RNA interference in curing linear keratosis in equines

By
Stephanie Neild

PASS WITH MERIT

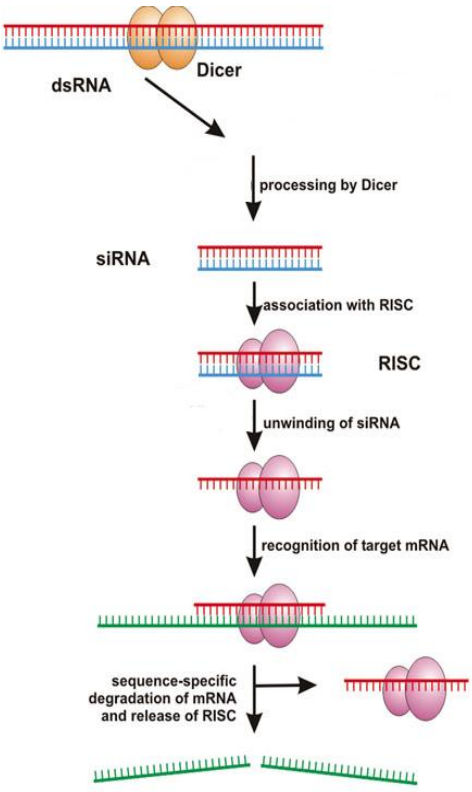
Research paper based on pathology lectures at Vet-medlink 2009

RNA interference is a relatively new discovery in genetics (1998) even though it is taking place in organisms all the time as a defence mechanism. I plan to write this paper on how RNA interference could possibly cure a disorder in equines, which is believed to be genetically based. Its symptoms include vertical linear areas of alopecia (hair loss), scaling and crust formation on the sides of the neck, shoulder and chest. It is not painful but it could destroy a horse's career as a show animal. I want to concentrate on this disease because I own a horse that suffers from the condition (Fig 1- although this is not a severe case of the disease it is very noticeable) and would possibly make it an easier topic think about. Another topic similar is cannon keratosis, which I will also be mentioning as it has similar symptoms.

Fig 1:This is an image of my horse. It clearly shows how the condition manifests itself

<u>*An overview of the genetic code*</u>

DNA is the genetic code. It codes for the sequence of amino acids that make proteins. It is made up of a deoxyribose group, a phosphate group (which form the sugar-phosphate backbone), and bases, either Adenine, Thymine (or Uracil in RNA), Cytosine, or Guanine. In mammalian DNA there are always equal amounts of A and T, and C and G. Base pairs are joined together by hydrogen bonds. This forms the double helix we are familiar with.

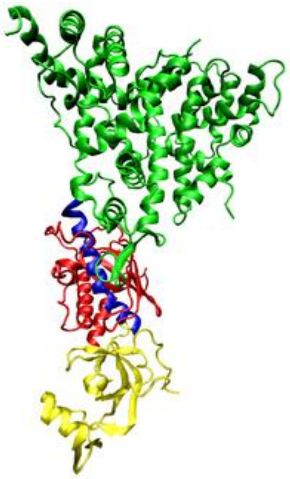


Interference response

RNA is a single strand of DNA. It is called RNA because it is made of ribose not deoxyribose. This is what it formed during transcription, as the DNA never leaves the nucleus. During transcription the DNA spirals separate using the enzyme RNA polymerase. Free nucleotides join to the exposed bases. This forms an exact complementary strand of the original section of DNA, which is called messenger RNA, or mRNA for short. The mRNA travels out of the nucleus through pores to the ribosome where it is translated into the sequence of amino acids that are joined (peptide bonds) to form proteins.

The idea behind RNA interference is that if the RNA never arrives at the ribosome then production of that protein could be stopped. This would mean that any gene could be switched off at any time making a cure for almost every genetic disease where it is the protein malfunction causing symptoms (for example, is would not cure cystic fibrosis) or any virus. Viruses only have a single strand of RNA as genetic material, so when the virus invades the cell, the virus could be switched off so it wouldn't multiply and infect other cells.

