

Possible applications of RNAi in the treatment of Chronic Myeloid
Leukaemia in dogs

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Abstract

In this research paper I will be talking about the possible application of RNAi to treat chronic leukaemia in dogs. RNAi is a system, a pathway, in which active genes can be effectively silenced and turned off in the post translation stage of protein synthesis. Using this process of silencing genes; oncogenes in active cancer cells, such as those formed in the bone marrow, can be switched off to prevent development and metastasis of the cancerous cells. In addition to this RNAi can also be used to support chemotherapy by reducing the chance of cell rejection of the drug by silencing the gene responsible for removing the cytotoxic drug from the cell. In conclusion the following paper aims to show that the application of RNAi therapies, or RNAi supported therapies has the potential to vastly improve the prognosis of a patient diagnosed with chronic leukaemia.

Introduction

DNA inside the nucleus is not directly used in protein synthesis. Instead it is unwound and copied by DNA polymerase to form RNA in a process called transcription. Because the RNA is used to transmit genetic information to the ribosomes outside the nucleus via the nuclear pores it is called mRNA (messenger RNA). When the mRNA leaves the nucleus it enters the small sub unit of the ribosomes (40s) where protein synthesis occurs. This is the process of translation and the final step in protein synthesis, both the ribosomes and mRNA are reusable. RNAi (RNA interference) is a recent advancement in medicine^[1] whereby the expression of a gene is moderated inside the cell. In plants it also has a role of anti-viral defence. Long dsRNA (double stranded RNA) are cut into smaller sections only 18-20 nucleotides (nt) long^[2] called siRNA (small interfering RNA) by the RNase enzyme Dicer. The siRNA then activates RISC (RNA Induced Silencing Complex) which binds to the siRNA, forming a ribonucleoprotein complex, and unzips the molecule. The anti sense strand of the siRNA is used by Slicer (a part of RISC) to identify mRNA with matching base sequences inside the cell. Slicer lines up the single strand of siRNA with the mRNA in a sequence specific manner and creates cleavage at the point of complementarity in the base sequence. The cleaved mRNA is recognised by the cell and destroyed. By this process the gene relating to that mRNA is never expressed and effectively turned off. In mammals there is an amplification step where an enzyme attaches to the region of genetic information in the mRNA matching that of the siRNA and creates a new single strand of siRNA that can be used by RISC. There is potential for vets to use this in the death of cancer cells where growth genes, that are over expressed, can be turned off.

This pathway also exists in mammals, but when long dsRNA enters a cell it triggers the complex and non specific interferon inflammatory response by the immune system which shuts down all protein synthesis^[1].

Leukaemia is a type of cancer that affects the stem cells in the bone marrow. There are two main types of leukaemia, chronic and acute. Acute leukaemia has a rapid onset whereas chronic leukaemia often develops over months or years^[3]. Leukaemia then has further subtypes

according to which type of blood cell is being affected. This paper will only be concerned with chronic myeloid leukaemia (CML) which affects the granulocytes causing them to become cancerous. Because of the over production and rapid growth of immature granulocytes the normal stem cells in the bone marrow have less space and are therefore reduced in numbers. This causes the symptoms associated with CML, such as: lethargy or tiredness (caused by anaemia from reduced red blood cell count), swelling of the lymph glands (where the cancerous granulocytes congregate), weight loss and in some cases a painful swelling of the spleen^[3].

Granulocytes themselves are part of the non-specific immune system. That is to say that they do not respond to specific antigens. There are three types of granulocytes: Neutrophils, eosinophils and basophils^[8].

There are 3 main stages to CML^[4]: Chronic, Accelerated and Blast. In the chronic phase the cancer is still slowly developing and may have been doing so for a few years before diagnosis as the symptoms are very mild and vague. In Accelerated phase the growth and division of the tumor is faster and less stable, symptoms may become more severe. The final stage can be called acute phase, blast phase or blast crisis. This is when the tumor has affected over 30% of the bone marrow and the blast cells have spread the cancer to other organs. In this stage of CML the patient will be feeling very unwell and prognosis is not good.

Discussion

As briefly discussed in the introduction RNAi is a system in which active genes are stopped from being expressed and therefore turned off. In this section I shall discuss how this can be harnessed by vets to combat cancer, more specifically how it can be used as a therapy for CML. I will also discuss the possible problems associated with the use of RNAi therapies in treatment of CML in dogs and how these problems can be overcome.

