

RNA interference – Discussion to show how
we could adapt current research to halt the
Foot and Mouth virus from replicating
within mammalian cells.

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PASS WITH MERIT

RESEARCH PAPER
BASED ON
PATHOLOGY LECTURES
AT VET-MEDLINK 2009

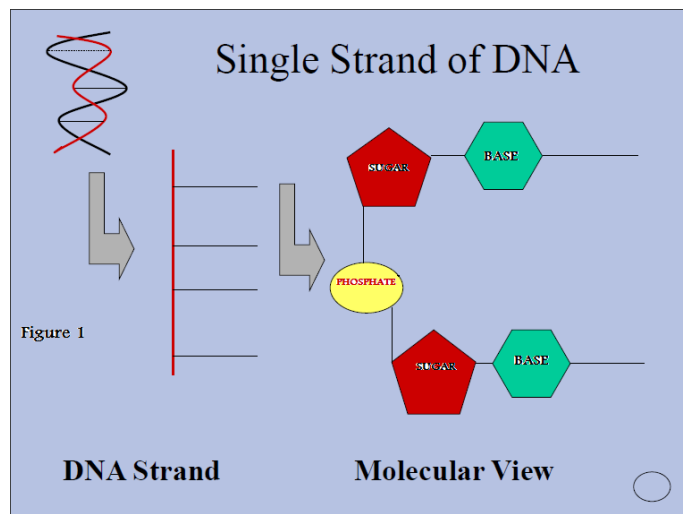
Abstract

Lots of diseases are caused by viruses, there are thousands of viruses circulating around the world all the time, some cause mild discomfort and some can be extremely painful and even cause death, so there is an obvious need for a cure. By developing cures we can save many lives and halt disease spread, this would increase life expectancy and save animals from pain. Through exploring other people's research developments and conducting a thought experiment, I have come to my own conclusion and personal discovery, of how RNAi could be used to cure a common cattle and livestock disease like Foot and Mouth. This theory could be put into practice and possibly need to be perfected to form a cure for any disease, this RNAi technique seems to be the most effective treatment to date and a current medical breakthrough. Research leading to a cure using the RNAi technique could quite possibly influence the way we look at treatments, thus revolutionise medicine and provide a universal cure for any disease.

Introduction

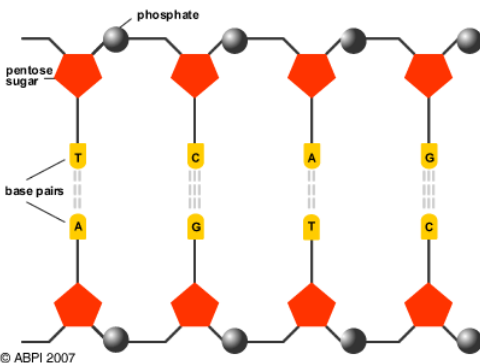
RNA interference is a way of controlling the actions and activity of cells; everything from protein production to defences against disease. To understand RNAi we must first understand DNA in cells; starting with the structure and position within the cells.

DNA (also known as Deoxyribose Nucleic Acid) is the genetic material of a cell, this acts like a 'brain' in the cell. DNA is found in chromosomes in the nucleus of a cell where it always resides; (unless the cell is undergoing mitosis); as DNA is far too large a molecule to escape through pores in the nuclear membrane. A DNA molecule is double stranded, it has a sugar phosphate backbone (figure 1) and complimentary base pairs chemically binding the separate strands of DNA together; (figure 2). Single strands of DNA are polymers made up of nucleotides; a nucleotide is made up of a base; a pentose sugar and a phosphate atom. The base pairs



