The Use of Gene Silencing in the Treatment of Cancer

BY

Peter Dickson

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ABSTRACT

RNA interference is able to terminate the expression of a gene. This is particularly useful in the treatment of chronic diseases such as cancer, the over expression of a gene that is not normally expressed. This paper discusses the use of gene silencing, through RNA interference, in the treatment of cancer. Delivering RNA currently poses a huge problem in the treatment of any disease using RNA interference. Possible applications of modern technology that could be used to aid this process, such as nanotransporters, are discussed. RNA interference has shown a very high potential in the silencing of genes involved in tumorigenesis and metastasis. The importance of not only targeting the cancerous cells but also the surrounding environment is highlighted. RNA interference currently poses many advantages and some disadvantages. However, with further research this technique has exciting potential to target many cancers affecting our population.

INTRODUCTION

The process of RNA interference (RNAi) involves the insertion of a double stranded RNA (dsRNA) corresponding to a particular gene's sense and antisense strands into cells. The dsRNA is degraded in vivo and the resulting fragments base pair with messenger RNA (mRNA) from the target gene causing it to be degraded. Consequently it terminates expression of the gene as shown in figure 1. (1)

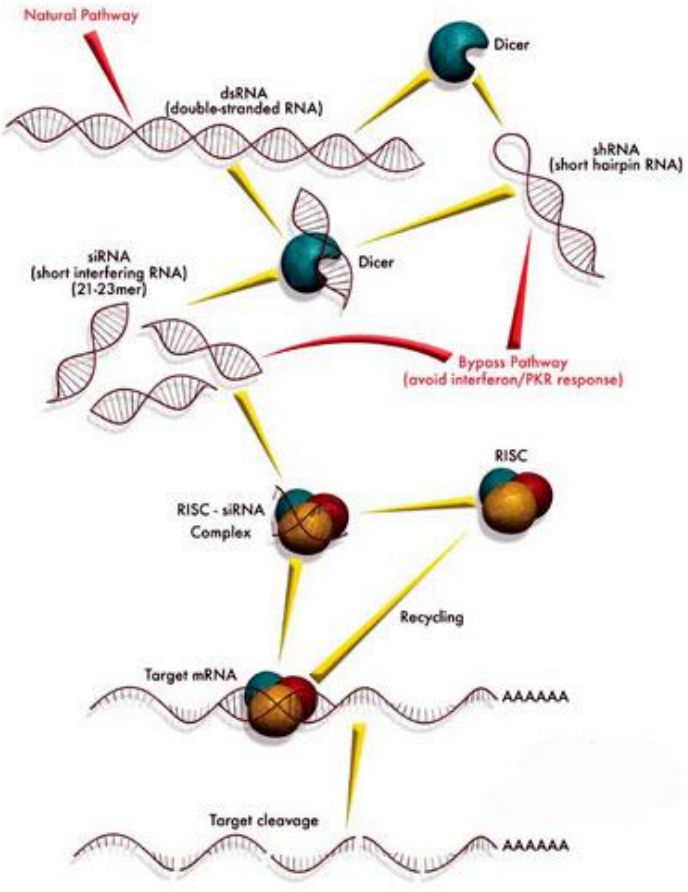

Figure 1: the mechanism of RNAi. (6)

RNAi is becoming an increasingly popular technique due to its success in the treatment of many diseases. RNAi has several important advantages over other methods for targeted inhibition of gene expression (e.g. antisense, catalytic RNA and DNA molecules, and homologous recombination). These include broad cross-species application, a gene specific mechanism of action allowing no or little off-target effects. Also the ease of manufacturing synthetic small interfering RNA (siRNA), since siRNAs are formed by a natural endogenous process, all cells carry the machinery needed to mediate gene silencing.(2) Thus RNAi can be used in order to combat chronic diseases such as foot and mouth, hepatitis B and cancer.