The method I believe appropriate for treating CML is to target the oncogenes that control the growth factors of cells. This can be done by introducing siRNA into the affected cells. The siRNA will have to contain the optimum sequence of bases as to target as many oncogenes as possible without causing 'off target' silencing of genes where the RNAi system attacks mRNA that is healthy but contains part of the base sequence found in the siRNA that has been introduced to the cell. Such silencing of genes can cause adverse side effects but can be effectively reduced with careful forethought and planning^[5].

Because the oncogenes have been silenced in the cell it will no longer have the ability to enlarge beyond its natural size and rapidly divide. This will bring about the death of cancerous myeloid cells.

Although to be effective RNAi therapies will also have to target various other cells in the body where the cancer could have spread to. To do this an effective delivery system for the siRNA is needed. Problems arise when trying to transport the siRNA to the effected cells because 'naked' siRNA breaks down quickly in the bloodstream and fails to meet the target cells. Possible solutions to this problem have been found in virus vectors. This is a practise by which siRNA is attached to modified viruses stripped of their harmful genomes. These viruses then travel to the required cells and excrete the siRNA in to the cytoplasm causing the RNAi pathway to be activated and not the interferon inflammatory response. Using this method has taken a long time to refine and will prolong clinical trials of the drug.

The idea for virus vectors was first conceived by biologists in the 1970's. Paul Berg used a modified SV40 virus containing DNA from the bacteriophage lambda to infect monkey kidney cells maintained in culture^[6]. The principle works by taking a virus and removing the harmful genomes (including those needed to replicate inside the cell) and then using further modification of the virus to manipulate the 'machinery' for transport of intended genetic material (siRNA) to the target cells. The process of using a viral vector inside a living organism is called *in vivo* and the name for the delivery of genes via viruses is called transduction. Because viruses have evolved to reach and take over host cells to replicate they have a very efficient method for transduction although some factors should be taken into consideration when choosing a virus. Low toxicity is a preferable factor as minimal damage to the host cell is expected, as is a high stability of the virus (resistant to change in the genetic information carried).

Levels of siRNA may need to be maintained in the cell in order for the oncogenes to be actively silenced. This can be done either by re-administering the viral vector containing the siRNA to the patient or by setting the virus containing the siRNA to a set number of replications inside the host cell.

A possible problem facing RNAi treatment in CML when using a virus vector is the triggering of an immune response by the body. Possible solutions are to modify the virus so it contains antigens preventing the immune system from responding. A second option is to use immunosuppressant drugs on the patient, but this could be dangerous as a symptom of CML is reduced immunity due to the vast number of faulty granulocytes that do not function in to properly. This also causes a lower count of other white blood cells due to the tumor affecting blood production in the bone marrow. Therefore it is unlikely that the second option will be used when combating this problem.

Unfortunately another problem arises when the oncogenes can further mutate and develop immunity to RNAi therapies. The risk of this happening can be reduced by swift application of treatments to ensure maximum amount of cancerous cells destroyed with the least amount of

healthy cells destroyed or damaged. By this method the chance in which the granulocytes can mutate to develop immunities is narrowed meaning reduced risk of resistance to the treatment. Combining RNAi therapies with other treatments is also an effective way of reducing the risk of drug resistance as discussed below.

But using RNAi alone, even with an efficient delivery system, will not be enough to put CML fully into remission. If the RNAi was also used to target genes that encoded for proteins that caused drug resistance from chemotherapy then the treatment would have a much greater and widespread affect on CML. A protein that has been found responsible for drug resistance, P-glycoprotein, can be stopped by silencing the gene responsible for its synthesis using the same methods outlined above.

