

USING RNAi IN THE PREVENTION OF THE  
INHERITANCE OF SICKLE CELL ANAEMIA AND  
OTHER GENETIC DISEASE

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## **Abstract**

RNA interference is a relatively new, importantly considered in molecular oncology. It is a process which regulates gene expression and corresponding proteins. In effect any gene can be stopped at any time. RNAi can be used to prevent the inheritance of Sickle Cell Anaemia by silencing the responsible gene during meiosis in embryos. Achieving this is much easier in females than in males, as males produce gametes throughout their life, so a solution should be found to regularly introduce small inhibitory RNA in the early stages of meiosis. I identify the stage in which siRNA should be introduced in both male and female meiosis and the resulting benefits for the embryo in later life, also highlighting ethical issues and other problems which may be held by the public and other bodies.

## **Introduction**

RNAi is the process of inhibiting gene expression and can be used to prevent and treat genetic disease. In humans the protein complex RISC (RNA-induced Silencing Complex) is a secondary response after inflammatory response to invading double stranded RNAs. RISC needs to be activated by short chain RNA referred to as small inhibitory RNA. This doesn't occur naturally in humans so the inflammatory response to for example a virus is always triggered. Though RNAi is significantly better than alternate gene targeting techniques further investigation is needed into the administration of siRNA and the duration of the effects. (*Journal of RNAi and Gene Silencing 2006*)

Currently RNAi research is taking place to find treatments and cures for terminal diseases such as cancer. Within cancer research RNAi is being looked into with its essential gene targeting ability to make an impact on tumour-host interactions and chemotherapy resisting tumours. (*Prospects of RNA interference therapy for cancer 2005*) Other research, in immunodeficiency diseases such as HIV-1 has found that RNAi is better us with long hairpin RNA. This is because the lhRNA is able to inhibit the HIV-1 which may result in synthesis of siRNA to further reduce the chance of viral escape. (*Inhibition of human immunodeficiency*)

Based on the idea of using siRNA to treat disease, by furthering the use of RNAi, theoretically we can prevent genetic disease being inherited initially. The theory is to administer siRNA to embryos in order to prevent their children in later life developing the genetic disease which the embryo carries the gene for. This will comfort expecting parents that their grandchildren will not have to endure long and strenuous treatment for a genetic disease they would have inherited, if it weren't for RNA interference. If siRNA is given to their developing child during the production of its gametes, the chance of their grandchildren inheriting the disease will be virtually zero, providing the siRNA is administered at the right time during meiosis in the embryo.

This could also be used in veterinary medicine to prevent diseases which have no current treatment while more research takes place. Also, it would aid the research in genes causing genetic disease, with the ultimate aim of providing future generations with a population free of genetic disease. This theory can be spread across a wide range of veterinary related issues such as wildlife conservation all over the world. Ridding of the genes causing genetic disease while the animal is still an embryo will mean more effective breeding. This could help the recovery of endangered species and rare breeds, if siRNA was introduced to animals in captivity, when released the animal can help repopulate the species and ensure that they are free of genetic disease.

Sickle Cell Anaemia is a genetically inherited disease which causes the red blood cells to have abnormal haemoglobin which after dissociating oxygen become distorted or "Sickle shaped." The abnormally shaped red blood cells are unable to flow through the blood vessels easily as they are stiff and sticky, (*National Heart Lung and Blood Institute*) this along with other contributing factors can result in clumping of the red blood cells and thrombosis in the blood vessels, causing episodes of severe pain for the patient. Also they suffer from chronic anaemia as the sickled red blood cells die after 10-20 days as opposed to normal red blood cells which are active for 110-120 days (*ornl.gov*). The red blood cells can't be replaced quickly enough therefore causing anaemia. In order to suffer from sickle cell anaemia a recessive allele must be inherited from each parent.

As only inherited by the recessive allele the embryo still has a 25% chance of inheriting sickle cell anaemia, but the embryo's children will be at significantly less risk if no risk at all. This is the aim; to reduce and eventually eliminate sickle cell anaemia in the human population. The female embryo begins to produce precursor egg cells at about five months after conception (*Gametogenesis and Gamete Interaction during Fertilisation*). However, before introducing siRNA it must be established at which stage of meiosis the introduction should occur. The answer is during interphase at DNA replication; this would ultimately trigger the RISC process (RNA induced silencing complex) in the meiotic cell therefore destroying the responsible gene for sickle cell anaemia, before the two new strands of DNA can be formed by DNA polymerase. This is achieved because the mRNA is destroyed which stops the sickle cell anaemia gene being replicated in the first place. The end result would be the female embryo having her complete number of precursor egg cells without the sickle cell anaemia gene and therefore ensuring that her child will not suffer from the disease even if her male partner in later life carries the gene, because as I have previously said sickle cell anaemia must be inherited from both parents.

