AN EXPLORATION INTO FUTURE DEVELOPMENTS OF RNAi AND THE ETHICAL ISSUES SURROUNDING THESE DEVELOPMENTS

BY
ALICE HARDY

PASS WITH MERIT

RESEARCH PAPER
BASED ON
PATHOLOGY LECTURES
AT VET-MEDLINK 2009

ABSTRACT

The RNAi mechanism can be used to silence genes ie. eliminate their effect. My project will explore whether this could lead to active choice of characteristics of an individual, by choosing genes to eliminate. I will focus on the specific examples of changing coat colour in horses, the "mean gene" in honey bees and hairlessness in Xoloitzcuintle Dog, caused by a mutated gene. I will explore whether this would be an easier or more ethically sound method to combat unwanted genetic traits than genetic screening of embryos, and any ethical issues surrounding this idea.

INTRODUCTION

The RNAi mechanism was discovered by Jorgensen et al (1990), when they were trying to enhance the colour of petunias. They introduced more copies of the pigment-producing gene to the petunias as they were growing, expecting them to turn a deeper purple. Most flowers produced the surprising result of turning colourless or partly colourless in the parts of the plant that grew after the genes were inserted. Jorgensen named this effect "cosupression".

Figure 1- The unexpected "cosuppression" effect in Jorgensen's petunia experiment.

RNAi stops the expression of a certain gene by blocking the corresponding messenger RNA, thus halting gene expression before translation into a protein sequence can occur. This was first discovered as a natural defence mechanism in plants and invertebrates. When the viral double stranded RNA (dsRNA) invades the cell, the cell can recognise it and initiates the silencing process. The alien dsRNA is identified, then cut into 21-23 nucleotide small interfering RNAs (siRNAs), each with 2-nucleotide overhangs, by the enzyme Dicer. These then bind to a nuclease complex to form the RNA-induced silencing complex (RISC), which splits the siRNA and uses the antisense strand to target, bond to and silence the corresponding mRNA by cleaving it.

This method, therefore, could be used to silence chosen genes by inserting the required dsRNA. However, in higher mammals, although the RNAi pathway is there, as the cells contain both Dicer and the nuclease required, the introduction of the dsRNA will provoke an inflammatory response, which shuts down the production of all proteins in that cell. To overcome this, siRNAs are introduced with a specific fingerprint to code for a specific gene, which does not provoke the inflammatory response. Richard Fire and Craig Mello won the Nobel Prize in 2006 for their work in nematodes, discovering this property, and the idea that this could turn off any gene.

This is particularly important as it could become a cure for genetically- related diseases, principally viruses and cancer, which is being looked into today. Any virus could be prevented from manufacturing proteins, silencing its effect completely. This is so revolutionary as there are many dangerous, as well as common viruses that need to be combatted, for example, HIV, influenza and even the common cold. RNAi as a treatment for viruses has the significant advantage that it can be used across many species. This is very useful for vets, as it would provide one treatment for a specific virus, but across all the species the virus affects. RNAi could be used as a cancer treatment by switching off the mutated genes that cause tumour cells to divide. This would be unique for each case, meaning subsequent mutations of the cancer cells' DNA could be dealt with. Cancer is a significant and dangerous disease to most organisms; a cure for cancer that could be used across species would be revolutionary in medicine and veterinary medicine.

Once this mechanism has been properly tested, this could become a very efficient method of controlling disease. It is clear that before something like this becomes widely used, there are several problems that need to be overcome: how to cope with mutations in RNA, keep siRNAs present when the cure is not immediate, how to prevent cells dying when the siRNAs are inserted and how to insert the siRNAs into every cell that needs them. Before any developments can be made, these problems need to be solved.

Even though it has its faults, this mechanism could prove revolutionary in science in more than the obvious ways of combating viral forms and cancer. For example, if RNAi can be use to silence any gene, it could be used to silence healthy genes as well as unhealthy or non- beneficial ones. One future use of RNAi could be to silence genes in cells at the blastocyst stage in an embryo's development, enabling you to actively choose genes you want to eliminate, and so dictate to some degree the characteristics of the offspring. This is very similar to the "designer babies" argument, and obviously would have many ethical and moral issues surrounding it. Is this method likely to be accepted in the light of the "designer babies" controversies, and is it more or less likely to be accepted than the idea of genetic screening? These are all issues I will explore in this paper.

