

Possible Future Applications  
of RNAi in  
Veterinary Medicine

By  
Emily Beachill

PASS WITH MERIT

Research Paper  
Based On  
Pathology Lectures  
At Vet-Medlink 2009

## **ABSTRACT**

RNAi is the mechanism by which endogenous genes can be suppressed, via cleavage of RISC and mRNA by the enzyme dicer. The potential for use of RNAi in veterinary medicine is massive. Some of the possibilities include combating viruses, treatments for cancer and use in veterinary research. Yet currently there are still problems to be overcome before widespread use of these treatments can ensue. In mammals, dsRNA produces an interferon response, so only the use of siRNA is viable. There is still a question as to how to administer the siRNA to all cells requiring it, and the question of safety, target specificity and side effects. Still, early RNAi research is promising, and RNAi is set to become a major tool in the future development of medicine. In this paper, I intend to give an explanation of current RNAi research and consider specific possible uses of RNAi in veterinary medicine, along with the problems that must be overcome before these treatments can be used.

## **INTRODUCTION**

In normal cellular function, a gene being expressed results in a protein. Genes are sections of DNA, which code for particular amino acids to make particular proteins. This genetic code is expressed in three-letter 'words', called codons, which are derived from a sequence of three nucleotides. Codons are transcribed from the DNA onto messenger ribonucleic acid (mRNA) by RNA polymerase. This then travels to the ribosome, where the codons are translated to form amino acids and so proteins.

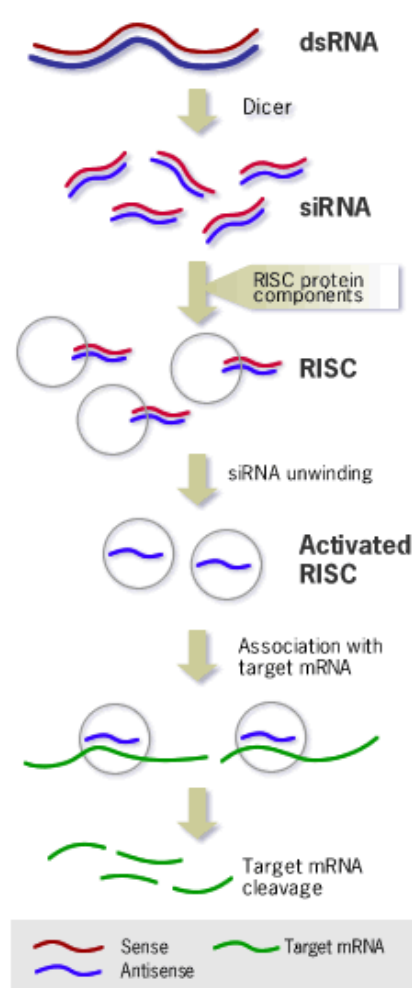


Figure 1 – diagram detailing the RNAi mechanism in plants.

One of the first observations of RNAi was in the results of experiments performed by plant scientists, in which copies of the key gene for pigmentation were introduced to petunia plants (Napoli, et al., 1990). The plants are normally of violet or pink pigmentation, and the introduction of more of the pigmentation gene was expected to deepen their colour. However, instead the resulting plants were partially or fully white. At the time this was called 'co-suppression of gene expression', and the exact mechanism was unknown. In 2006, Andrew Fire and Craig Mello won the Nobel Prize in medicine for their work on RNAi and their paper 'Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*' (Banerholt, 2006).

In plant cells, the presence of long double stranded RNA (dsRNA) triggers the RNAi pathway. dsRNA can be endogenous, or exogenous, coming from viral forms containing dsRNA. It is thought that initially the RNAi pathway provided defence in cells against viruses such as these. The presence of dsRNA activates the enzyme dicer, which cleaves the dsRNA into short interfering RNA (siRNA). These siRNA typically consist of 20-25 base pairs. In turn, these siRNA activate RNA Induced Silencing Complex (RISC). The siRNA are separated into single strands, and their bases pair with complementary bases of their target RNA, which induces cleavage of the target RNA by dicer.