In cancer research, RNAi is regularly being used as a tool to study and manipulate a particular gene and its function. The technology can be applied to cancer cells in a broad range of model systems, including animals. (3) RNAi could be used in many stages of cancer development. It could prevent the molecular changes that a normal cell undergoes to become cancerous, kill off cancer cells already present and prevent metastasis of the cancer.

Cancer occurs by two main methods: loss of tumour suppressors due to genetic alteration e.g. p53transcription factor, or the over expression of a gene which is not normally expressed (e.g. Ras- a small GTPase). It is the latter with which we are concerned here. Gene over expression can cause rapid cell division leading to the formation of a tumour which may be malignant or benign. Benign tumours do not spread to other body parts or invade other tissues, and they are rarely life threatening unless they compress fundamental structures or are physiologically active for instance, producing a hormone. In a malignant tumour the cells have the ability to spread to other parts of the body. Cells may break off the primary cancer and be carried round the body by the blood stream or the lymphatic system therefore destroying the surrounding tissue as shown in figure 2. (4)

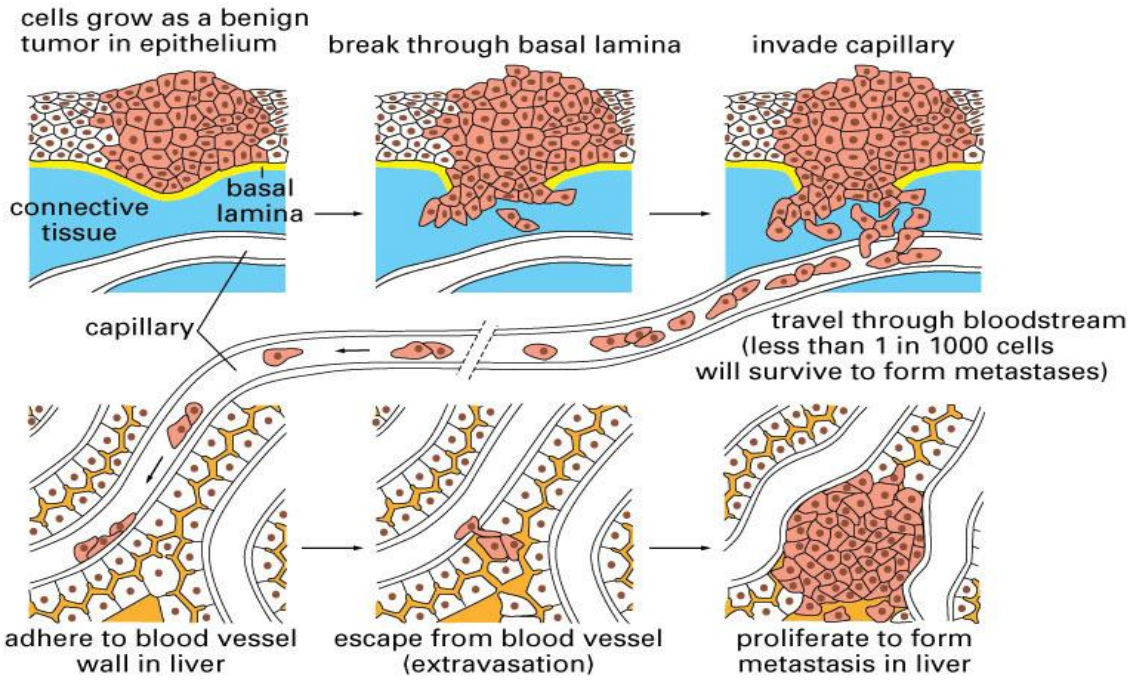

Figure 2: The spread of a primary tumour (metastasis) through a blood vessel. (5)

DISCUSSION

It is important to realise the many possible uses of RNAi in relation to the treatment of cancer. In this section I will discuss the delivery of RNA, the targeting of specific genes in the treatment of cancer and the general advantages and disadvantages of the use of RNAi in the treatment of cancer.