DISCUSSION

The current genetic screening method is a potential way to control the characteristics of an individual, by screening many embryos, and choosing the embryos with the desired characteristics. The test, called aneuploidy screening, involves taking a single cell from an IVF embryo for analysis. Many embryos are created, and only the ones with the required characteristics are implanted into the womb of the mother, normally two or three embryos to ensure one implants properly into the wall of the uterus. This has prompted the "designer babies" arguments, however at the moment screening is only used if the family has a history of a particular genetic disease, and the idea of choosing the characteristics of an individual is very contriversial and is associated with many ethical issues, which I will explore in the second half of this paper. RNAi could be used in combination with the current screening method to check for unwanted genes, and then eliminate them once they have been found. This could cut down on the number of embryos that need to be fertilised by IVF, as just one embryo would be needed if it is possible to silence the unwanted gene or genes. Using the RNAi pathway to do this could prove to be difficult, taking into account the problems above, however it is the next step forward in creating an individual with specified characteristics.

Using the RNAi pathway to silence genes could be used to turn off any gene in the early stages of life. The siRNAs coding for a particular unwanted gene could be inserted into germline stem cells at the blastocyst stage in development. By introducing the siRNAs early in development, the problem of how to insert the siRNAs into all the cells requiring them is solved; they only need to be inserted into one or two cells, which are then encouraged to divide normally by mitosis; each germline stem cell could potentially become a new being. All daughter cells would then silence the expression of the unwanted gene (see figure 2).

If using RNAi to silence genes on humans will be so controversial, it may be more acceptable to decrease the scale to lower organisms, which would be easier to conduct experiments on, as well as reducing many moral objections. Africanised Honey bees, for example, a genetic variant of our own European-origin honey bees, have what is affectionately called a “mean gene”. Research has been carried out to discover that this gene is in fact five different genes which control the Africanised honey bees’ more aggressive behaviour. If these genes could be switched off, not only could the bees become less aggressive, but the “mean gene” would be less likely to spread across to the “more pleasant” European honey bees. This illustrates the wide possibilities of RNAi and gene silencing, and how it could revolutionalise medicine as well as our knowledge of genetics. However, even on this scale there is still the issue that, without its aggressive behaviour, the bee would be less able to defend itself and the colony. With time, this could impact on the food chain and biodiversity could be reduced. However, silencing just one or two of these genes could be more practical at reducing the aggressiveness of honey bees; the aggressiveness could be reduced to a certain level, still leaving bees a method of defence. This could be very useful as the population of bees has been declining in the UK for about 20 years and if bees were less aggressive, more people would be inclined to keep them, reducing the chance of extinction. This would also be of benefit to the crop industry as bees are very important in the process of pollination.

