

THE PROBLEMS AND ETHICAL ISSUES
OF THE FUTURE OF GENE SILENCING

PASS WITH MERIT

BY

KATHARINE CLARK

BETHANY SELWYN

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Abstract

This paper discusses the main problems with gene silencing (by using RNA interference), before and after it is used to treat people and whether the gene is given to fetuses or adults. The main problems that we will be researching are: how the immune system can prevent the gene from entering the body effectively; ways of administering the gene, and how this can affect the patient; how the gene can be a short term effect and finally how not all problems can be solved at this current time. Also as the gene silencing (RNAi) field of research is rapidly expanding and progressing, we thought it was important to address the ethical issues involved currently, as well as the ones that may become more apparent in the future. Therefore, in the second section of our paper we shift the focus slightly to cover this topic- exploring the ideas of vivisection (animal testing) for HIV and also the future RNAi uses.

Introduction

The scientific theory behind our research is gene silencing, RNA interference (RNAi). This takes advantage of the process all cells must undertake to reproduce and survive- genetic copying and protein synthesis. Most living cells of a multi-cellular organism copy their genetic data during interphase so that when they undergo mitosis they are not losing chromosomes. First of all a small section of the DNA in the nuclear envelope of the cell is uncoiled by the enzyme helicase to expose the two strands of genetic data so that polymerase can break the hydrogen bonds holding them together. Next, using the rules of base pairing, free nucleotides that were previously floating freely in the nucleoplasm are bonded to one strand of the DNA, this is known as transcription. These free nucleotides join to form RNA which is free to leave the nucleus through nuclear pores. The enzyme lipase then rejoins the original pieces of DNA. The RNA that has exited (translation) the nucleus is now what we focus on, as it is now in the cytoplasm where many different things can be done to it. It can be cleaved by Dicer to form a short double stranded length of RNA known as small interfering RNA (siRNA) which can bind to form RISC (RNA-induced silencing complex). This uses the siRNA as a template to single out complementary strands of genetic data in the cytoplasm and splits them so that the cell can recognise them and destroy them, thus stopping their translation. [1][2] The implications of this natural process mean that if we can work out the correct siRNA to inject into the cells, and how to inject them, and how to copy this silencing into all cells in the organism; many cures for terminal illnesses such as cancer will be able to be developed.

The areas of veterinary medicine that will benefit the most from RNAi in the future are mostly the commercial farms. This is because farms will be able to prevent their herds/ flocks from suffering infections that decrease or destroy their yield. For example reference [3] shows how RNAi was used to reduce the transmission of the viral infection of *babesia bovis* into cattle. In human medicine RNAi is being used to develop many different cures and vaccines, for example a vaccine for HIV is being researched. Also huge leaps forward are

being made in respect of curing genetic disorders, the most successful so far being that of Macular Degeneration where people were given injections into their eyes to stop the over-expression of the VEGF gene, 25 per cent experienced a significant improvement in their vision. [13]

Where an example is needed to illustrate the point being made we will try to use the Human Immunodeficiency Virus (HIV) as this is a rapidly expanding disease that is affecting more and more people in the world today. Human Immunodeficiency Virus (HIV) causes Acquired Immunodeficiency Syndrome (AIDS). There are four stages of the virus; the incubation period which leads on to acute infection, next the latency stage and finally AIDS. HIV is a retrovirus meaning it contains two linear RNA molecules in each cell. It works by binding to a receptor cell: a T-Cell in the human blood stream, and injecting its RNA into the host cell. The viral RNA codes for the production of thousands of new viruses, these eventually burst out of the cell destroying it and therefore rendering it useless to fight the disease. This obviously weakens the immune system making it very easy for any other pathogens to enter the body, and affect the patient. Therefore there are no specific symptoms of HIV that make it immediately recognisable as the only real change that a person will feel is that they pick up common colds etc more easily than others.

