

**THE USES OF RNAi TECHNOLOGY ON THE VETERINARY
TREATMENT OF CANINES, WITH SPECIFIC FOCUS ON
HEREDITARY CANINE SPINAL MUSCULAR ATROPHY IN
BRITTANY SPANIELS.**

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ABSTRACT

An interest in canines led to my research into genetic disorders present in canines, in which I found monogenic and polygenic diseases. My search led me to quite a well known, polygenic condition, Hip Dysplasia; mainly found in large breeds such as German Shepherds. Although RNAi technology could, in theory, be used to eradicate polygenic diseases, it is more suited to diseases caused by one specific gene; while the technology is still in the development stages. This led me into further research on monogenic diseases in canines, where I came across an uncommon, but serious condition known as Hereditary Canine Spinal Muscular Atrophy (HCSMA). It is an inherited, autosomal dominant condition, newly recognized as a motor neurone disease. A condition in which motor neurons are lost in the spinal cord and brainstem causing progressive weakness with difficulty in supporting weight, reduced reflexes, abnormal gait and loss of muscle mass; eventually leading to paralysis. It is most commonly found in Brittany Spaniels, but has characteristics in common with human motor neuron disease. In this paper, I will explore how the use of RNAi can silence the gene which causes HCSMA which will, hopefully, eliminate the disease along with others like it.

INTRODUCTION

It was in 1995 when Guo S and Kemphues KJ first utilised antisense RNA to interfere with a gene's activity in *C. elegans* worms (1). But it wasn't until 1998 that RNAi was described, when Andrew Z. Fire and Craig C. Mello published their findings of a mechanism to silence sequence-specific genes; by injecting *C. elegans* worms with dsRNA (2). Then Zamore et al, in 2000, reported breaking up long dsRNA, by the Dicer enzyme, into shorter fragments; in *Drosophila* (3). The RNAi mechanism was further developed and a year later, in 2001, it was first described by Tuschl T and colleagues in mammalian cells (4). After continual development, in 2003, it was reported by Song et al, that siRNAs can be used therapeutically in whole animals; to protect mice from fulminant hepatitis (5). In 2006, Andrew Fire and Craig Mello were awarded the Nobel Prize for their discovery of RNA interference, and found that it was possible, by post-transcriptional gene silencing, to 'turn off' specific genes (6-7).

This worked well in plants and smaller organisms, but in higher animals a problem was discovered. It was found that chains of dsRNA, longer than 23nt, activate an antiviral response shutting down all protein production. Instead, shorter chains of dsRNA could be used (the best was found to be 21nt) with no such response (8).

Another problem found was, by delivering the dsRNA; the effect of targeted mRNAs depleted quite quickly, but is found to transfer to future generations; although the effect weakens. This would mean that if a canine with HCSMA was injected, the dsRNA wouldn't be able to reach all of the cells, so the gene silencing effect would gradually reduce (7). This means that siRNA would need to be constantly released into the canine, and a way would need to be developed in order for this to happen. Expression vectors have been recently developed to transiently produce a large amount of siRNA for a relatively longer period (9). This is a step in the right direction for overcoming this problem and, in theory, curing monogenic diseases.

RNAi is the mechanism in which the introduction of dsRNA causes degradation of the complementary mRNA. Current research into RNAi includes clinical applications such as "antiviral activity, treatment of cancer, and neurodegenerative diseases such as Huntington's disease" (10). Treatment of cancer is a large part of RNAi research, if the cancer gene was the target mRNA cleavage then it could be possible to 'kill' the cancer cells and in theory, cure the disease (10-11). The possibility of this could

open up new doors in both the medicine and veterinary medicine world, as monogenic diseases could be ‘turned off’ as only one specific gene needs to be targeted. Although polygenic diseases are further advanced than this, with continual technology developments, they too could be silenced. As polygenic diseases are caused by multiple genes, each individual gene could be silenced to, in theory, eliminate the disease.

