

RNA Interference – The basic biology of the future

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Abstract

The aim of this paper is clarify what RNA interference actually is, and to outline the importance of RNAi in the future development of medicines for both humans and animals. The core idea behind this paper is to focus on how being able to control and manipulate RNAi can be extremely beneficial to medicinal research and how it can affect the treatment of diseases such as HIV, Cancer and Foot and Mouth, which have habitually been thought off as terminal diseases. I have chosen to concentrate specifically on Cancer in this paper, and research how RNAi could be used to treat cancer. I believe that at the end of this paper, the reader will have a deeper insight into RNA interference and will be able to appreciate the importance of furthering research in the subject.

Introduction

The genetic material within a cell's nucleus was named deoxyribonucleic acid (DNA) in the mid-nineteenth century. Shortly after this finding it was discovered that DNA has a double-helical shape by Nobel Prize winners James Watson, Maurice Wilkins and Francis Crick. During this period of time the biggest concern was identifying how the DNA could control the goings-on within a cell. It was suggested that the single stranded RNA acted as a mediator.

Subsequent to the discovery of DNA and RNA, Richard Jorgensen, an American molecular geneticist stumbled across a surprising observation whilst trying to improve the colour of his petunias. In an attempt to deepen the purple colour of the flower, he and his team of scientists introduced a pigment-producing gene into the petunia. However the results the team achieved were not what they had anticipated. In place of a darker purple colour, many of the flowers were multicoloured and spotted, with some even appearing white. Jorgensen named the phenomenon he had seen 'cosuppression', due to the introduced gene and the pre-existing homologous gene both being suppressed. To determine whether this cosuppression occurred in only petunias or not, tests were carried out on other species of plant. The results of these tests provided evidence that many species display this characteristic, and that it is not isolated exclusively to the petunia.

These tests did not however provide an answer as to what the cause of this gene splicing was. To answer this question we must first understand the process by which DNA produces a protein. The first stage of two stages is the transcription stage, where the DNA separates via the help of RNA polymerase to copy its base instructions onto the messenger RNA (mRNA). The second stage is translation, where the mRNA reaches a ribosome within a cell, producing new proteins. This cycle is the key to RNAi, and produces an opportunity for scientists to be able change one of the most basic, yet complex pieces of biology known to man, the ability to turn off a gene.

Antisense RNA experiments lead to the discovery of Interference RNAi In 1998, the American scientists Andrew Fire (MIT) and Craig Mello (Harvard) published their discovery of a mechanism that can destroy mRNA made by a specific gene. Subsequently they were awarded the 2006 Nobel Prize in Physiology or Medicine. siRNA are small double stranded RNA molecules containing a "sense" and an "Antisense" strand - RNA interference results in the degradation of mRNA.

Using genetically altered strains of roundworms the scientists discovered genes responsible for RNAi, in which double-stranded RNA triggers the natural degradation of mRNA. During RNAi, the double-stranded RNA (dsRNA) degrades a homologous mRNA.

The RNA-induced silencing complex (RISC) is what controls the gene splicing process. It commences when the short double-stranded helix molecule reacts with the RISC, in the cytoplasm of a cell. The double-stranded RNA (dsRNA) is present in various viruses, which is why when dsRNA invades a cell in the body, it is identified and the DICER enzyme cuts the dsRNA, activating the RISC, which causes the RNA strands to open up and leads to the RNA being destroyed. Fig.1 represents the basic RNAi mechanism.

The introduction of dsRNA causes a gene knockout of almost any known gene by destroying the mRNA of the gene. The appliance of RNAi to mammalian cells has the potential to transform the field of medicine. The ability to control the expression of genes in mammalian cells holds enormous scientific, commercial, and therapeutic potential.

Theoretically an individual would be able to control what mRNA is degraded, subsequently removing a specific gene and the associated cells. This would be of great scientific importance in the field of medicine, both human and animal, as it would present a possible treatment for cancer and HIV which affect the DNA within a cell. Treatments involving RNAi would eliminate the need for risky procedures such as chemotherapy, which cause healthy cells in the body to be destroyed. The ability of small interfering siRNAs to suppress the expression of specific transcripts has proved a useful technique to probe gene function in mammalian cells. However, high production costs limit this technology's utility for many laboratories and experimental situations.

This theoretical ability to control what genes we have and what genes we can discard presents the average person with an extraordinary opportunity, the opportunity to remove genes which they do not like, but are not life threatening. For example a person could use RNAi to remove warts or moles from their body, or possible to even alter the colour of their eyes, hair or skin. This presents scientists and doctors with a moral predicament, whether it is ethical to allow a person to alter areas of their body solely for aesthetic purposes by changing millions of years worth of evolution.