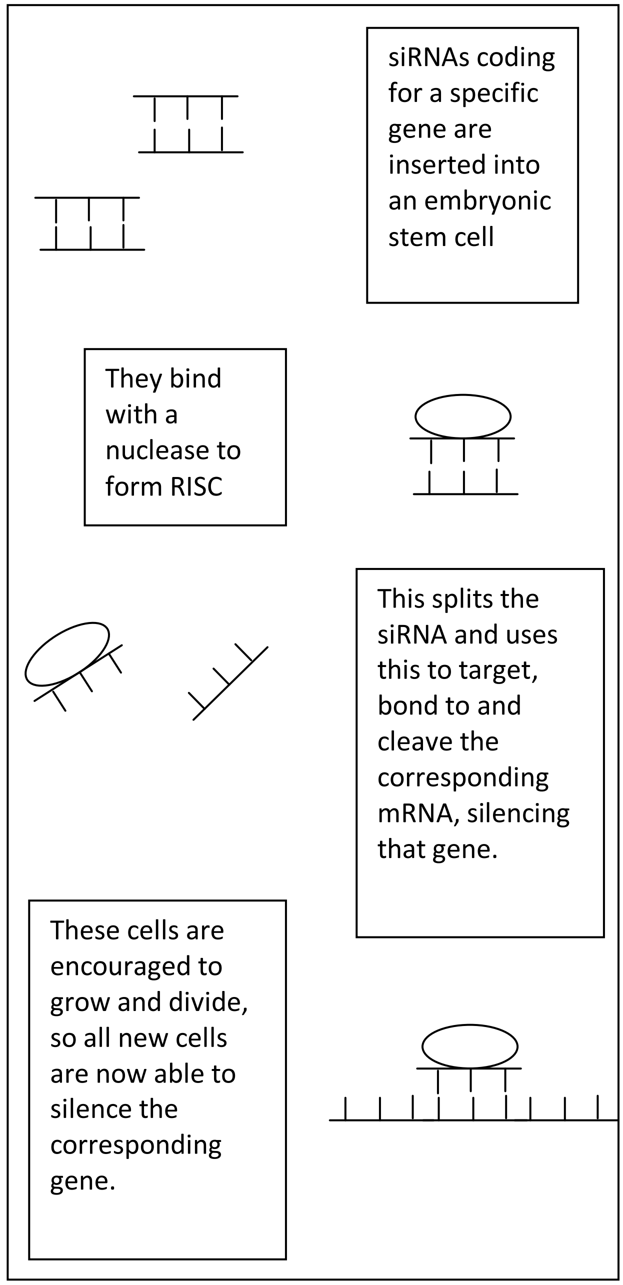
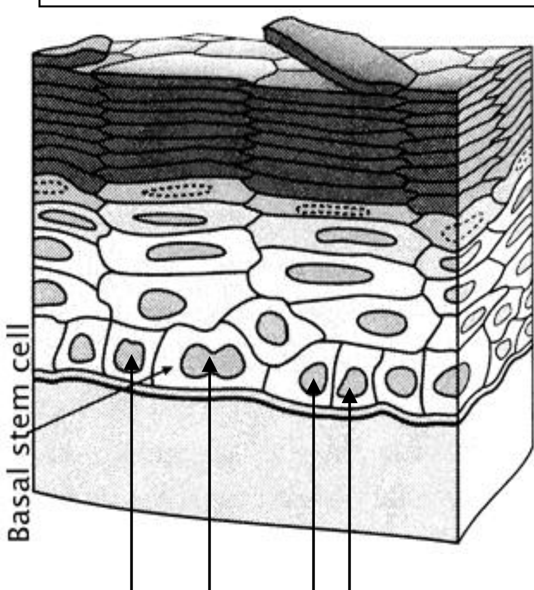


Figure 2- inserting siRNAs into germline cells

When it comes to “designer” organisms using siRNAs, it is not about choosing the genes we want, it is choosing the genes we do not want and eliminating them. It is interesting to think about what the effect would be if, as in the previous example, more than one gene codes for a characteristic, and we silence only some of those genes. Many more different combinations of characteristics could be created across many different individuals. Here, I will use the example of horse coat colour. The basis of nearly all horse coat colours; chestnut, bay, brown and black, are controlled by the two genes Extension and Agouti. The Extension gene controls the production of red and black pigment and Agouti controls the distribution of each pigment. About 10 other genes are involved in dictating the colour of the horse, by diluting the basic colours produced by the Extension gene or dictating the white patterns found on horses. A few are: champagne, cream, grey, pearl and silver. This is done by manufacturing different pigments; combinations of pigments provide the variety in coat colour. These genes are active in combinations, so in one horse three or four of these colour dilution genes could be activating the production of pigments, resulting in a huge variation in coat colours. If one or more of these genes was turned off, the horse would not be that colour tint, thus allowing the owner to choose which colour they did **not** want their horse to be. This does not give us complete control over what the individual will look like, however it gives us some control that could be used in the future of RNAi techniques; we can, to a degree, dictate how an embryo will develop. It would be interesting to see the different effects of different combinations of dilution genes switched off, as it is the combinations of genes that result in such variation in coat colour. Slightly different combinations of pigments will produce slight variations in coat colour.

