

POTENTIAL APPLICATIONS OF RNA INTERFERENCE  
TECHNOLOGY

BY

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## ABSTRACT

RNA interference is an innovative technology that could potentially cure many diseases, stop viruses and save endangered species. It is a natural process, initiated by the enzyme Dicer, where siRNAs are loaded onto the RNA-Induced Silencing Complex which destroys the homologous mRNA. This inhibits gene expression and could revolutionise the way we treat disease. The purpose of this paper is to explore the possible future applications of RNAi treatment and the limitations faced by researchers into RNAi. It explores the possible treatment of four conditions (Feline Immunodeficiency Virus, Hepatitis B virus, Lethal White Syndrome in foals and Charcot-Marie-Tooth disease) with RNAi and identifies possible ethical issues involved.

## INTRODUCTION

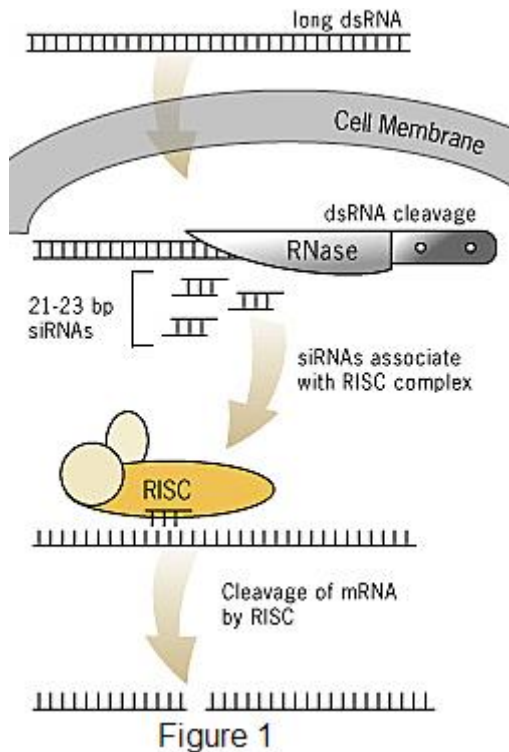


Figure 1

RNA interference (RNAi) was first described in 1998 by Fire et al who inserted double-stranded RNA (dsRNA) into the nematode worm, *Caenorhabditis elegans*, and found that it triggered a sequence-specific silencing process (1). This is initiated by the RNase type-III enzyme, Dicer, which cleaves dsRNA into smaller fragments and loads it onto the RNA-Induced Silencing Complex (RISC). This cleaves the fragments and degrades the homologous transcript, preventing expression of the encoded protein (2), as shown in Figure 1. However, in vertebrates, the presence of dsRNA triggers an interferon-based inflammatory response which leads to cell death (3). This means the use of long dsRNA strands to induce RNAi in most mammalian cell types is impossible. However, smaller strands of dsRNA, called siRNA (20-25 nucleotides in length), are able to be used in RNAi without an anti-viral response (4). This discovery

stimulated a lot of research, and in 2003 gene silencing was first done in vivo when Soul et al used it to protect mice against fulminant hepatitis (5). It has since become clear that RNAi is a powerful genomic tool.

Further research into the use of RNAi in veterinary medicine found that it significantly increases the latency period of ALS (amyotrophic lateral sclerosis) and delays the progression rate of the fatal motor neurone disease (6). This therapeutic potential was also demonstrated in many other neurodegenerative diseases, including Alzheimer's, spinocerebellar ataxia and Huntington's disease (7). Additionally, RNAi has been shown to be effective in protecting mice against respiratory and vaginal viruses (8) (9) and effective in inhibiting the replication of the foot-and-mouth disease virus by

significantly weakening the cytopathic effect (10). These are a few examples of the wealth of research involving RNAi in the veterinary field.

RNAi research has many important advantages over other methods of gene expression inhibition (e.g. antisense therapy, catalytic RNA and DNA molecules, and homologous recombination) as it is effective with a broad range of cell types, is gene-specific and is a natural process meaning all cells contain the machinery needed to mediate gene silencing (11). Given these advantages, RNAi has become a powerful tool and, most recently, has been used in cancer therapy research. There is now a wealth of research suggesting that RNAi could be a successful treatment for cancer. It was found that plasmids expressing siRNA against the oncogene, c-Myc, significantly reduced its expression in MCF-7 cells and reduced tumour growth (12). Additionally, it was suggested that RNAi could enable drugs to be tailored to individual patients which could potentially improve cancer survival (13).