A large number of inherited canine diseases are polygenic, some have unknown inheritance, here is an example of one of the monogenic diseases that could be eradicated with the use of RNAi; laryngeal paralysis where there is loss of function in the laryngeal muscles that normally open the larynx when an animal breathes in which results in the obstruction of the airway causing loud and laboured respiration. The breeds mainly affected by this are the Dalmatian, Bouvier des Flandres, Siberian husky and bull terrier (12). Hereditary Canine Spinal Muscular Atrophy is another monogenic disease which mainly affects Brittany Spaniels, and is the disease I will be exploring in this paper.

DISCUSSION

The monogenic disease HCSMA is most common in Brittany Spaniels and has “features in common with human spinal muscular atrophy” (13). As the disease is monogenic, I believe it is a good area for development into how it can be overcome through RNAi technology. It is a good starting point, as research could benefit both the world of medicine and veterinary medicine.

HCSMA is a fatal, motor neuron disease which drastically reduces the animal’s quality of life. It is caused by mutations in the antioxidant enzyme, SOD1 (14) which results in the degeneration of the motor neurons in the spinal cord and brainstem. The consequence of this is loss of muscle mass and eventually paralysis.

Currently, the only way to stop the disease is to stop breeding dogs that are either, affected by or are carriers of the disease. But by introducing testing for HCSMA, it is becoming easier to find which canines are carriers, so they can be treated accordingly; but this will only work if enough owners use this test on their animals. It could be an essential thing among breeders also, as if they test all breeding stock and determine which are carriers, those canines could be treated with RNAi, or at least not be bred with other carriers of the HCSMA gene.

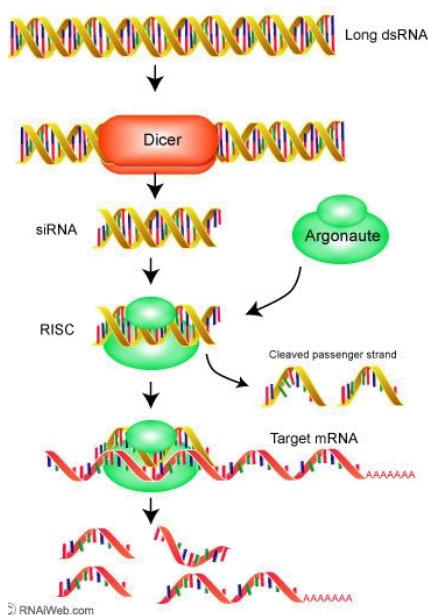


Figure 1

I believe that it is theoretically possible to eliminate any possible monogenic disease in canines, possibly in any mammalian species, due to the genes involved in the silencing process. Using the process in figure 1, we would silence the specific gene causing that disease. If the HCSMA gene were to be silenced, siRNA would have to be produced by fragmenting dsRNA, by the Dicer enzyme. The siRNA, which then binds to the RISC, is unwound and provides target specificity to RISC through complementary base pairing of the guide strand with the

target mRNA-containing the HCSMA gene. The target mRNA is then cleaved, by the enzyme slicer, and consequently taken out of mRNA. Therefore translation cannot take place on the specific base sequence which codes for HCSMA. As a result of this, the amino acids, and subsequent proteins, would not be produced from the HCSMA gene, so the code for the production of proteins of HCSMA never reaches translation.

If this could be attained it could open up new opportunities in the veterinary profession. Breeders would not have to worry whether their animals are carriers of the HCSMA gene, as all of the breeding stock would be injected with the dsRNA which contained the complementary base pairs for the gene in target mRNA. To stop the silencing effect from lessening, expression vectors would need to be used to constantly express the siRNAs, which would eliminate the gene, as the siRNAs would readily seek out the complementary base pairs which would be on the target mRNA HCSMA gene. This technique should remove all HCSMA genes in a canine's body. This can be proved using DNA tests, which means every new born canine need not be tested. Because if the parent canines had been treated using the RNAi mechanism, then the gene should not be present in the offspring; as the silencing effect will continue on into future generations.

Although clinical tests have been completed and no adverse effects have been found, there are still risk factors to consider. By silencing one gene, is it possible that another could be affected; if the genes were somehow linked, and if new defects do arise, could these cause further problems. If this were to happen, it would have to be considered as to whether it would be in the animals' best interest to continue silencing one gene, and let the animal live with the defects, or stop the gene therapy, and let the disease continue in future generations of canines. But hopefully, further research will eliminate this possibility, and if not, future preparation for this must be considered.