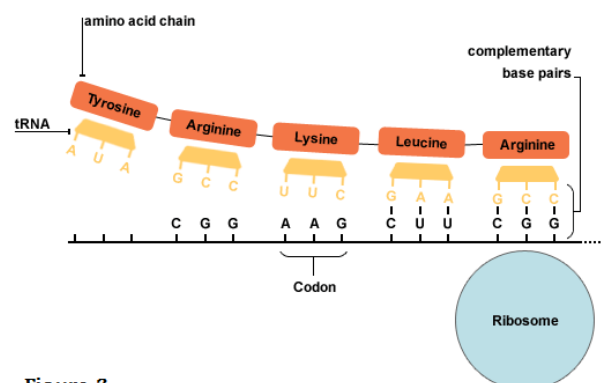
form hydrogen bonds between themselves and other base pairs which will determine secondary, tertiary and quaternary shapes of the DNA. Bases in DNA are the key to which amino acid is linked to another, i.e. the base coding for amino acids is in a sequence-specific manner. There are four bases in total, Adenine; Guanine; Thymine; and Cytosine (Thymine in RNA is Uracil), each base type can only ever combine with one other base type as they are very specific and complimentary shaped; (e.g. Cytosine and Guanine can only combine to each other and the same with Thymine and Adenine). What DNA does is code for genes and these genes code for proteins; which allow and control thousands of reactions in a cell.

Base pairs in a small section of DNA



unwinding and separating them into two strands, the guide strand is then used as a template where the DNA-polymerase enzymes attach the complimentary base of a nucleotide; which is 'floating' around freely in the nucleus; to the DNA strand. The enzyme bonds the adjacent nucleotides

DNA form proteins by first being transcribed into pre-mRNA and then to mRNA, when mRNA is formed the enzyme DNA-polymerase moves along the strands of DNA



together forming pre-mRNA; the pre-mRNA is then spliced so that only the useful genes within the original DNA strand will be carried through to protein production. After this stage the mRNA is translated at the ribosomes with the aid of tRNA attached to amino acids (figure 3). This translation process could be halted if we could stop the mRNA from ever reaching a ribosome or if the mRNA has been destroyed, meaning any gene could be stopped and we could control everything a cell does internally, also the external viruses which invade cells wouldn't be able to replicate themselves if we could halt translation.

This defence known as RNA-interference has already been discovered in plants and invertebrates.

The RNAi pathway is induced in plants and invertebrates when (dsRNA) double-stranded RNA invades a cell e.g. from viruses. When this happens the RNA is identified as foreign and serves as a substrate for the enzyme DICER,

then DICER chops the dsRNA into smaller segments known as small-interfering RNA (siRNA) which goes on further to activate and bind to another enzyme known as RISC (RNA-induced-silencing-complex). RISC is an enzyme which separates the strands of siRNA and bonds around the backbone of the guide strand, this formation then moves to meet complimentary mRNA either from the cell's nucleus or from a virus invading the cell (figure 4). Once the siRNA has been bonded to the mRNA it breaks the mRNA strands into pieces, then the cell containing nuclease enzymes destroys this broken down RNA. Obviously this pathway of protection could be very harmful if the cells begin to destroy mRNA which they require to survive but since this process is so sequence-specific the odds of this happening are very unlikely unless we synthetically manufacture siRNA which has the correct sequence.

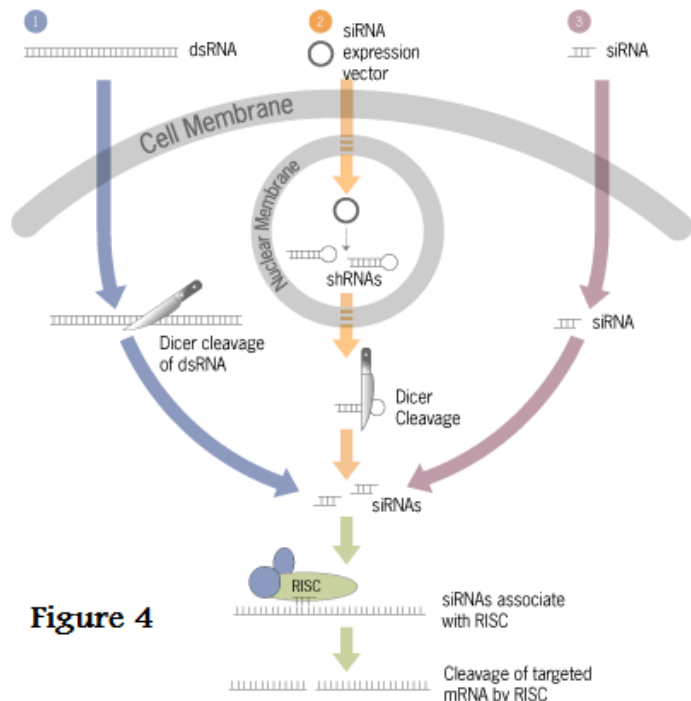


Figure 4

are very unlikely unless we synthetically manufacture siRNA which has the correct sequence.