Gene Therapy and Chemical Modifications:

Cancer often involves mutant genes that stimulate uncontrolled cell growth. In the last few years, researchers have silenced over a dozen known cancer-causing genes with RNAi. However, most of this success has been with cell cultures in a lab. Delivery poses the key hurdle in moving from the lab to the veterinary practice. Currently there are two main methods of delivering the siRNA in vivo. (7) One strategy is gene therapy; this uses a viral vector to deliver the siRNA to the cells of interest. Alternatively, the reagent can be chemical synthesis causing a change in the siRNA properties through the chemical modifications. This makes them more stable and retains them for longer periods of time in the bloodstream. The modifications also change their properties facilitating uptake. With the broad applicability of RNAi to a diverse range of animal diseases in a wide variety of organ systems, both delivery methods are being developed for specific therapeutic targets.

Exposure, Cytoplasmic Uptake and Nanotransporters:

The delivery of RNA has two main hurdles which must be overcome. The first is one of exposure. That is, if the RNAi compound is injected will the tissue targeted be exposed to the RNAi? If the RNA is injected it enters the blood, where it may be taken up by the kidneys and removed. In most cases, standard injections don't have a chance of turning off genes with standard RNAi compounds. A second hurdle is cytoplasmic uptake. Essentially overcoming exposure problems doesn't mean the RNA can get through the cell mebrane and into the cytoplasm of cells. This is a separate issue, as is the stability of the RNAi compound itself. This means that RNA needs to be carefully designed and chemically stabilized. Nanotransporters could potentially solve this problem. Nanotransporters are chemicals of specific size that are mixed with an RNAi compound forming minute particles for delivery into target tissues. The nanotransporter has a core to which layers are added by chemical synthesis. The final layer has a positive charge meaning it can attract and bind negatively charged RNAi compounds. Specific injection of nanoparticles into cancerous areas of an animal can effectively treat the cancer. The use of nanoparticles also solves the problem of gene targeting as long as the injection is accurate. (8)

Use of RNAi in Tumorigenesis and Metastasis:

RNAi could be used in the silencing of genes involved in tumorigenesis and metastasis. Tumorigenesis is caused by a mutation of the genetic material of normal cells, which upsets the usual balance between proliferation and cell death. This results in uncontrollable cell division and the evolution of those cells by natural selection in the body. The uncontrolled and often swift proliferation of cells can lead to benign tumours; some types of these may become malignant tumours (cancer). Malignant tumours can and will invade other organs, spread to distant locations (metastasis) and often become life-threatening. More than one mutation is necessary for tumorigenesis. Only mutations in those specific types of genes which play vital roles in cell division, cell death, and DNA repair will cause a cell to lose the power to control its cell proliferation. In order for cells to start dividing

uncontrollably, genes that control cell growth must be damaged. Proto-oncogenes are genes that encourage cell growth and mitosis, whereas tumour suppressor genes discourage cell growth and development, or temporarily halt cell division to perform DNA repair. Typically, a series of several mutations to these genes is necessary before a normal cell transforms into a cancer cell. Proto-oncogenes promote cell growth in many different ways. Many can produce hormones that encourage mitosis, the effect of which depends on the signal transduction of the receiving tissue or cells. If the proto-oncogenes could be silenced so that these hormones were not produced once the primary cancer had been found then growth and metastasis of the tumour could be prevented. (11)

Use of RNAi to Silence Cyclin D1:

The majority of deaths associated with cancer are due to the metastasis of the original tumour cells to sites far from the initial or primary tumour. Metastasis is the process by which cancer cells travel throughout the body. So how do metastases form from primary tumours? For cells within a primary tumour to become metastatic several events must occur and failure of any one step will put a stop to metastasis. Tumour cells must detach from the primary tumour mass, boost their mobility, enter the circulatory system, survive transport within the circulatory system, exit the circulatory system, and successfully colonize a new tissue. Metastasis is very inefficient; millions of tumour cells might enter the bloodstream each day, but only a small portion ever succeeds in successfully colonizing another tissue. This suggests healthy tissues are very hostile to invading cells, and tumour cells must overcome multiple barriers in order to successfully metastasise. If the gene cyclin D1, which promotes the detachment of cancer cells from the primary tumour, could be silenced through RNAi then metastasis would not occur. (10)