### **Treatment of Male Embryos**

Males are born with precursor sperm cells so siRNA would have to be introduced during foetal development, but do not begin producing mature sperm until puberty. This gives them the decision to have treatment, to prevent the inheritance of sickle cell anaemia in their child or not. However, at the embryonic stage if the child is found to be male and a carrier of sickle cell anaemia it could influence the decision of whether to abort the child or not. Treatment for male carriers of sickle cell anaemia would be significantly longer than the embryonic insertion of siRNA for females, as males produce gametes throughout their life.

Achieving this is possible through several ways: Continual treatment during adult life or taking sperm and replicating it in a lab with siRNA then storing it until the male is ready to have children. Continual treatment would be more inconvenient for the patient and expensive if this is used for a large number of patients. Also the frequentation of the administration of siRNA would be a deciding factor in whether the treatment would be worthwhile. Precursor sperm cells (spermatogonia) are diploid cells like that of the female precursor egg cells and exist in the seminiferous tubules in the testes. A way would have to be found to introduce siRNA into this area of the testes before the spermatogonia mature, preventing the messenger RNA from transferring the sickle cell anaemia gene to the daughter cells, during mitotic division of the precursor sperm cells therefore stopping any of the four new haploid sperm cells after meiosis containing the gene. The process from spermatogonia to mature sperm cell takes three weeks so the time of introduction would have to be accurate in order to sufficiently minimise the risk of passing on the sickle cell anaemia gene. (*Gametogenesis and Gamete Interaction during Fertilisation*)

The other suggestion I put forward is storing (freezing) sperm cells, either at the precursor stage before metaphase or mature sperm cells. If at the precursor stage they could be already without the sickle cell anaemia gene if taken from an embryo or child before the sperm begins to mature, or stimulated to divide and then introduce siRNA to stop the gene being in the new haploid cells after meiosis as with in the previous treatment. The limiting factor on this treatment is expense again as it will be more costly than treatment of the patient themselves and the length of time the sperm is able to be stored for. It has been proven that the sperm can be frozen for 12 years and still produce healthy pregnancies (*Sperm Bank Directory*) so this treatment is viable and convenient for the patient. This method could also be used for precursor egg cells from a female child if the parents of the embryo thought it unethical to have treatment before the baby is born. An ethical issue related with this treatment would be similar to the views toward IVF and the elimination of genetic diseases would be seen as playing God. People are already living longer in modern times and taking out another factor causing natural population control would result in problems such as; overcrowding and economic and environmental deficiencies in the future.

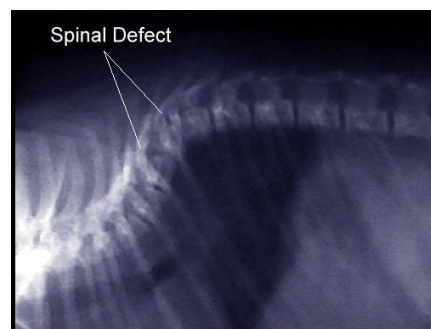
If these techniques are developed they could be used for practically all genetic diseases. If the disease only requires inheritance from one parent's dominant allele such as Huntington's disease only the embryo with the dominant gene would have to have treatment. This reduces cost and the ease for the patient, because only the carrier parent will have to be treated with the siRNA. However, to carry out the research and then treat millions would have with it a huge expense, is the money worth it to eradicate all genetic disease?

### **RNAi in Veterinary Medicine**

This theory can also be applied to veterinary disease and research is being carried out in various aspects of veterinary medicine where RNAi can be applied. The Veterinary University in Georgia is focused on research using RNAi to prevent and treat viral respiratory disease. (*Viral Immunology: Tripp Laboratory*) Examples of veterinary genetic diseases that could have prevented inheritance are: for equines; Severe Combined Immunodeficiency (SCID) that is common in Arabs is complete inability of the adaptive immune system to accumulate an appropriate immune response, this is usually due to absent or uncharacteristic T and B lymphocytes. Also, Glycogen Branching Enzyme Deficiency (GBED) which especially affects American quarter horses, this genetic disease results in the lack of the enzyme necessary for glycogen storage and the horse's brain, heart muscle, and skeletal muscles are unable to function resulting in rapid death. (*VetGen*) Both of these diseases cause quick death and if known carriers of the disease could have their foals treated with siRNA continuation of the disease could be stopped.



**Figure 1-** A Manx cat with spinal deficiencies as a result of Sacrocaudal Dysgenesis, causing inability to use its hind legs efficiently.



**Figure 2 –** Showing a spinal defect in a Manx cat with Sacrocaudal Dysgenesis.

However, the causal gene of SCID has already been recognised and with a DNA test precautions breeding can be put in place. This is already making difference by preventing foals being born with the disease. (*VetGen*) My theory could also be used to treat various genetic diseases in canines and felines: Cerebral Ataxia in dogs causes degeneration of the cerebellum as shown in figure 3 in comparison with figure 2 which is that of a normal dog, and can result in reduced movement and the inability to walk. In some breeds it can also cause other parts of the brain to degenerate (*AKC Canine Health Association*). For felines Manx Syndrome or Sacrocaudal Dysgenesis can be fatal if two of the causal genes are inherited by one kitten and the kitten is spontaneously aborted before birth. Sacrocaudal Dysgenesis or Manx Syndrome is the cause of Manx cats having a partial or non-existent tail though the gene can also cause spinal defects resulting in incontinence or un-coordination of the hind legs as shown in figure 1, if siRNA was used this would increase the population of Manx cat and reduce spinal deficiencies in their breed.