So, knowing we can synthesise sequence-specific siRNA this type of defence sounds ideal to stop the effects of any disease; maybe cure it? However the first problem we encounter is that humans and most vertebrates do not employ the RNAi defence, instead they induce the inflammatory response where the cells shut down all protein production stopping all actions off the cell which would cause the cell to die. The good news is that humans and other invertebrates do possess the ability to use RNAi as their main defence system, we can trigger RNAi without triggering the inflammatory response and then we can develop a cure for any disease such as cancer, bluetongue and influenza virus adaptations; such as bird flu and swine flu.

With any immunity and defence against disease the effects aren't permanent, so how can we maintain high immunity and prevent the infected cells from returning to the inflammatory response? Any cure has side effects such as damaging healthy cells or slowing down the body's defence system leaving the animal prone to other infections, so once we have synthesised the siRNA cure how can the cure be delivered all the cells requiring them quickly and efficiently to reduce risk of further infection?

Discussion

Foot and mouth disease (FMD) is caused by a virus; once the virus has entered the body it invades cells and infects these cells by replicating more of the FMD viruses in large quantities, thus causing the host cell to 'burst' (lyses) releasing the viruses into the tissue fluid and blood capillaries

around the infected cell. FMD can be transmitted a number of ways including; close contact animal to animal spread; long-distance aerosol spread; and fomites or inanimate objects, usually fodder and motor vehicles. The clothes and skin of animal handlers such as farmers; standing water; uncooked food scraps and feed containing infected animal products can transport the virus. Cows can contract the FMD virus from the semen of infected bulls as well.

This disease is spread by the FMD virus coming into contact with the receptors of the body cells and dissolves its way through the membrane. Once the virus is within the host cell the protein coating dissolves and the viruses RNA is then released into the cell, this is then synthesized the same way as the host cell's mRNA to form other proteins and duplicate RNA of the virus, to then produce more and more FMD viruses until the cell ruptures releasing the viruses into the surrounding area's; (Figure 5).

If we can halt the transcription of the foot and mouth disease RNA then we could stop the virus from spreading, causing further cell death and reduce symptoms of the disease such as high fevers, swelling of the genitals, blisters inside the mouth and on the feet and more severely to stop dramatic weight loss which can lead to all sorts of other body malfunctions starting with malnutrition. This process of RNA interference (described in the introduction) will halt the virus production, however, it is not a cure and the virus will continue to survive inside the cell unless the cell can recognize the original virus (and the viruses already produced before RNAi was activated) as foreign and destroy them.

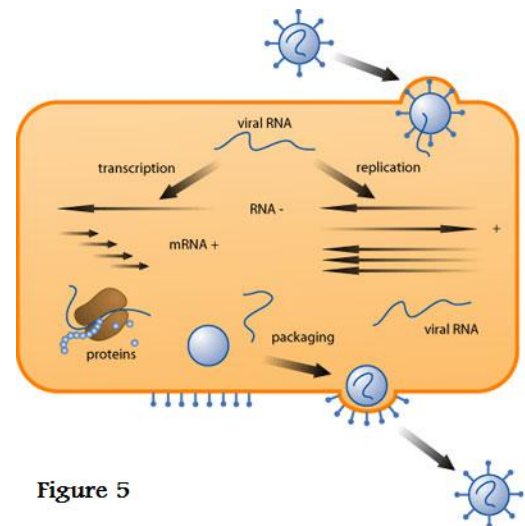


Figure 5

The first issue to address is to activate the secondary immune response in higher animals,