RNAi in Support of Chemotherapy:

On the other hand rather than targeting the cancer, some RNAi therapies may help defeat cancers by supporting chemotherapy. Drug resistance is a huge problem in chemotherapy, preventing success in between 20 and 50 percent of all current treatments. In many of these failures, the guilty agent is a protein called P-glycoprotein. This protein removes drugs from diseased cells. In 2004, a group of scientists at Imperial College London showed that RNAi can stop the production of this protein in multidrug-resistant leukaemia cells, restoring their sensitivity to existing drugs. This means that gene targeting of the cancer would not be necessary.

Advantages of the Use of RNAi in the Treatment of Cancer:

One advantage of RNAi is the ability to target virtually any protein A key limitation of most medicines is that they can only target certain classes of proteins. Targets for currently available small-molecule drugs include G-protein-coupled receptors, ion channels, enzymes and nuclear hormone receptors. Despite a great deal of effort, attempts to find small-molecule drugs targeting other types of proteins have been largely unsuccessful. The array of targets for protein drugs, such as monoclonal antibodies, is also restricted mainly to cell-surface receptors or to circulating proteins. In contrast, given the sequenced human genome, it is feasible to design small interference RNAs (siRNAs) for any gene and its mRNA transcript. This capability opens up the opportunity for using siRNA drugs for proteins that do not fit into the so-called "druggable target classes." Therefore enabling the treatment and prevention of all kinds of cancer. Further, specific diseases may be

caused by the mutation in a single allele, in which case a certain siRNA can target the disease-causing mutation leaving the regular allele intact. Therefore, RNAi therapeutics could be designed to target any gene in the genome involved in the cause or pathway of disease. RNAi also has the ability to act ahead of today's treatment With RNAi therapeutics it is possible to obstruct the uncontrollable replication of cancer cells. This has the potential to present greater efficiency in cancer control and intervention. The RNAi approach is able to avert the causes of cancer as compared with today's medicines that simply treat the disease. RNAi is able to harness the natural pathway of mammals RNAi is a natural pathway involved in regulation of gene expression in all mammalian cells. This natural pathway can be harnessed to create a new class of ground-breaking cancer treatment. Since this pathway is catalytic, RNAi therapeutics can be readily designed to be powerful and highly selective. (9)

Disadvantages of the Use of RNAi in the Treatment of Cancer:

The main disadvantage of RNAi in the treatment of cancer is the problems associated with the delivery of the RNA into the animal's body as already discussed this problem could be overcome by the use of Nanotransporters that are accurately injected into a cancerous area of the body. Unfortunately, currently, the production of these nanotransporters may be complicated, time consuming and expensive. However with increased use in the near future cost should fall as they become more widely available and people become educated in their use.

CONCLUSION

The use of gene silencing in the treatment of cancer is an interesting and potentially valuable method of treating many diseases a vet may face. Hopefully in the future knowledge and application of the subject will expand making effective methods available to all. The potential use of nanotransporters for the delivery of the RNA is a key method in overcoming the delivery problem. In addition perhaps more research should be conducted not towards the use of gene silencing to combat cancer itself but to prevent the spread etc through the surrounding tissue for example sealing off blood vessels to prevent metastasis. While the use of RNAi in the treatment of cancer may be expensive it still remains cheaper than other methods of cancer treatment and the cost should hopefully decrease as it becomes more widely used. In conclusion gene silencing shows a lot of potential in the treatment of cancer provided suitable research and investment are incorporated.

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