

APPLICATIONS OF POST-TRANSCRIPTIONAL GENE SILENCING AND ITS RELEVANCE TO VETERINARY MEDICINE

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ABSTRACT

The aim of our project and conclusion was to explore the way that gene silencing can be used in the veterinary (and medical) world. RNA interference could be more commonly used in the future as new ways of application become available. It may even change our lives completely as people in the veterinary profession or just as pet owners and patients.

INTRODUCTION

Post-transcription gene silencing, or RNA interference (RNAi), refers to the technique of switching certain genes on and off in a cell through the use of double stranded RNA (dsRNA). This helps to control gene expression and can potentially change a characteristic of a living organism. Post transcriptional gene silencing is the most common type of gene silencing studied currently.

RNAi research began in 1990 with Richard Jorgensen during his experiments with petunia flowers. He noticed when he altered the genes for pigment to gain a darker shade of colour in the flowers, instead of producing deeper purple flowers, the position of the different pigments on the petals changed and bands of white areas were observed. This effect was called co-suppression.

In 1995 Guo and Kempfues continued this research by injecting round worms with both sense and anti-sense RNAs to switch off target genes coded for in the RNA molecule. Continuing the time line, in 1998 Mello and Fire discovered that by combining sense and anti-sense RNA they could produce a double stranded RNA. This was much more effective than the conventional single stranded RNA as it was much more stable. The dsRNA can be administered either via ingestion, by soaking the subject in a solution containing dsRNA molecules (only possible for very small species), or by injecting the specimen with the dsRNA.

During genetic replication processes or, in fact, protein synthesis the DNA of organisms is transferred for growth, repair and general upkeep of the organism. Considering the vast amounts of information contained, it is a wonder that more mutations don't occur. Also the complex structure and replication process means that there is opportunity for errors. Yet mistakes rarely occur. When they do, mutations can result in evolution by natural selection. Given the changing climates across the globe many organisms will need mutations to save the ecosystems on the brink of extinction. We may be able to help restore balance that we have, so far, destroyed.

The importance of this relatively new technology is arguably one of the most important of the last five decades as it could lead to treatments for inherited conditions. If used on a regular basis in veterinary for example, the gene interference could be used to treat allergies, more easily treat and simplify dermatology cases and also other diseases such as cancer. This could also be a breakthrough for human medicine too. The possibilities are more or less endless and the future prospects are bright as a result. There is a very optimistic outlook for treatment of disease. The bacterial or even viral related conditions could be less painful and traumatic with more ease when treating; benefiting vast numbers of animals. After the methods can be perfected and practised, their owners would find the technology more viable financially in the long term cases.

DISCUSSION

BACKGROUND SCIENCE

Gene silencing is basically the amazing science of making things go wrong in the way that suits us. The basic principle is to stop protein synthesis part way through the process and swap pieces of information for bits that we want.

RNA (Ribonucleic acid) is a single stranded version of the DNA molecule and is a “direct product”¹ of the genes that it contains. It is used for transporting the genetic code that would otherwise be contained within the DNA in and out of the cell nucleus (in most Eukaryotic celled organisms). This is vital for the cell’s function and for replication. During the process of protein synthesis, messenger RNA (mRNA, the product of translation) travels to the ribosome where transcription is carried out and where amino acids are bonded together to make the components of polypeptide chains.

Furthermore miraculous is that many living organisms have their own ways to eliminate changes and prevent the damage caused by mutations. One such way is to do with the RNAs. This involves the smaller forms of RNAs such as the small interfering RNA (siRNA) and the micro RNA (miRNA) which can bind to other bigger RNA strands and increase or decrease their function and ability to carry out their job; for example preventing the translation of mRNA to produce a protein. In living organisms RNA interference would be used to protect the genetic information in cells from invading genes - viruses and transposons which can affect the genome dramatically if they are not carefully controlled. (Transposons are mobile parts of the DNA which can move and change the entire code causing mutation and also controlling the speed at which the replication can successfully take place and so controlling the amount of DNA in the genome.)