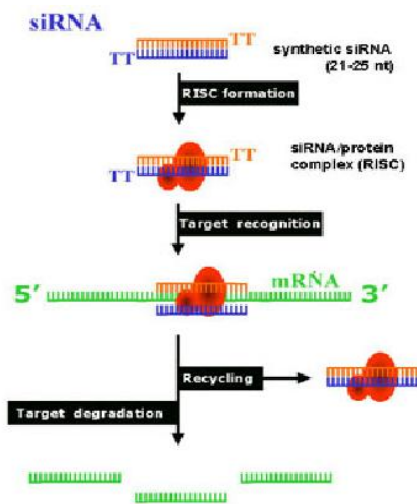


Figure 6

without triggering the primary inflammatory response like a virus would do. This can be done by one distinct and proven pathway of introducing/injecting short double strands of RNA into the cells, generally smaller than 23np (nucleotide pairs) in length. These RNA structures could be either siRNA or micro-RNA which activates RISC and DICER enzymes within each cell injected, and then the cell will destroy viral RNA and cease production of the proteins being synthesized; (Figure 6). Once the siRNA is introduced into the cell with the specific fingerprint of the foot and mouth virus the RISC enzyme is activated. RISC then copies the fingerprint of the siRNA and uses this specific sequence to bind to target mRNA (when silencing a gene), in viral cases the RISC will bind to the matching RNA of the virus, breaking the RNA down further, this cleaved RNA strand is then broken down by other enzymes within the cell thus silencing the expression

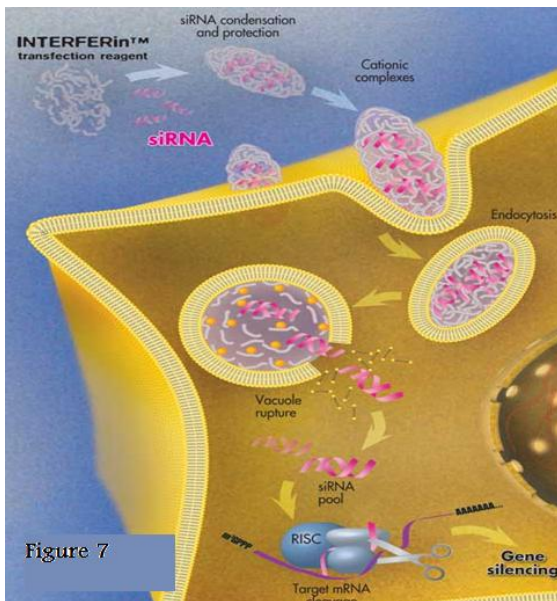
of that gene or viral infection.

So why hasn't this type of immune response been widely used before?

There are many problems with this type of viral defense such as, it is very expensive to manufacture specific siRNAs as laboratories and experienced scientists are needed in large quantities. We also need ways to inject every infected cell with an siRNA form of a virus, and we would also need to keep producing different strains of a siRNA for a disease; because a virus' RNA mutates and these mutations makes the previous siRNA completely useless as the sequence of nucleotides no longer matches the viral RNA; so the RISC enzyme can no longer bind to the target RNA. So how do we

know exactly which strain of siRNA we need to synthesize without taking samples and being specific to one organism at a time? Finally, this method of RNAi doesn't always cure a disease so how do we destroy this virus? If we can't destroy it then how do we keep the transcription process halted in an animal; to stop the effects of a disease until a cure has been found; or the animal dies through another route such as heart disease or fragility of the body usually caused by multiple mutations in DNA due to old age.

The main way we could introduce siRNAs into cells is transfection, this method is proven to effectively activate RNAi and the effects of siRNAs in target cells are shown, the effectiveness of transfection depends on many factors mainly how long the cells are subjected to the siRNA and how concentrated the siRNAs are within the mixture of substances injected into the body of the animal; (Figure 7).