Skin cells do not produce the pigment that the silenced dilution gene coded for; the coat in this area is a different colour to the rest of the coat



siRNAs coding for one or more of the 10 colour- dilution genes inserted in the base layer

Figure 3- how using the RNAi mechanism to change coat colour could work

This idea could lead us to be able to change certain characteristics when the animal is fully grown. siRNAs coding for one of the ten colour- dilution genes that are active in a particular individual could be inserted into the basal stem cell layer; the dividing layer of skin cells. As these cells divide by mitosis, many new cells with the ability to silence that gene would be produced. As skin cells gradually move closer to the surface before sloughing off, eventually the modified cells will reach the surface. This, in theory, should stop the dilution pigment being produced in melanocytes (pigment producing cells) in that area as the signal to produce a particular pigment, the protein, has not been made, resulting in an area of coat that is a different colour to the whole horse. There are some problems which need to be overcome in order to carry this procedure out, most importantly how to insert the siRNAs into a large enough number of cells so that the effects would be visible on the surface, and the fact that, because the basal layer is constantly dividing, many of the modified cells would be replaced over time, so for the effect to become permanent and visible over the whole body would be very challenging; and there is nothing to suggest it would be desirable in the first place. There could be beneficial economic and social effects, however, more pressing are the negative ethical issues surrounding genetics which are to be explored later, as meddling with genetics to change the coat colour could be seen as pure vanity and not a justified use of this technique when we could be using it to cure disease.

One occasion where the modification of coat colour, or in fact, the silencing of any gene would be desirable, or at least more ethically sound, is when the colour dilution gene has more pressing side effects, for example the gene responsible for the frame-overo white pattern is associated with a disease of newborn foals called Lethal White Overo Foal Syndrome. At the moment, there is no cure for it;

fatality is certain and the horse has to be euthanised. This, as well as illustrating how useful it would be to silence a gene, also shows how potentially risky RNAi treatments could be; here, silencing a gene could produce an array of side effects, as the gene may have more than one significant function, so extensive tests would need to be carried out to ensure, when silencing a gene, the gene does not have any other significant effect.

Another incidence where the mechanism could be used to identify and silence faulty, disease- causing genes is canine ectodermal dysplasia, or CED, (hairlessness in dogs) in the Xoloitzcuintle Dog, or Mexican hairless dog, which is caused by a mutation in the FOX13 gene. Three quarters of this breed of dog are born hairless. Hairlessness, apart from being unsightly and bringing bad economic effects in the west, gives the dog problems with their teeth; they either have no teeth or their teeth are very weak. This is due to the genetic link between teeth and hairlessness. The dogs of this breed which are born with hair do not suffer these conditions; they contain two healthy versions of the FOX13 gene, whereas hairless versions contain a mutated version of the gene. If this mutated version of the gene was silenced, the healthy version of the gene would be able to produce the correct protein, the abnormal protein would not be produced so would not affect the functioning of the body, and the dogs would not be hairless. Over time, this characteristic could be bred into dogs, resulting in significantly decreased number of hairless dogs. However, this seemingly- beneficial use of the RNAi mechanism still has contriversial issues surrounding it; in the east, hairless dogs are considered sacred and they have been bred for their hairlessness. Also, apart from silencing the mutated gene, it is very likely that more of the required protein would need to be introduced to the dog, as they would then only have one gene producing the protein. Perhaps my point is, then, that humans rarely agree, and even when life changing science is discovered, there are always issues surrounding its use.

ETHICAL ISSUES

There are many ethical issues associated with the whole concept of designer babies. The most prevalent at the moment is the “sex- select” controversy. In humans, parents are allowed to select the gender of their child if their family are associated with a genetic disorder only relevant to one sex, for example haemophilia; in this case the parent may choose to have a girl instead of risking having a boy with the disease. However, many people believe genetic engineering has begun a slippery slope, and that genetic engineering in any form is wrong. However, genetic engineering and even cloning in animals seems to be partially acceptable, and certainly more acceptable than in humans. If RNAi is used to create individuals according to our tastes, animals may be first in the firing line. Some say using animals like this is wrong as they should have the same rights as us. Many others see scientific progress as necessary; that, although the use of a treatment may be unclear now, it may be needed for future disease or development. Many scientists say science is a “quest for knowledge”, and the point is that we **can** carry out a procedure, not necessarily that we will.

Would using the RNAi pathway be more or less acceptable than genetic screening? On the one hand, you do not have to produce many embryos as, using siRNAs and the methods described above, it is possible to silence dangerous as well as unwanted genes in a single embryo. The issue of spare embryos having to be discarded seems less significant as it is not necessary to create many embryos until you find the one with the desired characteristics, instead to create one and modify it. However, the reality is that many embryos would need to be created to account for chace as well as human error, so using the RNAi pathway to alter embryos does not really eliminate the main contriversy of IVF and “designer babies” which is the problem of what to do with spare embryos. Many people view embryos as alive, and as a person as they believe life begins at conception, and many more at least see them as having the potential to be alive, and by discarding spare embryos we are in effect denying a possible life. Some people say that genetics and genetic engineering in any way are wrong. People with strong religious views, may say that meddling with cells and DNA is "playing God". Their arguments may be particularly strong here as we are actively changing the individual's characteristics; in genetic screening we are simply identifying whether a specific gene is active or not.