This shows that the potential of RNAi is huge and offers extensive possibilities. The technology could be used to eliminate viruses but also therapeutically to solve chronic disease conditions that are currently almost impossible to treat. Many viruses that are widespread in veterinary medicine could be inhibited by RNAi.

## DISCUSSION

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Feline Immunodeficiency Virus (FIV) is a ubiquitous virus which affects cats and resembles human immunodeficiency virus (HIV). It can suppress the immune system and is the causative agent in feline acquired immune deficiency syndrome (FAIDS). It depletes the number of a particular white blood cell, making the cat vulnerable to secondary infection, and can cause a cancer called lymphoma (14). Although the virus is often not fatal, it can be transmitted to other cats and once illness begins, it is almost always terminal. Currently, there is no effective vaccine for FIV, and developmental research has similar problems to HIV. Therefore I think RNAi research on this virus would be particularly useful as it would benefit both medicine and veterinary medicine.

If RNAi technology was applied to inhibit the replication of FIV, a successful method of delivering siRNAs would have to be devised. Currently the best way of delivering siRNA to cells remains a significant challenge. Methods include plasmid vectors which express siRNAs transiently and stably in the cells however these are not a good screening method and are unable to directly label expressed siRNAs. PCR templates are also a popular method as they reproduce quickly however optimising PCR conditions is not time efficient. A heavily researched method is viral vectors (lentiviruses, mouse stem cell viruses and adenoviruses) as they have almost 100% infection and infect dividing, non-dividing and hard-to-transfect cells however they do not integrate into the genome, take time to generate and cannot directly label siRNAs (15). Other methods are electroporation, increased cell membrane permeability through application of an electric field (16), and antibodies, which controlled viral replication and cell loss in mice infected with HIV (17). This shows that there are many possible methods of delivering siRNA complexes into cell's requiring them however as they need perfecting, it demonstrates the need for further research.

A successful delivery method has the potential to be able to stop chronic diseases, e.g. Hepatitis B virus (HBV), from entering a cell and replicating. Hepatitis B binds to an unknown receptor on the surface of the cell and uses reverse transcriptase as part of the replication process. This turns single-stranded RNA into double-stranded RNA which activates the Fas receptor and mediates apoptosis (18). If the receptor that the virus binds to is identified, RNAi could potentially block the receptor so the virus would be unable to infect the cell meaning it could be a very effective vaccine. This use of RNAi technology could solve many other chronic diseases including FIV, which also uses reverse transcriptase to kill cells.

This targeted action of RNAi could also be used to treat hereditary conditions. For example, one condition relevant to medicine and veterinary medicine is Lethal White Syndrome (LWS), in horses, which has similar etiology to Hirschsprung's disease in humans. LWS is a genetic disorder that causes the intestinal system to not develop properly. A mutation of the Endothelin receptor type B (EDNRB) gene mistakes isoleucine for lysine so the EDNRB cannot fulfill its role in the development of the embryo (19). It is fatal and means the foal cannot pass meconium, shows symptoms of colic and dies within the first few days of life. This can be a huge emotional and economic loss to owners.

As the gene that causes LWS is identified, the potential of RNAi to prevent it is huge. To silence the EDNRB gene, siRNA would have to be found that contains the complementary base pairs to the mRNA containing the gene. Once found, the siRNA would have to be delivered into the cell via a successful delivery method, it would then activate the RISC complex, as shown in Figure 1, and associate with the EDNRB gene. This would inhibit proteins being made from EDNRB and therefore stop that disease from reaching translation. This could potentially have a massive impact on the veterinary and medical profession. However, using RNAi in this way would also immobilize the EDNRB gene from fulfilling its role in embryo development which could cause the same effect as LWS if it is the only gene that can complete certain functions in development.