A double stranded RNA (dsRNA) is delivered into cells. This triggers a response and it is broken down by the processing enzyme DICER into siRNA. Typically, it is split into around 21-25 base-pairs per fragment. The small RNA molecules are known as guide RNAs and can be amplified producing many of the same. The guide RNA can be double or single stranded, some of which is integrated into a nuclease complex called RNA Induced Silencing Complex (RISC).

Guide RNA directs the RISC to a homologous mRNA due to complementary base pairing. The mRNA is denatured by exonucleolytic or endonucleolytic cleavage (the way the mRNA is broken apart). “The cleaved RNA can be recognised by the cell as being aberrant and then destroyed. This prevents translation from occurring, silencing the expression of the gene from which the mRNA was transcribed.”² (This is as written on the Nature Publishing Group animation under the 2006 copyright – created by Arkitek for *Nature Reviews Genetics*). It is yet to be confirmed exactly what the RISC is composed of, but it is known to be a RNA-multiprotein complex. However, through looking at its functions it has been deduced that it consists of: Helicase to unwind the siRNA, nuclease to cleave the mRNA, and RNA-dependant RNA polymerase (RdRp) to amplify the gene silencing signal.

¹ See Glossary reference 2.

² This was taken from an animation on the Nature Reviews website listed in Glossary 5 & 6.

The RISC molecule is activated by the presence of the siRNA (or guide RNA). The guide RNA leads the RISC to the messenger RNA emitted by the nucleus containing the genetic instructions from the nucleus. The RISC breaks down the mRNA and it is stopped from reaching the ribosome in the cell. As a result the ribosome does not make certain proteins.

Post-transcription gene silencing is a natural reaction occurring inside the cells of certain higher order organisms. The process serves the purpose of defending the genetic material of the cells from a viral attack, or invading RNA from other sources. This process allows the RNA injected into the cells from the virus to be destroyed before they cause any damage.

In plants and some roundworms, the RNAi pathway is inherited and plays an important role. In plants it is thought that RNA interference is also used to prevent self-pollination by itinerant parts of DNA. However in mammals this pathway is missing and the effects need to be artificially triggered by introducing siRNA into the mammalian cell.

USES: HAIR COLOUR

One possible use for gene silencing would be to change the hair colour of animals such as dogs to make them look more pleasing to the human eye.

Hair colouring in animals is caused by two different types of melanin: eumelanin and phaeomelanin. Eumelanin is dark, although its exact colour is dependent upon the protein, which may vary, and phaeomelanin covers a wide variety of colours from pale cream to shades of yellow, red to mahogany and tan. It is thought that there are mostly between two and four gene series that determine where on the dog and along the hair the eumelanin and phaeomelanin will appear, although there may be as many as six in some breeds. There are currently ten generally recognised gene series coding for colour, and there may be more. These are agouti (A), brown (B), albino series (C), blue dilution (D), extension (E), graying (G), merle (M), roaning (R), white spotting (S) and ticking (T). This knowledge may be used in the distant future for an informed choice of which gene series should be changed.

It would, therefore, be possible to use gene silencing to suppress the gene coding for a specific colour in a certain area of the animal, to give what may be considered a more desirable appearance. One example where this could be used is to give Shetland Sheepdogs or Rough Collies a full white collar. This could be achieved by locating the relevant gene series which is coding for a colour other than white in the collar of the dog and then injecting an identical siRNA chain into the cells around the collar area, triggering the interferon response. This would mean that new hair that grew in that area would not have the colouring present, leading to the hair being white. We can be sure that, without any melanin colouring the hair, it would be white because the characteristic white hair of albino animals is a result of a failure to produce tyrosinase, which is essential for the production of melanin.