The RNAi pathway may be a much more specific way to combat genetic conditions however it could prove to be difficult to carry out. Instead of scanning through the whole DNA of an organism in search for a specific gene, it is necessary to create a supply of the corresponding siRNAs and insert them into all the cells requiring them. Producing siRNAs could prove difficult as the genetic code has to perfectly match with the corresponding mRNA, otherwise it will not silence the correct gene, perhaps resulting in adverse effects, making the animal's condition worse. The people who carried out the process to manufacture siRNAs would have to be very skilled, as one base in the wrong order could lead to huge changes in the organism. Some people may be averted from this idea as the siRNAs would be inserted with the intent of changing the function of a gene, and they may have concerns about the consequences. Also, many of the treatments involving the RNAi pathway may not be "permanent". The individual may need to be "topped up" with more siRNAs to continue the effect when the gene is silenced. The cells may need a constant supply of siRNAs, or need regular treatment to ensure the levels are adequate in cells, which would mean regular insertation of siRNAs into the required cells.

As described above, using horse coat colour as an example, it may soon be possible to alter an organism's characteristics when it is fully developed. Obviously it is currently impossible to insert siRNAs into every cell in the body requiring them, but there is the possibility of finding an efficient method to insert siRNAs into many cells. For example, many liposomes containing the siRNAs could be inserted into the body at specific places- the liposomes would then fuse with plasma membranes of the target cells and the RNAi response would take place. Advances in technology may soon allow us to do this.

Many people may be attracted to the idea that they could change their pet, to a degree, whenever they wanted (granted, at a price, and with considerable effort), however the majority view will no- doubtedly be that this is morally unsound; it would not be fair for the animal to keep going through change, and again there is the argument that we are "playing God" and exploiting animals for our own vanity and curiosity. Is it worth meddling with the DNA of an organism so we can choose their characteristics? Some say we have been doing this for centuries with selective breeding; we have chosen which animals to breed together to enhance certain characteristics over many generations. However, it has never been done so we are actually entering cells to change their genetics; this idea may not appeal to some people.

CONCLUSION

Perhaps we have to accept that sometimes our "quest for knowledge" must be overcome by our sense of morality. Perhaps we should never have complete power to choose the characteristics of an organism; even if we used selective breeding in the past, however developed science may be, as our consciences stop us. This paper has shown how contriversial new therapies involving genes and DNA are, but has also shown that RNAi could prove to be very useful in the future, whether when combating viruses, cancer, mutations in genes or purely silencing genes for our own interest or benefit. It is safe to say that none of the procedures described in this paper will be possible for a significant number of years, firstly because the RNAi treatment need to be properly tested to eliminate its problems, and secondly because its use raises serious moral and ethical issues.

REFERENCES

History of RNAi discovery and RNAi mechanism

http://www.ambion.com/techlib/hottopics/rnai/rnai_may2002_2.html,
http://www.medicalnewstoday.com/articles/53273.php
http://www.vet.uga.edu/id/tripp/RNAi.php

Figure 1- The unexpected cosupression effect discovered by Jorgenson et al

http://www.easypedia.gr/el/articles/r/n/a/%CE%95%CE%B9%CE%BA%CF%8C%CE%BD%CE%B1~Rnai_phenotype_petunia_crop.png_32a6.html

Information on aneuploidy screening

http://news.bbc.co.uk/1/hi/health/3123633.stm

Information on the "mean gene" in bees

http://www.accessexcellence.org/WN/SUA12/bee498.php

Information on the genetics of horse coat colour

http://www.vgl.ucdavis.edu/services/coatcolorhorse.php
http://www.horse-genetics.com
http://www.uky.edu/Ag/Horsemap/

Figure 2- Drawn by author using Microsoft Word 2007

Figure 3- skin cell layers annotated by author

http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=dyncan&part=A280

Information on CED

http://generalmedicine.suite101.com/article.cfm/the_genetic_mutation_that_causes_hairless_dogs