However, RNAi could potentially be used to cure diseases that are not caused by a defect in the gene but too much of it or increased expression. One such disease is Charcot-Marie-Tooth disease (CMT) which is an incurable progressive hereditary condition that causes damage to the peripheral nerves and affects an estimated 23,000 people in the UK (20). CMT1A results from the duplication of the gene on chromosome 17 that carries the instructions for producing the peripheral myelin protein-22 (PMP-22). An overabundance of this protein causes the structure and function of the myelin sheath, a fatty insulating layer surrounding the peripheral nerve cells, to be abnormal (21). RNAi could be used to inhibit the production of PMP-22 in CMT1A by stopping the duplicate from expressing so there is the correct number of PMP-22. Modulation of PMP-22 expression could end a very painful and common neurological disorder.

As with all gene therapy, there are ethical issues to consider. The gene, e.g. the EDNRB gene or PMP-22 gene, could also be linked to another gene which would

subsequently cause other defects which could be worse e.g. making it fatal to mother and baby in EDNRB. Additionally, the ability of RNAi to treat hereditary conditions and chronic disease conditions would bring many tests on animals to identify the genes that cause specific conditions which could leave them deformed as scientists identify what gene has been eliminated. Although the genes for LWS and CMT have been identified, there are thousands of conditions where the cause is unknown leading to this issue.

However, RNAi could be used to strengthen endangered populations. Native birds of Hawaii are now under threat, with ten unique species extinct, due to avian malaria and avian pox. The devastation to the native bird population could potentially be stopped by RNAi as it could potentially help them gain resistance to avian malaria while eliminating avian pox and with the disease eliminated, Hawaiian bird populations could eventually regain strength. Additionally the Tasmanian devil population has come into a 'critical status' and faces extinction due to an aggressive parasitic cancer, Devil facial tumour disease (DFTD) (22). The research conducted into using RNAi on other cancers could be applied to DFTD and prevent this species from going extinct.

Although there is a risk that if RNAi is applied to strengthening populations and trying to eliminate disease, it could cease the process of natural selection leaving huge populations and overcrowding. This would inevitably lead to animals dying of a lack of food, water and shelter as there is so much competition and also humans having to cull huge numbers of animals to maintain the population. For example, deer herds in Scotland have been steadily increasing leading to starvation, inhumane suffering and destruction of Scotland's environment. The Scottish Wildlife Trust says that it would be more humane to cull them (23). Additionally, huge populations of kangaroo in Australia mean many have to be culled every year to minimise destruction (24). These are some examples showing that for animals that already breed prolifically, the complete eradication of disease by RNAi could be devastating.

## CONCLUSION

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The use of RNAi technology to cure diseases is revolutionary and its contribution to medicine could be huge. Its potentiality to treat viruses (14) (17), chronic disease conditions and genetic conditions (6) (7) proves that the scope of its possible capability is extensive and, theoretically, could be done easily thus solving many conditions that are incurable. We know that RNAi has been successful in many cases, e.g. in ALS (5) and foot-and-mouth disease virus (10), but the application to future developments brings problems.

HBV treatment with RNAi is possible but the siRNA would have to be able to target every cell that the HBV binds to and would therefore either have to stop the HBV binding to any more cells or if already infected, it could be used to eliminate the protein the virus needs to replicate. This could be done by silencing the gene that expresses that protein. This would also apply to FIV, and therefore HIV.

Using RNAi to cure LWS is more complicated as it is a mutation. The mutated EDNRB gene would have to be silenced from expressing without stopping it from

performing its usual function of regulating several critical biological processes, including the development and function of blood vessels, the production of certain hormones, and the stimulation of cell growth and division (25). The RNAi would have to recognize the complementary RNA sequence, by base pairing, and then cleave it thus reducing mutant protein synthesis and its toxicity. This is also shown in RNAi treatment of ALS (6).

Knowledge about the molecular switches that regulate PMP-22 gene expression is limited meaning that although RNAi treatment of CMT has huge potential, if the siRNA does not have a complementary base pair to degrade then inhibition of the gene's expression cannot happen. However, once the molecular switches are identified and more knowledge of regulating PMP-22's expression is gained then RNAi could be a successful therapeutic therapy.

The outlook for RNAi treated conditions looks promising. In principle, the only drawback is the current limited knowledge of some diseases which, if overcome, would allow RNAi to take off. However, the likelihood that owners of pets would pay inevitably high sums of money for RNAi treatment is debatable, particularly if it's available for relatively minor problems like FIV. However research into FIV could allow breakthroughs in HIV treatment which would presumably be readily paid for and offer support to many sufferers.

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