Discussion

To start with, one of the problems with RNA interference is the immune response of the human cells. The immune system works by identifying foreign pathogens and blocking their ability to attach to a host cell. To do this the white blood cells in the blood produce antibodies which bind to antigens on the surface of the pathogen, therefore blocking the pathogens from binding to receptors on the host cell. The immune system also destroys the pathogens by using neutrophils and macrophages to engulf them. This indicates a severe problem when trying to administer the siRNA that will help eradicate the faulty genes into the cell; any foreign body entering the cell will be identified and broken down as soon as possible.

In the year 2000 scientists hoping to produce a vaccine for HIV had a massive breakthrough; they managed to successfully deliver a small active part of the HIV virus into the cells of mice. This induced an immune response allowing the mice to fight the virus more efficiently and effectively when infected with full HIV later on. To do this the researchers injected some siRNA of the HIV virus into a weakened version of the rabies virus cell. The rabies virus acted as usual to inject its own viral DNA into the host cell of the mouse, but because this had been replaced with the HIV RNA the cells produced antibodies to fight that virus instead of rabies [4]. One side effect that needs more research is the constant threat of viral variation. If the HIV (virus) mutates, then the protein coat surrounding it is different from the other versions of it. As it is this that the antibodies recognise to fight the infection, if the virus mutates the previous antibodies manufactured by the cell have no effect in fighting the HIV. Also, if the weakened rabies virus mutates it

may then become harmful to the mouse. Scientists are now trying to control the variation of the viruses and also see how effective this administrative method is in humans.

Another main set back with using RNAi to treat and cure disease is the occurrence of multi-gene syndromes. Many disorders, such as cystic fibrosis [5], affect just one gene so would be relatively simple to treat with RNAi as just one defective gene would need to be identified and silenced. However many other disorders are a result of a mix of genes being defective such as when DUOX2, PAX8, SLC5A5, TG, TPO, TSHB, and TSHR genes are defective hypothyroidism is caused. [6] To treat this disease many of the defective genes would need to be sited to prevent those being copied further which will need a huge amount of research, time, money and effort.

Another problem is that the gene silencing may only be short term. This is because it is not certain that the silencing complex will stay in the body for the rest of the patients' life. This causes real issues, as, if the patient has to have a replacement gene every 5 years, as the gene doesn't stay in the body for a long time, it would take a lot of money and time from the healthcare system and patients may just decide to stay with their current treatment.

Also there is the problem that if the mRNA is silenced in somatic cells [7], when the patient is an adult, the change would not be passed down into their children if they decided to reproduce. This is because the process only prevents the cells from copying the faulty gene into other cells but it may still be passed on to the child. This means the child would have to either live with the effects of the faulty gene or receive the same treatment as the parent. However if the foetus receives RNAi treatment it is very likely that they will not carry that gene anymore, and therefore in the family genetic line the abnormality has been eradicated.

Now we move on to the more ethical side of RNA interference, discovering what different parties believe and which issues will become more relevant in the future.

Firstly the issue of vivisection has a massive impact on the work of researchers as there are many groups that feel strongly about animal rights and their involvement in testing for new drugs etc. So why are tests and research carried out on animals? In the United Kingdom it is a legal requirement for all new drugs to be tested on animals before humans, and for researchers small mammals will obviously have a more similar reaction to the drug with humans than plants or insects would because their genome will be the most similar to ours.

To demonstrate this ethical hurdle that researchers of RNAi have to overcome we will use the example of the Human Immunodeficiency Virus (HIV). Researchers have held many different conferences concerning the development of a vaccine suitable for humans to prevent the virus from attacking T-cells in the immune system, e.g. the conference entitled

“The continuing HIV vaccine saga: naked emperors alongside fairy godmothers” on which an informative paper was written by Kendall A Smith [8] .

To justify their actions on animals, researchers are working in the name of alleviating pain and discomfort of people who are HIV positive. However many of these people, despite realising that the very drugs that keep them alive must have been tested on animals, either are against animal testing or remain neutral on the issue. This was shown in September 2006 when an organisation was formed by US groups which had the aim to oppose the organisation PETA (People for the Ethical Treatment of Animals). Their efforts were however worthless as many people who were HIV positive at the time were opposed to animal testing themselves and therefore would not give their support. [9]

Another reason why animal testing is essential is because of the nature of the drug itself- it is a vaccine that they are developing therefore to test its effectiveness it is necessary to expose the ‘vaccinated’ individual to the virus and test immune responses. As there is no cure as of yet for HIV it is completely unethical to do this sort of test on a human individual as, if the vaccine is unsuccessful, this person will have to continue their life with HIV and the high risk of developing AIDS. Therefore it is much safer to infect an animal with the virus.