A breeder may want to do this to give an animal more desirable features if they are to be able to sell it for more money; although this seems light hearted it could become very popular. Another reason for owners wanting to do this would be to try and improve their chances of winning if they intend to show their animals; but this may cause controversy as direct interference with the breed's original genes may be considered cheating. However, to make this viable, a quick and cheap method of silencing the desired genes would need to be developed.

Before this could be attempted there are many drawbacks to be taken into account. Continuing with the example of giving a Sheltie a full white collar, there would be millions of cells which would need to be injected with the siRNA. Injecting every cell individually would be a very long and expensive process. One alternative could be to synthesise a solution which allowed the genes to be absorbed from the fluid surrounding the cells. Ingestion or cream based formulas may be a good idea for less intrusive treatments, or the cells could be infected by a man made virus that targeted specific cells and contained the necessary information. However, there would be many ethical issues to consider.

USES: DISEASE/ DERMATOLOGY/PRESERVATION OF SPECIES

The RNAi technology could be used to treat disease and help with allergies and dermatology cases. Looking for advantageous features such as immunity due to genetic reasons could lead to helpful tools. By comparing genes of a healthier animal with another that is susceptible to disease could allow those specific genes to be identified and modified. For example, inbreeding has led to many complications that result in weakness of immune systems – giving us animals with other sought after characteristics but with the prominent illnesses and problems. Selecting specific genes for modification would effectively be a form of cross breeding, but with the original animal traits that are required or wanted. This would also speed up the process of evolution.

With the changing world in a delicate state and inevitable crisis on the way for many animals across the globe, we may be able to use this technology to help them. Species in rapidly changing habitats need to adapt quickly otherwise they may die out if they cannot be preserved, as much of their ancestry does not equip them to become versatile. By looking at and understanding the genomes better we can start to identify which genes and characteristics they will need in their struggle. The RNAi could be used to exaggerate genes that they may need in their current and also new climates, enabling them to avoid extinction. Even giving the animals a slightly improved ability to cope may be a key to their entire species' survival. The changing climate across the globe has put many ecosystems on the brink of disappearing forever, such as that of the polar bear. We may be able to restore the balance to the habitats and populations of living organisms and undo much of the harm caused by the disturbance we have, so far, contributed to.

Acne is an example of a dermatological problem which gene silencing could be used to prevent or at least reduce. It can affect animals (such as cats) around the chin and the muzzle, and one of the main causes of acne is oily skin. If gene silencing could be used to reduce the amount of natural oils being produced by the skin cells, it is likely that the severity of the acne would also be significantly reduced. However, as the natural oils are essential for maintaining skin condition, you could not stop the production of all oils by gene silencing. Instead you would need to find a compromise by silencing the gene coding for the production of skin oil only in the areas worst affected by the acne. However, as there is no way of undoing the gene silencing, it would need to be done a little at a time, and it should only be considered as a last resort for very severe cases.

Another use for gene silencing would be to help treat hormonal skin diseases³ caused by the over-production of the hormone. Most diseases caused by excess hormone levels will be displayed by a

³ The website in glossary reference 8 was used to gain more background information about this area of uses.

thickening or thinning of hair which would normally be evenly distributed over the animal's body, and may also affect the bodily functions of the animal in other ways, potentially having serious consequences. Gene silencing could be used to treat these problems by reducing hormone production. However, care must be taken because the hormones also play an essential role in the bodily functions of the animal, and so their production cannot be reduced too much. One approach to ensuring a balanced hormone production is achieved would be to only silence a small proportion of the cells at a time. Then, after each procedure, a blood test would check the hormone levels, and if still raised, further silencing could be carried out to further reduce their production. This would provide a permanent cure, eliminating the need for the long-term administration of drugs to control the hormone levels. However, due to the risks of reducing the hormone production too much, this should only be considered as a last resort for very severe cases.