RNAi was first discovered in petunias when researchers wanted to make them a deeper purple colour. They thought that adding more of the gene which controls the purple colour would make the plant have a deeper colour but when the plant grew it was found to have less of the purple colour and instead it was replaced by white. This is an example of co-suppression. Some cases of this resembled the effect of RNAi. It has been shown that co-suppression happens when a single strand of a particular gene



Dicer structure

is added to the original DNA. This it thought to make the transgenes (dsRNA) cause silencing of both the transgene and the homologous endogenous gene (mRNA) therefore inducing specific-series silencing of the gene and eliminating the colour rather than darkening it. RNAi works in a similar way but instead of the single strand injected it is a double strand (dsRNA). A single strand is not shown to work in mammalian animals because it is known to degrade and therefore become ineffective in gene silencing. This silencing takes place after the dicer (a member of the RNase family of nuclease enzymes that specifically cleave dsRNA) cuts the injected dsRNA into 21-25 RNA species called small interfering RNA, or siRNA. This siRNA then bind to the complementary mRNA and tags it for destruction so the gene is silenced.

Discussion

As mentioned earlier, Linear keratosis in equines is a linear, usually vertical area of alopecia and crusting on the neck and shoulder area. Cannon keratosis is similar except it is located on the cannon bones instead of the neck. There is a brindling effect that is connected to linear keratosis and this is hereditary and therefore it is known to be genetic. It is usually apparent in lighter breeds and in particular, thoroughbreds and quarter horses. As of yet there is no known cure for the disease but through RNAi research there may be one in the future.

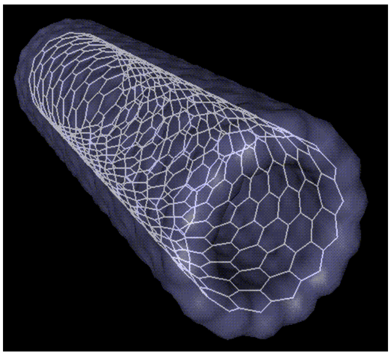
Mammals are slightly more complicated than plants. They have the dicer and the RISC (RNA induced silencing complex) interference response that works in the same way as plants, but, there is also an interferon based inflammatory response which shuts down all protein production not just the silenced faulty gene. The inflammatory response occurs when a strand of dsRNA is detected in the cell. It only occurs when the strand is more than 23 nucleotides long. A solution to this would be to inject a dsRNA shorter than 23 nucleotides. The inflammatory response is not stimulated. Instead the dicer and RISC are stimulated, which destroy the mRNA and any identical to it, silencing the gene so the protein cant be made. The dsRNA is introduced into the cell. It binds to the dicer and it chopped into short strands. This separates then one strand is loaded onto a RISC complex. This then binds to the post-transcription mRNA through base pairing and identifies it so it is cleaved and so that particular protein can't be synthesised. The remaining mRNA carries on to the ribosome for translation, and protein production continues as normal.

Linear keratosis is known to be genetically based. If the gene is isolated it could be copied and the dsRNA injected into the cell. The interference response would then take place destroying the gene that has been added, and the original endogenous one on the mRNA. Problem solved!

…Or maybe not. This would work if it was just one cell affected, but by the time the disease has become noticeable there are millions of cells with the faulty gene. It would be impossible to inject every cell with the dsRNA. One solution would be to take some affected tissue cells, inject dsRNA with a very thin hollow glass needle and culture them so that they undergo mitosis without the faulty gene. Then implant the cultured cells back into the horse’s neck as a skin graft to allow granulation (new skin growth). This would replace the damaged cells and multiply to form new skin and because the cells are ‘self’ there would be no chance of the graft being rejected. A disadvantage of this would be that it might leave scarring and would not improve the overall look of the horse when it isn’t causing the horse any trouble in the first place. There is also the risk of infection as it is an open wound, which would further damage the tissue and contribute even more to scarring. Also if it doesn’t work then some dsRNA will need to be kept so some of the cultured cells could be stored by chilling/freezing until needed again. This may be a possible solution.