Figure 2 – Normal Dog

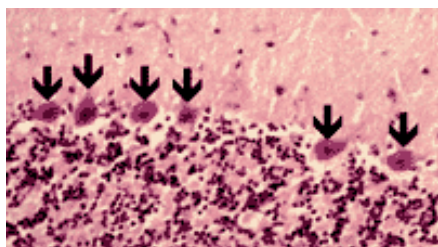
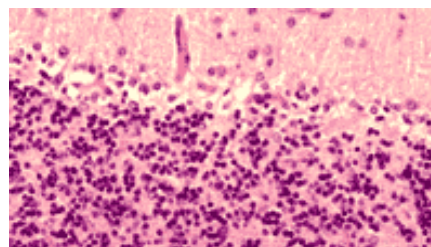


Figure 3 - The Purkinje cells which normally line up between the layers of the cerebellum (arrows) are lost in hereditary ataxia.



### **RNAi Use to Conserve Endangered and Rare Breed Species**

Another use for my theory would be in the genetic diseases of endangered species in captivity, or rare breed species. This would be feasible even though the risk would be higher, because of the limited time you can keep a wild animal under anaesthetic. Especially in larger animals such as elephants and rhinos as if they fall wrongly while anaesthetised they can put their lungs under pressure from other organs and suffocate, also the recovery is more difficult for wild animals. Although, if genetic disease could be eliminated as a factor for making a species endangered we would be able to have a higher population of endangered species in captivity helping the overall situation of the species in question. Genetic diseases in endangered species such as the Sumatran Tiger and Red wolf would be similar to domesticated animals so identification of the disease would be much easier, this means that the concentration of the siRNA administered to the embryos would be similar to that of a cat or dog. This reduces the fatalities in these animals and helps us repopulate rare and endangered species in their natural habitats.

Of course experimentation and research would have to take place to continue my theoretical ideas is necessary, and developing research in the future on the causal genes of various genetic diseases would mean that the prevention of inheritance of sickle cell anaemia and other genetic diseases could be used for many years.

## **Conclusion**

In conclusion, siRNA can be introduced to the embryos of carriers of genetic diseases to prevent the inheritance of genetic diseases to the embryo's future children. This technique of intrauterine introduction of siRNA could be used in human male and female embryos and animal male and female embryos. More complications arise within animals because of the wide range of different species, but using this method within rare breeds and endangered species would be a great breakthrough in science and conservation would improve.

An issue with the administration of the siRNA to a female embryo though, is what it would entail for the mother of the embryo during pregnancy and whether she would be willing to allow her baby to have the siRNA administered. The siRNA would have to be introduced into the embryo's ovaries potentially meaning an intrauterine procedure unless the stage at which the embryo's precursor eggs develop was at a very early point, and then keyhole surgery could be used with an endoscope to insert the siRNA. This is feasible as prenatal surgery on fetuses has been performed successfully on humans and animals (*Intrauterine repair of Cleft lip in mouse fetuses*). Another problem with the surgery would be if blood transfusions were needed, the baby could reject a different blood type therefore endangering it and the mother. This could be solved by minimal interference such as keyhole surgery to make blood transfusions unnecessary.

Also, defects in the efficiency and duration of effectiveness of the siRNA must be further tested. It is no use administering the siRNA to the embryo to find that not all of the embryo's gametes are free of genetic disease. If the siRNA has stopped being effective after a short period of time. Future development may find that the siRNA may have to be given more than once if this is the case. More research and development in this area must be carried out in order to apply my theory and put a stop to inheritance of debilitating and potentially fatal genetic diseases.

Problems associated with siRNA introduction into animal embryos is the differences in gestation of different species and again the interference with the mother during pregnancy, meaning the time of introducing the siRNA must be established for each individual species which will be treated. Though this is a problem, gestation periods are known for a lot of animal species but it could be more difficult if you wished to treat an endangered species such as tree kangaroos that have not been studied to an extent. There is also the issue as within humans of treatment throughout life, but in animals it may be more cost effective to store the sperm from that particular animal and if wishing him to breed with a female in another zoo the sperm could be sent there. Also, if gametes without genetic disease could for several years be stored for endangered species, this would mean if extinction ever were to happen the species could be reinstated if gametes were taken from all known animals of the species in captivity. Again this all involves cost though which is a barrier to be overcome by support from the government and other bodies globally.

Also economical and ethical issues, such as "Playing God" would have to be addressed, this may include being selective of which patients to treat or to wait until the country is in a good enough economic situation to begin further research and set up trial clinics. Charities may also be formed in conjunction with the research, raising further funds to initiate NHS clinics as well as private ones. Regarding ethical issues not everyone should be treated if they object to it. It is expected that people will have protests and oppose the research and procedures taking place but scientific endeavours are about the progression and bettering welfare of humans, animals and other living species.

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