This method has the potential to introduce siRNA to all cells requiring them but also to all cells around the injected body parts which would activate the RNAi response and if the siRNA has the same sequence of nucleotides as mRNA within the cell then the introduction of foot and mouth siRNA could stop the cells from expressing certain genes and thus cause the RNA to mutate causing expression of faulty or 'nonsense' genes which no longer code for anything they just take up space and energy within the cells. However this type of interference within cells is very rare as the siRNA is manufactured in a sequence-specific manner to match the dsRNA of the foot and mouth disease virus, this rarity decreases as the FMD virus mutates; changing the combination of nucleotides in the RNA which gives a greater chance of eventually

matching the nucleotide chain combinations. So by circulating siRNAs all around the body we have a great chance of halting the transcription process in all infected cells providing the siRNA can enter the cells efficiently thus depending on what membrane-entering substances the siRNA is combined with.

Current research shows that mutations in viral RNA is completely random and we can't artificially match these changes unless we are very lucky, destroying mRNA of a cell is constant and there aren't different strands of the same genes so it is easier to maintain gene silencing inside cells, however viral diseases are external and mutate far more rapidly, creating lots of different strains of a disease, so when viral RNA mutates we wouldn't be able to control the effects of the disease, and as we have deactivated the primary inflammatory response temporarily the virus is free to replicate inside the cell and spread over the rest of the body, so this method of curing a disease/ halting the virus from replicating would actually leave the body of the infected animal open to other forms of the virus if we created the wrong siRNA complex, thus rendering the body completely useless and possibly causing a disease to have a far worse effect than what it should have done.

In order for RNAi to destroy a viral infection firstly the spread of the viral RNA would need to be halted which results in the virus being contained within a few cells, this is done through RNA interference by introduction of FMD siRNA into the cells, once inside, the interferon response is activated so the RNA; with complimentary base pairs of the siRNA strands (attached to RISC); are cleaved so then the sections of the viral RNA are seen as aberrant and destroyed by an enzyme in the cell. Once all the RNA is destroyed or broken down in the cell the virus can not replicate and the original RNA from the virus is also cleaved; and destroyed when recognized as aberrant. This doesn't always cure a disease though because not all cells will allow siRNAs to enter, cells like nerve cell are very sensitive and if they become infected we would struggle to introduce siRNA into

them, this means the disease wouldn't be cured. However, if we could keep levels of siRNA in the body then after a certain period of time the viruses in the nerve cells would multiply that much, then burst out of the host nerve cells into other types of cells where siRNA can enter and activate RNAi within those cells, to disintegrate the viruses. Obviously this type of cure would be potentially dangerous in case the viruses progressed into other nerve cells and cut out the nervous system in the body, which could lead to Axonotmesis; a nervous disorder caused by nerve damage possibly through a viral infection. Eventually the viral disease would be killed off through RNAi however the longer it takes to cure a virus infection this way the more prone a person is to undergoing another disease caused by effects of the targeted virus, and this secondary disease could possibly cause disability or even death.

The actual process of creating siRNAs is inexpensive and they can be produced in masses which is great for the economic side of drug production, but these siRNAs need to be combined with other chemicals which can transport themselves inside cells, so the choice of chemical to combine the siRNA to can be expensive. Some forms of viruses can also harbor synthesized siRNA which then introduce themselves into cells through invasion, this process is more effective than the usual transfection process used in RNAi at the present time, but with all these methods there is no guarantee of success so repetition must be covered for in the budget, thus making each individual treatment more expensive than it could be. As the animals which are mainly infected by foot and mouth disease are cattle, the farmers who own these livestock will be intending to sell the animal or the animal's by-products, if these animals were left to die or killed the farms would lose out on thousands of pounds and in case of an epidemic billions would be lost, despite the fact most animals recover and FMD is a non-fatal disease. In 2001 in the UK £8bn was lost through supporting farmers and agricultural land to eradicate the disease thus making the process of RNAi (although it could be highly expensive) more feasible and gain backing from animal welfare groups and from the government to save more in their budget. Like any new course of treatment for a disease it would need to be tested first before being mass produced and widely used to ensure it is safe and can be used with little or no side effects. The trial period would be expensive as it would involve infecting an animal on purpose to test the cures, once it has passed the laboratory stage it can be trialed on farm animals which could then pave the way for veterinary surgeons to be frequently sewed if the administration wasn't effective or if the animal still proceeded to die. There are many different ways in which this process would become expensive very quickly, including those environmentalists with ethical issues against animal testing trying to halt the production or discovery of the cure.