Another possible use for gene silencing could be to cure dermatological conditions caused by allergies. These are caused by the immune system over-reacting to an allergen and producing antibodies to fight it, leading to an allergic reaction. However, if the area of the body in contact with the allergens (e.g. skin)⁴ didn't send the message for the antibodies to be produced, then there would be no immune response. Stopping antibody production would not be an option because the animal's body needs to be able to produce antibodies to fight disease. However, gene silencing could be used to prevent the messages being sent for the production of the antibodies and histamine. This would provide a permanent solution to the problem and remove the worry of the animal having an allergic reaction each time it is near the particular allergen.

⁴ Some inspiration for our ideas came from the website in glossary reference 7.

ETHICAL ISSUES

With all new cutting edge technologies there are always ethical issues with their use. Today we are meddling more than ever with the genetic make-up of individuals, and we have to constantly ask ourselves whether this is acceptable.

Another ethical issue comes as we are planning to use this technology as a treatment to genetic diseases in animals. Some people argue that as pet owners we should not decide on the behalf of our animals. This becomes a problem as we have no way of ascertaining the wishes of these animals, so it would be up to the owners to make the decision if they wanted this new technology to be tested on their respective pets or livestock.

In RNAi we are injecting genetic material into the cells of animals in order to trigger the immune response. This could be a problem as we are inducing a response by external means, and influencing the response of the cells. This is coined by religious groups as 'playing God' and they are opposed to this as they believe that life should take its natural course, and we have no right to decide its course.

RNAi interferes with the expression of genes in the cells of the animal or plant being treated. We are actively changing the genetic make-up of cells which raises ethical issues; for example we could suppress genes which are responsible for eye or skin colour. While on the surface of it this appears to be perfectly reasonable, it could be used in conjunction with embryonic screening (where the genome of the embryo is scanned pre-birth). This may perhaps lead to 'designer pets'⁵, where people may decide what their animal will look like. This can be considered as unethical as should we have the right to decide what future generations of the natural world are made to resemble, and should it just be left to natural evolution? For animal breeders, it would enable greater control when breeding their animals as they could in effect choose which features they wanted to enhance in their pedigree animals; and they would not have to employ selective breeding to get the same results. For example a dog breeder could breed pugs with even flatter faces, or horses with better leg muscles for jumping, without having to wait years for this to occur naturally. This is very important as this could worsen the problems at the present and this could more than likely cause more undue suffering for the animals concerned.

If you consider livestock that are used to make a living, it could be extremely beneficial to be able to produce animals for their desired purpose. An example of this is dairy cow herds. At present breeds such as the Jersey, Guernsey and Swiss dairy cows are used for good quality milk from healthy cows. At the same time a breed such as the Hereford may be used in the mixture because although they don't produce as high quality milk, the cows produce bulk - larger quantities to give lots of milk with good quality nutritional values. This may mean that farmers could use cows with good yield - benefiting the human population. Again there is a down side as the other breeds may be lost and the heritage and traditions with them. Another ethical and emotive problem is the amount of testing and possibly the procedures are not fair to put onto the creatures. If it was going to be used could it be pain free? Would it involve treating existing livestock or breeding completely new sets of dairy cows? Would it be cost effective or even worth putting the animals through?

Added concern about RNAi is that it has many potential applications, which may or may not have been discovered yet, and its prospective use in treating genetic diseases is amazing but could become a cause for concern. New technologies should be treated with caution or we could risk being carried

⁵ A potential spin off from 'designer babies' in humans.

away with wonderful whirlwinds of new ideas. The technology should be subject to evaluation by an ethics panel to decide how plausible and necessary the developments are in this era.

As well as religious and moral concerns, social concerns have a part to play in the ethical issues surrounding RNAi research. There are concerns that research on RNAi will have to be carried out on animals before it is ultimately allowed to be used and tested on humans. Animal fundamentalist groups are opposed to this as they believe testing on animals is not ethical as it causes unnecessary suffering for the animals. However these concerns are not likely to sway the scientists or the politicians as the benefits of this new technology far outweigh the drawbacks that testing on animals brings up, but then would it be worth it or is it better to use what we can in moderation?