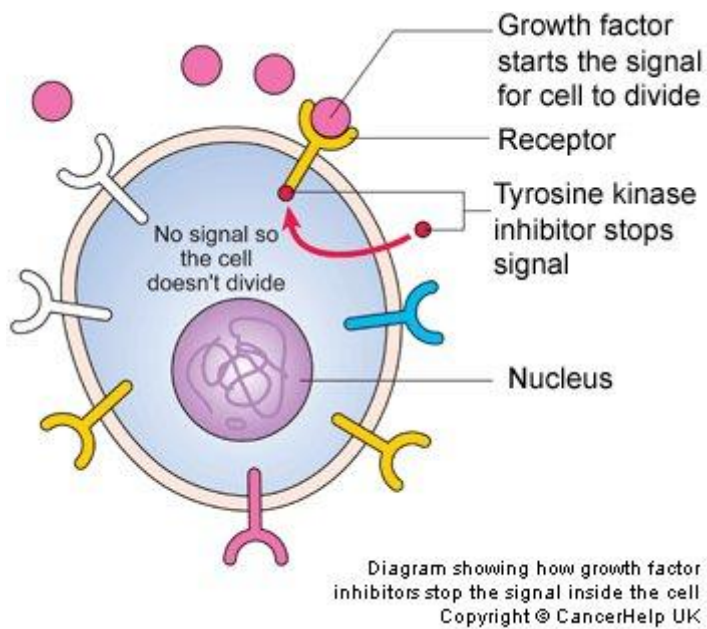
Ethical issues surrounding the use and development of RNAi therapies:

There are many ethical issues scientists face when developing new drugs or testing out new theories, the same can be said for RNAi. Whilst some research can be carried out on cultured cell samples it is necessary to use models such as mice to produce more accurate simulations of the possible effects on mammals, and more specifically dogs. And due to the specific nature of the RNAi therapies and genetic variation between species it will be necessary to carry out tests on the recipient species , this is because mice are too genetically different to make accurate predictions of the affect of the treatment on dogs. Because of the need to test on dogs further questions are raised about the importance of this new treatment and the ethical justification of the clinical trials involved.

As well as the above mentioned issue of clinical trials there is also the issue of interfering with the cell's natural function. As seen in genetic engineering certain religious and ethical groups, such as certain denominations of Christians, are strongly opposed to manipulation and/or modification of genetic material inside the body. Although significantly less opposition is faced in use of this treatment for animals then there is in that of human treatment. It is still an issue that needs to be addressed by the global community on weather research into RNAi for use in animals should be allowed. An argument against the use of RNAi in veterinary medicine is the inability of the dog to choose to take the treatment. As a consequence of this lack of free will the choice is ultimately left to the owner putting them in a position of power which is often abused. Whether intentional or not, in some cases the application of RNAi to prolong the life of a companion animal (dogs) with CML in the blast phase and with a terminal prognosis is ethically incorrect and in these cases euthanasia should be considered instead. Although it should be stressed at this point that the development and application of RNAi therapy in CML will increase the survival rate and reduce the need for euthanasia.

Considering the reasons mentioned above it is understandable that RNAi therapeutic treatments will take a long time before becoming commercially available to vets.

Alternative treatments for CML, in addition to chemotherapy, are biological therapies that employ growth factor blocking agents that inhibit the signals inside a cell telling it to divide (as shown below in figure 1). Tyrosine kinase inhibitors are usually given at the chronic stage of CML and one such drug is called imatinib. Though these drugs also come with an onset of side effects some of which are weakness, tiredness, loss of fertility, diarrhea and a skin rash^[7].



(Above: figure 1, a diagram showing growth factor inhibitors.)

Conclusion

It has been shown that the potential for RNAi as a therapy for cancer is promising. RNAi can be both used for targeting oncogenes and in support of other therapies such as chemotherapy, to decrease drug resistance. The siRNA cannot be delivered directly to the cell but a viral vector is able to effectively transduce the siRNA into the cell. And the use of RNAi is much more effective if used in conjunction with other cancer therapies. Considering the ethical issues and the possible advancements for both veterinary and human medicine I believe that the development of RNAi to combat CML is both a worthwhile and justified application of science.

References

http://en.wikipedia.org/wiki/RNA_interference#cite_note-Raoul-119 ^[1]

<http://www.nature.com/focus/rnai/animations/animation/animation.htm> ^[2]

<http://www.cancerhelp.org.uk/type/cml/about/symptoms-of-chronic-myeloid-leukaemia> ^[3]

<http://www.cancerhelp.org.uk/type/cml/treatment/staging-for-chronic-myeloid-leukaemia#cm> ^[4]

<http://www.nature.com/gt/journal/v13/n6/pdf/3302690a.pdf> ^[5]

http://en.wikipedia.org/wiki/Viral_vector ^[6]

<http://www.cancerhelp.org.uk/about-cancer/treatment/cancer-drugs/imatinib> ^[7]

<http://www.medterms.com/script/main/art.asp?articlekey=8780> ^[8]