

Could RNA Interference Be The New Technology That Eradicates All Chronic Diseases And Viruses?

BY

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ABSTRACT

Genetics that is what makes each of our characteristics different. DNA lead to animals inheriting great characteristics which make them so memorable for example stripes on a zebra, however it also create destructive dilemmas with which scientists are puzzled on how to solve. Now however a new scientific discovery has shook the foundations of the science world. The discovery that RNA interference or gene silencing can stop devastating diseases, there is even the possibility that gene silencing could eliminate genetic inherited diseases. After successful tests on mice for human genetic diseases like inherited blindness the veterinary world is opened up to this new and very controversial technology. This paper will discuss the uses of RNA interference in treating Foot and mouth disease, one of the most feared contagious animal diseases in the world, the complications and ethics surrounding it as well as other uses which could be presented in the future.

INTRODUCTION

The key part of a cell for performing its function is it's nucleus in which DNA is contained. In DNA three nucleotides form a triplet, and then triplets consequently form a gene, which then codes for a specific amino acid, furthermore making a protein. The steps in which DNA must undergo to make a protein are as follows

1. Double stranded DNA is transcribed to make single stranded mRNA, this is so that the DNA can send it's genetic material to ribosomes so that fundamental proteins can be made for the cell to function
2. The mRNA is then translated by the ribosomes to make a protein
3. This protein cans them for different things in the cell and sometimes the cell is dependant upon what proteins it contains to what its function is.

These are functionally important for cells in what they are capable of doing. But DNA does not move out of the nucleus. Instead the cell uses mRNA to transport these important chemical instructions to other organelles in the cell, this is where the name messenger RNA derives from, as it ferries genetic material around the cell. To do this the DNA must undergo a process known as transcription. Firstly a protein called RNA Polymerase transcribes a gene from the DNA onto the RNA. Then the RNA is exported through the nuclear membrane pores. The messenger RNA takes the gene to ribosomes where it is converted into proteins which are the beginnings of many different cells such as brain and muscle cells. If a virus such as Foot and mouth was to invade a cell it would insert its own RNA into the cells cytoplasm, the cell then recognises it and sends out an enzyme nicknamed DICER which makes the double stranded RNA smaller and easier to handle. Then a group of proteins called RNA-Induced Silencing complex or RISC for short separates the double stranded RNA into two single strands. RISC then looks for matching RNA to make dsRNA, blocking ribosomes which stops the viral invasion. This is what scientists have been trying to recreate and engineer for human diseases and now animals diseases.

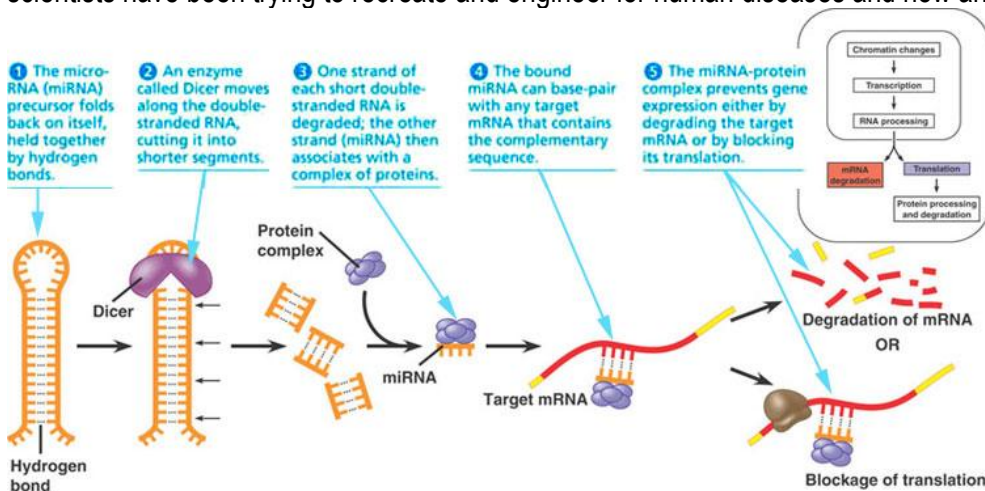


Figure 1 on the left shows the process of DNAi

RNAi History

Initial knowledge of RNA began in the 1950's with the discovery that once a cell is invaded with a virus it injects double- stranded RNA. This led to more research and findings regarding RNA interference which are detailed below.