Most animals will recover from FMD and lead normal lives, maybe cattle will produce less milk, however they would be considered healthy. But to stop this disease spreading the government will burn all infected animals, section infected areas off and ban trade and transport of livestock to ensure the disease is contained. This seems drastic at first and totally barbaric against the infected animals however, the high fever and blisters would make it very painful and uncomfortable to walk around; eat and drink; and even painful to sleep. Also this disease can also cause secondary infections, if the blisters burst; which is quite common thus more pain will be inflicted on the animal, the foot and mouth disease is also known to lead to myocarditis which is inflammation of the heart muscle. Another issue with allowing the animals to recover is that some of these animals remain asymptomatic meaning they show no signs and no symptoms of the disease but transmit the FMD virus to other animals.

All this does support the killing of animals affected by the Foot and Mouth disease but if we could offer an alternative route; one which cures the animals and does so in a humane manner we could please everyone. By using RNA interference we can stop the symptoms of this disease and the animals will eventually recover from FMD without any pain or discomfort (besides injection), even though a few animals would need subjecting to testing, there would be an overall decrease of deaths and reduction in pain and total eradication of the virus. Eventually we would be able to cure any disease through the same methods, we would also be able to halt gene expression within a cell the

same way as we halt gene expression of a virus, meaning we could live longer on average due to reduction in deaths due to disease and we could even halt malfunctioning/mutated gene expression; and manipulate the characteristics of every animal; eradicating deformities.

Conclusion

RNA interference research could quite possibly be the solution to the vast majority of problems related to every species; including humanity. From a veterinary point of view this type of cure seems perfect as animals do not possess the same legal rights as humans, meaning we can test and almost force this cure upon some species with little resistance. As this type of research could harm animals before we perfect the cure, we must be conservative in the early stages and administer this treatment to terminally ill animals and those which are already sentenced to death, this is because of animal welfare, and we do not wish to inflict unnecessary pain on an animal, but do the opposite. If we can administer treatment which is the better of two evils e.g. the animal is dying painfully with a slight chance of survival, against administering possibly a life saving drug which might temporarily inflict more pain, then many people would agree with this treatment method.

The key to success with RNAi is siRNA, these trigger the interferon response which is needed to suppress a virus and perhaps stop a disease. We can produce siRNAs in large amounts in laboratories without harming any animals or going through a costly procedure, thus meaning this method of treatment is environmentally friendly so far. The problems and issues show when RNAi is initiated within test animals because then side effects and what is considered as unnecessary pain is administered.

This process could be administered to animals carrying the FMD virus, destroying all the genetic material within the virus, thus halting the production of the virus' genetics, eventually all animals will be free of FMD, so not only will animals survive a lot longer but we could be able to completely eradicate the virus thus causing this disease to become extinct. Once we have cured a strain of FMD we can then focus on curing other strains and even other diseases to eventually form a world without disease. Viruses are being created all the time by evolving and mutating, so we would never destroy every virus however we could make it almost impossible for a virus to harm or kill an animal.

Some diseases are caused through faulty gene expression originating from DNA mutations e.g. Huntington's disease. If we could stop a disease by halting transcription of a virus' RNA then we could also halt transcription of our own genes, if we could halt the production of faulty genes then we could stop these genetic mutations from expressing themselves, this is just an example of what else we could do with RNAi technology. Just like scientists had discovered how to alter gene expression in plants around the 1980's, we could alter our own gene expression to produce the same results. RNAi was discovered by trying to make petunias a deeper purple, by injecting more of the purple pigment genes into cells. A few of the flowers turned out as planned however some turned out to show un-pigmented areas of the flower, this is now known as co suppression. By using this information we could be more selective about characteristics of a species without using the selective breeding process, if we were able to control which genes were suppressed too we could halt co suppression and finish with the result we expected almost every time.

So to summarise, we could cure the symptoms of any disease and eradicate any virus; we could 'turn genes off' to discover which genes code for what expression; and we could theoretically create an animal with the perfect required characteristics; and everything in-between... meaning the possibilities are quite literally endless!

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