If this disease is definitely hereditary then when a mare is going to be put in foal and is a carrier the disease, the oocyte could be removed from the uterus. It could then be fertilised in vitro and implanted back into the uterus. It could then be removed again and the dsRNA could be injected into the blastocyst when the embryo is about 5 days old (consisting of about 30 unspecialised cell) meaning that all of the cells can be targeted. This would mean the gene could be silenced before cells become specialised therefore completely removing the faulty gene from the gene pool to prevent the condition being passed on through generations.

Another way of getting it to the correct cell could work much like a magic bullet used for cancer treatment. An enzyme could be implanted at the affected tissue and the dsRNA could be injected into a liposome which is like a vesicle consisting of only a phospholipid bilayer, coated with something that only that enzyme would break down and then injected into the blood stream. When it travels past the area with the enzyme, the coating is broken down and the dsRNA is released and absorbed into the cells in close proximity to the enzyme.



A very new delivery method has been discovered which involves attaching the dsRNA to carbon nanotubes. This was discovered when trying to use RNAi to combat HIV/AIDS. At first the idea was to load the virus protein coat with the dsRNA then inject it into the bloodstream so it will get into cells. But there was a major fallback. The virus couldn’t enter T-cells which is where the HI virus is located. Nanotubes however, could enter the T-cells and ‘you have more control over the drug if you give it as a drug rather than as a viral vector’. The nanotubes could be loaded with the dsRNA for the linear keratosis faulty gene and injected into the blood stream. It binds to receptors on cells and the dsRNA is released into the cell. The nanotubes however will have to be made for specific cells eg. Skin cells, as it has to be complementary to bind to receptors.

There are ethical concerns involved with this research into RNAi as this is altering the natural course of the mRNA from the nucleus to the ribosome. Things could also go wrong and the wrong dsRNA could be injected into the cell, which could cause different, necessary genes to be silenced, which could cause harm to the animal. The delivery method may be unsafe depending on the delivery method chosen, so putting the animal at unnecessary risk. It may not be seen as a necessary treatment to some as the horse isn’t in pain in any way and can live a perfectly normal life. It would only be for cosmetic reasons. Animal rights activists would be against the idea of the experimentation on animals that would have to happen for the treatment to become legitimate. This is the time when things are more likely to go wrong and before techniques become refined.

There are other treatments for the disease but they do not cure it, they just hide the symptoms for a short period of time. One treatment is hydrocortisone cream applied to the affected area. This removes the crusting when the horse is next washed thus reducing the visibility of the condition for competition purposes. Another option would be to have hydrocortisone injections under the skin, which has slightly longer lasting effect than the cream. These are only temporary and the crusting soon returns when cream stops being used or the injection wears off. The RNAi could cure it completely so there is no need for further treatment, and potentially no scar tissue. This treatment may not take priority, as the condition does not affect the horse’s wellbeing. Research into other areas of veterinary medicine may come above this condition in priority as they could be immediately life threatening such as colic or laminitis.

Conclusion

It is clear that there are negative aspects to the treatment but it is possible that there are many positive outcomes that could apply to linear keratosis and other mammalian genetic disorders. This could even go to the extent of curing the most life threatening human diseases although there would be serious ethical concerns as it is believed by human rights activists that ‘we should not mess about with human life’. The treatment also has to be economical to provide. If it is too expensive then horse owners will not want the treatment and only very few will be able to afford it. Because it is seen as cosmetic then insurance companies wont want to cover the cost so the price of the treatment must be cheap.

In years to come it may become possible to use RNAi as a routine treatment in treating genetic disorders depending on how much development there is in this treatment. At the moment the treatment hasn’t been tested and there may be side affects which haven’t been expected, for example, how to get the carbon nanotubes out of the bloodstream. This will have to be overcome before the treatment becomes possible.

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