The founders of this exciting discovery which is RNA interference were Craig Mello and Andrew Fire. In 1998 they published their paper on the cells ability to silence a specific gene(see reference). Their experiment included the use of worms and injecting them with two opposite types of RNA, sense and antisense. They found that the worms began to twitch similarly to worms without the muscle protein. They were able to deduce that the cause of this was that the two different strands in fact bonded to form double stranded RNA. This initiated the cells interference response and the blocking of the production of that gene that the RNA coded for thus the loss of the muscle protein injected into the worms. They received a noble prize in 2006 for there significant contribution to the scientific world. Although there acknowledgement was not until some time after there initial report of their findings scientists were still working during this time on building upon this new discovery.

Further research and testing was done into Mello and Fire's work, by such scientists as David. P. Bartel whose studies were presented in 2000 in possible his most well known paper [2] Cell paper (Zamore PD, et al., RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. His findings were not necessarily from him as it was down to Tom Tuschl and Phil Zamore who created a way to study RNA without cells. Tuschl investigated further and found that if he was to inject RNA of 21-23 nucleotide pairs long that this infact triggered the interference response but not the inflammatory response, this important technical advance intrigued Bartel's lab so they thought to study further.

H. Robert Horvitz, was able to identify 4 main killing cells in which can lead to neuro degenerative diseases in which we can now experiment with the use of RNA interference to combat the cellular death which as explained in his report contributes to the end result of cell death. His work has created the possibility of curing Alzheimer's, Huntington's and Parkinson's disease just to name a few. Also animal diseases such as rabbit haemorrhagic disease, which is when a rabbit is infected with RHVD it suffers severe kidney damage causing in a lot of circumstances death. Although new technologies like stem cells could possibly, in the future, be used to replace a damaged kidney, the cells identified by Horvitz would still be present in the body so the new kidney and other cells of the body could still be destroyed and consequently cause the death of the rabbit. However the development has now enabled the possibility to block these certain genes which code for that specific cell and its contents. The cell is no longer synthesised and it can no longer cause cellular death and it would then be possible to rehabilitate the animal.

Luk Van Parijs has begun to investigate the uses of RNAi. Specifically the one that could make such a fundamental difference to the medicine profession both human and animal. The uses in stopping cancer. In his experiments he is targeting immune cell survival techniques. By doing this you could stop a cancer cell from being malignant aggressive, stop it from being destructive and stop it from being incurable.

Phil Zamore a biochemist at the university if Massachusetts made important contributions to the scientific world in 2003 when he explained that small interfering RNA sequences could in fact stop the occurrence of HIV in humans. This could in theory be extremely useful as it has been suggested by scientists that the RNA fragments left behind after RNA interference could in turn be passed onto progeny cells. Leading to the eventual wipe out of the HIV virus from countries which could in turn lead to less poverty. Know Zamore has founded the Alnylam

Pharmaceutical company which are experimenting in the making of RNA interference drugs which development programmes could propose the use of an injection or tablets in the future.

Research involving mice has been very successful in controlling the HIV virus. It is a very complicated process in which first required the isolation and identification of the cell in which the HIV virus targets, to be specific a T- cell. A protein was attached to the surface of the T-cells in which dotted the surface of the T- cells allowing the HIV virus to gain entry. To do this was progression for the scientific world let alone the fact that the mice reacted in the same way as humans would to the disease. The mice were specifically lacked their own immune systems so they could be injected with human blood stem cells. This helped the research to be more reliable as it is more like a human's response. Silencing RNA was then injected which halted the destruction of the T-Cells and stopping the virus from either invading the cell, or once invaded spreading to others in the body. This is fundamental proof for the fact that RNAi can be used to treat diseases which as yet have no hint of being cured. Although there results look promising there is still further testing to be done on amounts of the RNA to use, method of inserting siRNA into the cells needed and more investigating needs to be done into the possible problems of using RNA to solve such diseases such as gene co-suppression.