With regards to saving or not saving species, many people believe that the changes in climate, due to global warming and human activity should be left to take its course. The fine balances in ecosystems have changed many times over history and many people think this should be allowed to happen. Even if magnificent animals are lost, it is natural and so inevitable at some stage. On the other hand, why should animals be left to suffer and quite possibly die out completely? If we haven't caused the global issues we have certainly contributed in which case we should act against it and repair what we have disturbed. Also, who are we to decide what is kept and what is not? Why should we choose how species should be changed and what happens if we are wrong?

As this technology is relatively new there has not been enough time for the long term effects of it to be discovered. It could be the case that blocking the mRNA from reaching the ribosome could lead to mutations in the DNA of the cell – especially if we ourselves do not get the target response required, potentially causing worse long term problems. No research on this has of yet been carried out. RNAi may not even function on the scale we are hoping as work so far has been carried out on plants and simple organisms (eg ringworms). Certainly problems begin to arise when we scale it up, such as how it would be possible to suppress a gene in all of the relevant cells. We haven't yet found a solution to this although a cream or ingested substance may be effective. We have to decide if it's worth spending lots of money on research for a technology that may or may not be as effective as we initially hoped.

Other serious problems may arise while we try to treat a simple condition such as diabetes. This already has treatments that work and although they involve constant monitoring to be effective, it may be less problematic to find a completely new method. Providing alternatives to tablets and injections would be fine but there is a lot that could go wrong when considering such detailed codes. One small mistake could cost the organs targeted which may become malformed or mutated although a solution to this may be found.

If RNAi was used to treat genetic diseases in humans the cost would soon become an issue. Decisions would have to be made in regard to the provision of this treatment on the National Health Service, or whether it should only be made available to people going privately. As the application of this treatment involves state of the art equipment and facilities it would probably be made available to paying customers only. This raises the issue whether we have to right to withhold potentially lifesaving treatment from the average person, and therefore end their life when treatment was available. As it stands, most people would be unable to afford the sheer cost of using RNA to treat their particular disease.

CONCLUSION

In this project we have presented the basic technique of post-transcriptional gene silencing, and the methods of targeting the specific cells that are being altered. We have also put across several potential uses for this new technology, and the ethical issues that arise when this technology is put into practise. With only a limited amount of time we have covered a few possible applications of RNAi, however there are likely to be many more uses which will become apparent over the next few years when more research is done into higher-order organisms such as humans and animals. A few possible uses that we have identified are combating disease, reducing the effects of acne, changing the hair colour of show animals, combating allergies and in dermatology. However, as we have demonstrated, using the technology in this way - for our own benefit - is bound to bring up some ethical issues that need to be resolved before more research is done into post-transcriptional gene silencing but this can be said for almost all medical treatments we use. Technology is a never ending cycle of research and improvement - now we can move forward and improve lives with what we find.

GLOSSARY

These websites were used for extra information and for inspiration to write about some of the topics in our project. There are reference labels at relevant points.

1. http://en.wikipedia.org/wiki/RNA_interference
2. <http://en.wikipedia.org/wiki/RNA>
3. <http://bowlingsite.mcf.com/Genetics/ColorGen.html>
4. <http://hopepage.usask.ca/~schmutz/dogcolors.htm>
5. <http://www.nature.com//focus/rnai/animations/animation/animation.htm>
6. http://www.nature.com/nrg/journal/v2/n2/animation/nrg0201_110a_swf_MEDIA1.html
7. http://www.aad.org/media/press/_doc/SensitiveSkinFactSheet.html
8. <http://www.lowchensaustralia.com/HEALTH/skin.htm>

We also gained support and inspiration from colleagues, friends and family.