Figure 1 (Ambion) – above left – shows this process. If the dsRNA was endogenous, target mRNA would be destroyed, inhibiting translation and the production of protein for a particular codon, thereby silencing a particular gene. If the dsRNA was exogenous, target RNA from the virus will be destroyed, thereby protecting the cell. This process is demonstrated visually in the animation produced for Nature Reviews by Arkitek.

In mammalian cells, this viral defence is not employed, although RISC and dicer are present. The presence of foreign dsRNA triggers a complex interferon response, which inhibits protein production within cells. However, siRNA can use the RNAi pathway, triggering the interference response whilst avoiding the interferon response. Therefore if RNAi therapies were to be used in mammals, only administration of siRNA would be effective.

Suggested therapeutic uses for RNAi have included use in combating viruses, use in treating cancer and use against neurodegenerative diseases. Currently, further research is needed before RNAi therapies could be used. There are problems with the specificity of siRNA. These problems often arise when dsRNA used contains repetitive sequences, and so the siRNA produced from its cleavage can base pair to several mRNA with similar sequences. This would inhibit the expression of several genes, which may lead to severe consequences. It has been estimated that 10% of siRNA may produce this effect (Shibin Qiu et al). There are also problems in administering siRNA to all cells requiring it, to generate effective therapy. Another concern may be that over use of RNAi pathway may damage the cell machinery, affecting normal protein production. Further experimental tests would have to be undertaken to address such problems before RNAi could be applied therapeutically.

## **DISCUSSION**

The future development of RNAi therapies in medicine and veterinary medicine could be a major advancement of recent times, as the number of diseases that could be effectively prevented or treated is huge. One way in which RNAi could be used therapeutically in veterinary medicine could be to treat viral diseases, such as foot and mouth disease.

Foot and mouth disease (FMD) is a highly contagious infection that affects cloven-hoofed animals, including major domestic species such as cattle, sheep, pigs and goats (World Organisation for Animal Health, 2002). It can be fatal, and often in outbreaks large numbers of livestock must be slaughtered in an attempt to prevent the spread of the disease. Therefore economically in the farming industry, foot and mouth is a serious disease.

The virus that causes foot and mouth disease (foot and mouth disease virus – FMDV) contains a small, single stranded RNA genome. Upon contact with the host cell, FMDV will bind to a receptor site on the cell membrane, which results in the infolding of the membrane. Inside the host cell, the single stranded RNA is translated at the ribosome, producing new viral RNA and viral components, and also inhibiting the endogenous production of proteins. The new viral RNA and other components form new FMDV viruses, which will bust out of the host cell, destroying this cell and leading to possible further infection of cells.

RNAi could be used here to prevent or treat this virus. Administration of siRNA homologous to the viral RNA could prevent the translation of the viral RNA at the ribosome. RNA matching the siRNA codon sequence would be cleaved by dicer, thus preventing the synthesis of new viral RNA. This treatment would prevent production of new viruses, so would reduce the risk of further infection. It would also destroy any viral RNA present in the host cell, preventing further damage to the host.

The limitations of this therapy may be that it would be hard to ensure dicer cleaves every single viral RNA; therefore further infection may take place. The siRNA would have to be administered more than once to the cells over a period of time, or kept at a low constant level, to ensure that all viral RNA is destroyed. However even then it could not be guaranteed that all viral RNA is destroyed, although production of new FMDV would probably be low enough for the animal's immune system to combat it. Also, as yet there are not any ways of administering the siRNA suitable to several, practical administrations over a period of time.

It may be possible that in times of outbreak and so likely potential infection, a vaccine containing the FMDV specific siRNA could be administered to animals at risk. Therefore if infection occurred in these vaccinated animals, there would be a ready supply of siRNA to combat the viral RNA. This may reduce the severity of the spread of a foot and mouth disease outbreak.

Limitations would be that there are several strains of FMDV virus, and the siRNA could only be specific to one, so would only protect against one. Also, there is currently a vaccination available for foot and mouth disease, so an RNAi based vaccine may be redundant. The current vaccine uses inactive FMDV, which allows the animal's immune system to recognise the antigen for this particular virus and produce memory cells, which prevent future foot and mouth disease in the animal. An advantage of using siRNA is that viral RNA is actually destroyed, whereas the current vaccine merely prevents the animal developing clinical signs of the disease. If the current vaccine is used, the country cannot claim FMD-free status even if the vaccinated animal does not become infected, so trade restrictions will apply (DEFRA 2008). With a siRNA vaccine, if the vaccinated animal does not contract FMD, the country can still claim FMD-free status, so this could be an advantage to using siRNA.

