusion; Antibodies and Carbon Nanotubes are both possible ways of delivering siRNA to specific cells by using their Cell surface receptors. For humans these could be delivered on a regular basis via injections into their bloodstream, for domesticated animals this would also work. However, this process would be difficult for wild animals. More research is needed into ways of coping with mutation and rejection of the treatment.

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