It isn't just the risk factors that have to be considered, ethical implications also need to be addressed. With RNAi, a specific gene must be found, in this case HCSMA, so for this specific gene to be silenced, it first has to be found. There is research which has already identified this (15) but there is still the issue with the numbers of animals left deformed through the process of trying to find which specific gene controls what in the animal. This means each individual gene must be silenced in order to find out what has been turned off.

By developing RNAi, there is the possibility of 'curing' monogenic diseases. If it is proven that monogenic diseases can be alleviated relatively easily, then the next stage will be developing RNAi further to eliminate polygenic diseases. It would mean that all diseases (which can be helped using RNAi) would be eliminated; although this would seem to be a good thing, we would be destroying natural selection. This could have undesirable effects, as the population of animals would develop to be out of control. This would become a problem if, for example, a disease-free dog was to become stray and breed with other stray dogs; as the RNAi passes down through generations, so any offspring produced would also be disease-free. Eventually, all strays will be disease-free, so the numbers will increase. This could lead to culling, a process of manual selection, to cut numbers back down; this is, once again, something we need to consider. Is it better to have a population of animals disease-

free and having to cull large numbers to keep the population down, or let natural selection remove the need to cull by using disease to kill a number of animals?

But, at the opposite end of the scale, RNAi could also be used to strengthen the population of some species; such as the red squirrel. If RNAi were to be used to remove disease in the red squirrel, it would help increase numbers against the grey squirrel. RNAi could also be introduced in zoos and specialised breeding programmes to increase the numbers of endangered animals. In addition, this would be beneficial in conservation work, as once the numbers of species are increased, some could be reintroduced into the wild; giving them a chance to become a stable population. This could then become standard practice with endangered animals, as using this technique would produce disease-resistant animals which would have more chance of success on being released into the wild.

CONCLUSION

Using RNAi in canines is, in theory, a simple concept; obviously putting the theory into practice is much more complex. But it is possible to see how such a technique could be used to help monogenic diseases in canines. Through the process shown in figure 1, it can be seen how such a technique is possible and how monogenic diseases, such as HCSMA, could be a thing of the past. The silencing process can last for at least one generation (16), which means the offspring of the treated canine will not have the gene present; and therefore will not have the disease. So by using expression vectors, the silencing effect should continue over future generations.

On the other hand, as evidence suggests, it is quite likely that the silencing effects will reduce given time (16). Although this is excluding the use of expression vectors to continuously release siRNAs, so further developments are needed to see how accurate this is. If, in the future, it turned out that the effects do not diminish, the potential for the use of RNAi is vast. But if future developments show the effects do gradually weaken, then a solution must be found to alleviate this problem.

A possible solution could be to keep track of the silencing effect through the generations of several canines, to work out the loss of its effectiveness. This could be done by using DNA tests that are already in practice to test each generation of canine. Then when the test shows a positive result for HCSMA, or any other monogenic disease, then an extra dose of the dsRNA and expression vectors should be given to enable the silencing effect to continue. This could be a solution to the possible problem, and will ensure that the affected genes were kept silenced.

Although we have found a solution to the possible problem, there is also the question of will the breeders agree to the RNAi treatment? As it could be a costly treatment, and breeders may not want to know if their canines are carriers as it may harm their reputation. If it is known to people that a breeders stock are potential carriers of a serious disease they may be unwilling to buy an animal from that breeder. This could have a knock on effect, as breeders may be unwilling to disclose whether their animals are carriers, which could result in more offspring either affected or as carriers of HCSMA.

The principle of RNAi is quite clear, and simple in theory, although limited in the canine species by the number of monogenic diseases, the future of HCSMA does not seem bright. The treatment would not only be very costly, especially for breeders, but they also wouldn't want to make it known if any of their animals were affected by or carriers of HCSMA. The right thing to do would be to get all of their canines tested, and then treated accordingly, but as a reality, we know this won't always be the case; because in these times, we have more of a desire for money than the care and wellbeing of others.

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