DISCUSSION

As knowledge about RNA interference is growing I feel that I may be able to suggest a use for this in today's current world which could make a large difference. I also aim to explain how I would expect it to work and I will account for problems that may occur.

What In The Future Could Be Cured By RNAi?

Foot and Mouth disease is stored for up to 4 months in the lymph nodes of animals. Spread by dead carcasses, particularly in the case of pig swill. In 2001 the UK went into turmoil as 2000 cases of foot and mouth were reported after over 40 years of being free of the disease. It spread rapidly eventually reaching 50 reported cases a day. Culling was the only combat to slowing down the spread of the disease. The symptoms, fever, blisters on mainly the tongue and feet, were not always presented. The use of a laboratory was the only method of confirmation to whether an animal had the disease. Animals that did not present the symptoms could still be carriers in their saliva etc, so they would be culled as well. Agricultural farms were left livestockless as not only cows were infected but also pigs and sheep. It didn't only affect agriculture but tourism as well as deer were also able to catch the disease. Around 10 million sheep, cows and pigs were culled and their carcasses burned to halt the disease. It is estimated that it cost the UK £8 billion.

Firstly you would need to decide whether you would experiment with cultures in a Petri dish, small rodents such as mice or guinea pigs or larger specifically bred animals such as pigs. For many, this would pose ethical issues as some people may not agree with the use of animals in which they would propose the Petri dish method however, previously explained above; mice pose as suitable models in this experiment. I would specifically use mice that had no immune system of their own and inject cow, sheep and pig stem cells into the mice. The use of all 3 animals' stem cells would give a larger view of the results.

Previous research has been done into the proteins which are involved in the stability and the life cycle of the disease. It has been suggested that VP1 is a protein which plays the main part in the binding of the cells. So to fight this disease I would focus preventing the protein VP 1 from reaching the ribosome and making the protein. At current vaccines and culling are the only options to fight this disease. However in a lot of cases this vaccine still leads to the culling of animals because it is so difficult to tell what animal is infected. The vaccine is illegal to use in the EU unless it can be proved to halt the spread of disease and isolate in a sort of ring pattern. So scientists are keen to develop RNAi technology which will fill this gap in the market. Also what makes the vaccine so ineffective

is that there are 7 different strains of the virus and the current vaccines will not be readily available if a new strain was to present itself.

RNA interference involves stimulating a cell to interrupt the making of certain proteins as explained above. I believe that in this we could possibly prevent the spread of disease and cause a resistant population to susceptibility of the Foot and mouth disease.

HIV is one of the most deadly diseases affecting the world today with just over 40 million cases. It doesn't only kill people who have been infected with it but also leaves children parentless and families disintegrated. At current there is no cure against HIV but efforts are focused on reducing the chances of spreading the disease more specifically in third world countries, where HIV is most abundant. The virus is very hard to treat as it remain hidden for a while and then suddenly re-emerge, this means that treatment would need to be over a prolonged period of time, which means more money that just isn't available to the third world countries. A current treatment is a group of drugs called HAART these are used to prolong the onset of AIDS but become ineffective as the virus mutates, because the drugs have to be used for such a long period of time, and become resistant. This is why there is such excitement for the new technology of RNAi.

Scientists can develop siRNA's to disrupt the proteins in HIV or it could disrupt the synthesis of the protein which allows the virus access through the cell membrane.

One dilemma is that HIV mutates rapidly to become resistant to a lot of treatments at the moment. Their protein structure changes which means that certain specific siRNA's would become ineffective however one possible remedy to this problem could be the use of siRNA's that are complementary to the mRNA of all HIV virus. There would need to be extensive tests so to check that no co-suppression occurs or an immune response to the siRNA's classed as medicinal side effects.