Another application of RNAi in veterinary medicine could be to treat cancer. RNAi could be used to inhibit expression of oncogenes; these are genes that contribute to a normal cell becoming a tumour cell. Often, an oncogene is expressed at high levels, or is mutated, which results in it becoming active. Many oncogenes are involved in gene expression or cell differentiation. Some of these oncogenes result in neoplasia. This is the irregular proliferation of cells. (Brenner S. et al 2002) This excessive cell growth results in the growth of the tumour, and the possible spread of the cancer.

One oncogene associated with the proliferation of tumour cells is the gene that codes for survivin (Pennati M. et al). The survivin protein has been found in high quantities in human tumours, but is absent in non-cancerous differentiated cells. Survivin inhibits apoptosis (programmed cell death) in cells. Apoptosis is not caused by trauma (as necrosis is), but is instead regulated in a way to benefit the organism. For example,

a cell infected with a virus may undergo apoptosis, which will prevent the spread of the virus in the organism. When a protein like survivin inhibits apoptosis, the result is uncontrolled growth of the cell, resulting in a tumour.

If the gene responsible for the expression of survivin could be identified, siRNA could be produced and administered to inhibit the expression of survivin. This would reduce tumour growth, which may prevent spread of the cancer. If cancer was spotted early and treated with survivin gene specific siRNA, it may be possible to reduce the severity of the cancer growth, and so increase the chance of survival. This RNAi treatment for cancer may have advantages to current cancer treatments available, such as chemotherapy. Chemotherapy targets all cells that divide rapidly, inhibiting their proliferation. This affects not only tumour cells, but also non-cancerous cells in the bone marrow, hair follicles and digestive tract, so results in unwanted side effects. A siRNA treatment would not result in these side effects, as it is specific only to the production of survivin. It has been shown RNAi can be used to effect survivin production (Beauchamp L. et al).

Again, before this treatment could be used, an effective method of delivering siRNA to all cells requiring it for a period of time would have to be researched. Also, survivin may not be the only protein produced by an oncogene resulting in proliferation of a specific tumour, so siRNA specific to survivin may only be partially effective. However, if all oncogenes and their proteins in a tumour could be identified, treatment containing siRNA for each could be administered.

Other oncogenes that could be targeted include genes that code for Ras type proteins. When these proteins are activated, one of the results is cell proliferation. A mutation in a Ras gene that leads to it being permanently activated can therefore cause uncontrolled cell growth and division, resulting in neoplasm. Ras genes with activating mutations have been found in 20-25% of human tumours (Julian Downward, 2003).

RNAi could be used to inhibit expression of mutated Ras genes in the same way it could be used to inhibit the expression of the survivin gene, instead using siRNA specific to the mutated Ras gene. This treatment for cancer would experience the same limitations as the treatment targeting survivin, but also it may be more difficult to identify the siRNA required to inhibit a mutated Ras gene, as there may be different mutations.

A further possible use for RNAi would be in veterinary research. By inhibiting expression of a particular gene via the RNAi pathway, scientists can decrease the amount of protein produced by this gene. By observing how this decrease affects a cell or organism, scientists can better understand the function of a particular gene and its product. As well as increasing understanding of veterinary genetics, this may lead to developments in treatments for genetic diseases, or in diagnosing what causes a particular genetic disease.

Experiments using RNAi in this way would have to ensure that the siRNA they introduce to the cells they which to observe is very specific to the gene they wish to inhibit. If the siRNA inhibited more than one gene's expression because of similarity to another's mRNA, results would not give an accurate picture of the function of the

gene of interest. Again, to observe the particular affects of decreased expression of a gene, first the gene of interest would have to be identified so that complementary siRNA could be produced.