However there is a big problem with HIV testing. As the name suggests the virus is specific to humans and a different virus is needed to infect monkeys (Simian Immunodeficiency Virus, SIV) or other primates that are being used in labs. Animal Activists could argue that in fact this will not help as it is not the same virus and therefore will not behave in the same way as HIV, a vaccine that is effective here may not be in humans and may even cause harm, especially as there are so many gaps in the knowledge of RNAi where some experiments have come out with completely different results to what was predicted (e.g. the Nobel prize winners Andrew Fire and Craig Mello were very surprised by their results of what affected the worms they were testing [10]). Scientists may argue that they are more similar than you may think as it is possible that HIV developed from SIV [11] and also that a test that has similarities is much better than no test at all, putting humans at higher risk.

One of the four principles of biomedical ethics (devised by Beauchamp and Childress) states that for a person to have treatment they must have autonomy over the decision, meaning they must be provided with enough medical information so as to make an informed choice. Because of this we need to know as much as possible about the drug before asking a patient to make a choice about which method of treatment they would like to use. Therefore animal testing, coupled with computer and *in vitro* testing is essential so as to provide the most useful and relevant information to the patient.

Looking to the future, it may be that animal testing can be abolished completely due to a new type of testing drugs on humans, micro-dosing where amounts so small of the

product are introduced through the skin so that a whole-body reaction is extremely unlikely to occur yet important results can be obtained from doing it. [12] This would mean trials on humans can be carried out, with no harm to animals and the results are much more reliable as they will be more specific to the reactions of humans, not animals who may have slightly different gene make-up.

Secondly, the very idea of fiddling, controlling and changing genes within a cell awakens a whole host of questions. If we can cure blindness caused by macular degeneration (MD), a genetic disease in the retina [13], why not alter the colour of the eyes as well? Can we draw a line as to what is ethical to spend resources on to treat and what is simply personal preference and therefore not worth it? Perhaps an obvious answer would be to offer treatment with the aim to alleviate pain. However diseases like MD may not cause pain to the patient, they only cause an inconvenience in everyday life. This means it may be possible to argue that people, especially children, who are the victims of bullying, are suffering just as much as those that are blind. Therefore expectant parents could argue that they believe their child will get bullied if, though an extreme example, they were born with brown eyes. The doctors in this case could offer RNAi treatment to eradicate the dominant brown gene from the foetus on the basis that it is preventing the child from potential pain. This could lead to couples more or less 'designing' their babies before they are born, which sounds intrinsically wrong.

Also, allowing couples to choose preferences in their babies could destroy economies. In some societies it is preferable to have a son, or in places such as Portugal blonde hair is considered luckier than other colours, so if the parents are able to alter their child's genes they will choose the more desirable versions. This means there will be an imbalance of males so the society will not be able to reproduce as it did before; or there will be more blonde haired children and they may suffer the same problems as produced by inbreeding. The main concern with this type of choice is the lack of study done on it and how, to test it, one has to put the life of an unborn child, who cannot possibly give consent, at risk. It has the potential to turn into a major incident as Thalidomide turned out to be when it was given to pregnant women to suppress morning sickness but had not been trialled for this purpose, leading to the birth of many deformed babies.

Conclusion

In conclusion, this paper has explored the main problems and ethical reasons of issues when using gene silencing in the future. Although gene silencing is a promising potential cure for those suffering from diseases, there are many problems associated with it, possibly resulting in the benefits being outweighed by the risks. If we could research more about the gene silencing, without being hindered by animal rights activists and tight legislation we believe we would be able to see it playing a vital role in healthcare for animals and humans in the not-so-distant future. However as things stand, we must remember and respect the views of all parties involved whilst trying to overcome the main problems.

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