The use of RNAi could solve the problems posed by scientists. For example there would be a possibility of the virus mutating but by combating a protein which after tests could be found in all strains of the HIV virus would mean that it would still apply to a large variety of the strains if not all. The ability to produce some sort of siRNA which attack all forms of HIV virus would reduce the cost and particularly help the third world countries as they don't have the money to spend.

Problems With RNAi

A dilemma is still facing scientists, how to get the siRNA to cells which need it and keep it away from affecting other cells. These problems need to be solved thoroughly. If the siRNA were to interfere with other cells then it could cause co-suppression such as in the case of Rich Jorgensen and how he tried to make petunias a deeper purple. Unexpectedly he gained a white flower which led to the discovery of RNAi. This was because the gene that Jorgensen injected into the plant to give it a deep purple pigment actually were recognised by the plant as a viral attack triggering RNAi causing the plant to stop making pigment. Because of the risk of co-suppression pharmaceutical companies run extensive test which end up costing a lot of money. The money spent needs to be recouped from the selling of the vaccine, which may pose as a problem as the price may be rather high. Furthermore the cells exposure to this siRNA could trigger the cell to destroy any remaining RNA including messenger RNA which may present a like coding sequence on its surface. The cell would then be able to recognise this a lot faster like the bodies secondary immune response.

There are many possibilities on how to get the siRNA into cells:

1. One possibility on how to get siRNA into cells could be inserting the siRNA into platelets which would allow them to travel around in the blood and also get to cells which may be damaged by the disease, such as a blister opening. However this would not change the fact that the animal would have to be infected to have that blister and the chances or then getting rid of the disease is minimal.

2. Another possibility is to look at how certain bacterium works. For instance the cholera bacterium *Vibrio cholerae* is able to identify that it is in the small intestine, this is achieved by certain receptors. This could be mimicked using an artificial bacterium structure; antigens that are presented on the surface of infected cells could act as the receptors for an engineered bacterium structure. The flagellum is the initial movement aid this could then be used to propel the artificial siRNA carrier into the cells so the siRNA could be released. It may be possible to develop the artificial bacterium to not release the cholera bacterium but to release the siRNA. This process could be replicated for certain cells and diseases of the body using the antigens which are presented when infected as the receptors for the artificial bacterium/ the siRNA carrier.

3. As science advances there are further possibilities on how to develop a way of getting the siRNA into cells for instance a antibiotic like drug could be developed (which are being looked into at Alnylam Pharmaceutical company) which recognises certain cells by what enzymes are in there for example the DICER enzyme which could then trigger the release of the siRNA. Channel and Carrier proteins could also play a part. If you were to attach the siRNA to certain molecules which are able to get through the membrane by these proteins then this would enable the siRNA to get straight into the site of infection.

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CONCLUSION

RNAi is the new technology. There are possibilities that it could cure many diseases that at current are puzzling scientists and costing countries healthcare service's millions. It could cure a variety of human diseases such as Alzheimer's and Parkinson's disease as well as animal diseases such as Foot and Mouth. There is also excitement over the possibility of curing cancers which have previously been incurable.

The possibility of curing Foot and mouth could save the economy of many countries when it comes to another outbreak. By inserting siRNA's you could halt the protein production at the ribosomes of the protein involved in the disease's life cycle, VP1.

A huge benefit to the world would be the curing of HIV. I think this would be possible if research was to be carried out on the possible proteins in which to target to restrict the protein synthesis.

The importance and need for curing such diseases as Foot and Mouth and HIV has led there to be a large range of pharmaceutical companies who are attempting to develop these siRNA's. However there are problems:

- How to deliver the siRNA's into specific cells
- How to make sure viruses such as HIV do not become resistant

There are ways of combating these problems but do require an extensive research lab and experienced team, which I had no access to so my opinions and ideas are based upon a Gedanken experiment. Already it has been found that 21-23 nucleotide pairs long siRNA's do not trigger the inflammatory response which does mean that the human or animals immune system would not react and cause medicinal side effects.

References

RNAi animation from teacher's domain

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