There are some ethical issues raised by the use of RNAi. If RNAi were to be used therapeutically in human medicine, human clinical trials would have to be carried out. This raises ethical issues on the safety of RNAi to be tested on humans. Currently there are problems with off target effects, safe delivery methods and toxicity of RNAi therapies, and these would have to be overcome before human clinical trials could be ethically accepted, as otherwise the trials would probably result in more harm than good, and ethical principles accepted are generally based on not causing harm and promoting good. The same issues would apply if RNAi were to be used in veterinary medicine, but for the species targeted.

Another ethical issue may be that RNAi could be viewed as genetic engineering. This is a controversial issue that raises many ethical concerns. Some people are against genetic engineering as they feel we do not have enough knowledge to interfere with genes safely, and so therapies such as RNAi may be viewed as unethical from this perspective. Also, many people are against genetic engineering because of spiritual or religious reasons, and so may also feel RNAi is not ethical. However many people are for genetic engineering when it is used to treat and cure diseases and so benefits many people, and so from this perspective the use of RNAi therapeutically would be ethical.

## **CONCLUSION**

In conclusion, future developments of RNAi in veterinary medicine could include: treatments for viruses such as foot and mouth that work by preventing replication of viral RNA; treatments for cancer that inhibit the expression of oncogenes; and use in veterinary research in helping to increase knowledge of gene function. However there are limitations with all of these possible developments, and further research is needed before any could be developed. There are also ethical issues that must be considered, such as safety. One of the main problems is administration of siRNA to cell requiring it, as well as the need to keep a low, constant level of siRNA in the cells for the duration of the treatment. There is ongoing research into how best to administer siRNA (REFERENCE) that may solve this problem in the future. Another challenge is to identify the genetic code of the target gene, so that it is possible to produce complementary siRNA. If these problems can be overcome, RNAi could provide effective treatment for a number of diseases in veterinary medicine.

## Reference Sheet

Ambion, “The mechanism of RNAi” – Figure 1.

Available at: [http://www.ambion.com/techlib/append/RNAi\\_mechanism.html](http://www.ambion.com/techlib/append/RNAi_mechanism.html)

Arkitek, Nature Reviews ‘Animation of RNAi mechanism’.

Available at: <http://www.nature.com/focus/rnai/animations/index.html>

Baneholt, D. (2006) “The Nobel Prize in Physiology or Medicine 2006”

Available at: [http://nobelprize.org/nobel\\_prizes/medicine/laureates/2006/adv.html](http://nobelprize.org/nobel_prizes/medicine/laureates/2006/adv.html)

Beauchamp L, Batten D, Magdaleno S, Krebs J, Cheng A, Ford L, Gee M. and Khodier C., “Delinating the role of survivin in oncogenesis: an SiRNA study”

Available at: <http://www.ambion.com/techlib/tn/134/1.html>

Brenner S, Horvitz H.R. and Sulston J.E. (2002) “The Nobel Prize in Physiology or Medicine 2002”.

Available at: [http://nobelprize.org/nobel\\_prizes/medicine/laureates/2002/](http://nobelprize.org/nobel_prizes/medicine/laureates/2002/)

Julian Downward (2003), “Targeting RAS signalling pathways in cancer therapy”.

Available at: <http://www.nature.com/nrc/journal/v3/n1/abs/nrc969.html>

DEFRA (2008), “FMD: Disease control and vaccination”

Available at:

<http://www.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/fmd/control/index.htm>

Napoli C., Lemieux C., and Jorgensen R. (1990) “Introduction of a chalcone synthase gene into *Petunia* results in reversible co-suppression of homologous genes *in trans*”. *Plant Cell* **2**: 279-289.

Available at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC159885/?tool=pubmed>

Pennati M., Folini M. and Zaffaroni N. (2007), “Targeting survivin in cancer therapy: fulfilled promises and open questions”.

Available at: <http://carcin.oxfordjournals.org/cgi/content/abstract/28/6/1133>

Shibin Qiu, Coen Adema and Terran Lane (2005) “A computational study of off-target effects of RNA interference”.

Available at: <http://nar.oxfordjournals.org/cgi/content/abstract/33/6/1834>

World Organisation for Animal Health (2002), “Foot and mouth disease”

Available at: [http://www.oie.int/Eng/maladies/fiches/a\\_A010.htm](http://www.oie.int/Eng/maladies/fiches/a_A010.htm)