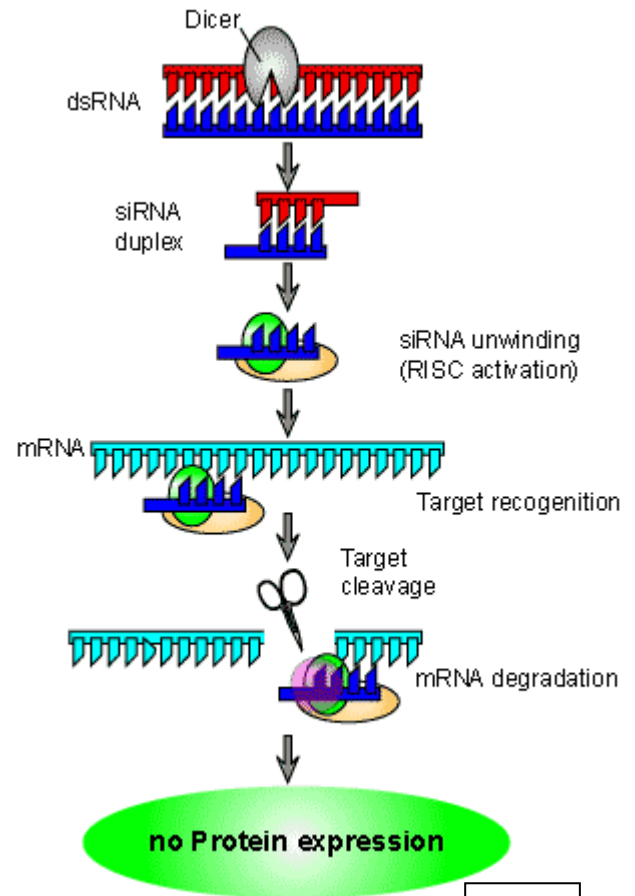


Fig.1

DISCUSSION

Currently RNAi is being used as a tool to study and manipulate a particular gene and its function. The technology can be applied to cancer cells or primary tumour cells and is not exclusive to just humans, but also to animals, making it relevant to both doctors and vets alike. RNAi is particularly useful for characterizing molecular targets, screening genes and looking at gene interactions with specific drugs, making it highly important in producing new drugs to combat DNA related diseases. In 2007, scientists at Abbot Labs used RNAi as a tool to functionally evaluate genes that play a role in maintaining human tumour cell survival. Scientists are currently studying the link between RNAi and changes in DNA in regulating gene expression in a many organisms, such as yeast, fruit flies and plants. Some scientists believe that RNAi plays a role in triggering potentially long-term changes in gene expression by modifying chromatin, the basic matter within chromosomes.

However one area of the research into using RNAi as a viable treatment for cancer is still very frail - the delivery. Delivery represents a major obstacle for the development of cancer RNAi therapeutics. This is even more difficult when treating metastases, which emphasises the need to find universal RNAi delivery solutions to reach cancer cells distributed throughout the body.

Another problem area for the use of RNAi is that before gene therapy can become a permanent cure for any condition, the therapeutic DNA introduced into target cells must remain functional and the cells containing the therapeutic DNA must be long-lived and stable. Patients will have to undergo multiple rounds of gene therapy in order to achieve a long-lasting effect.

Anytime a foreign object is introduced into human tissues, the immune system is intended to attack the intruder. The risk of stimulating the immune system in a way that reduces gene therapy effectiveness is always a potential risk. Furthermore, the immune system's enhanced response to intruders it has encountered before makes it difficult for gene therapy to be repeated in patients. This makes this it difficult to use RNAi in cancer cell cases, but could make it a possible use for those suffering from AIDS, as their immune systems would not be fully functional.

An example of a using RNAi to help cure a disease was when researchers at Sun Yat-sen University in Guangdong, China, and Intradigm in Maryland reported first successful use of siRNA against the viral respiratory disease SARS, in primates. Two siRNA sequences that matched bits of the SARS virus genome, largely protected macaques against SARS when given as nasal sprays shortly before, or four hours after, infection. Although the therapy did not completely prevent disease, it did limit viral replication and damage to the monkeys' lungs.

This is an example of how RNA interference could be used to help cure a disease which plagued large parts of the world, as well as affecting large populations of animals.

In early RNAi experiments, researchers saw some hints that the technique could induce an immune reaction or switch off the wrong gene or genes. In 2007, Dr. Mark Kay, the man to publish the set of first results showing that RNA interference could be an effective gene

therapy technique, confirmed those findings but also showed a possible way around those toxic effects by selecting particular RNA sequences.

More recent studies have expanded the use of RNAi beyond cell culture, showing that gene silencing can be demonstrated *in vivo* in mammals and that siRNAs can be targeted to specific tissues. The development of chemical modification to the siRNA molecule that enhances effectiveness, mRNA target specificity, and *in vivo* stability provide additional assurance for the use of RNAi in the development of therapeutics.

I believe with the current rate of increase in modern technology, the research into RNAi will increase, leading to further discoveries into how the treatment can be made more universally usable. I feel that once the difficulties surrounding the delivery of any possible drugs and the possible side effects and immune system responses have been thoroughly checked and approved, the RNA interference technique will become widely used in treating diseases for both humans and for animals.

As the technique can be used for humans and animals, adequate testing must be carried out before any drugs or treatments can be released, as the effects on humans may vary on other mammals.

As with any new medication there are some ethical issues involved with the research. For example, new drugs or procedures under study are not always better than the standard care to which they are being compared. The treatment may have side effects or risks that scientists do not expect, causing harm to the patient.

The major ethical issue involved with a procedure such as RNA interference is that it involves changing nature, and natural biology that has evolved over millions of years. Clearly no one knows what effect this could have in the future as research is still relatively new. However once more substantial research has been carried out, and more results collected, the field of RNAi could become one of the most important scientific fields in the world, as it relates to many other scientific and medical fields.

One of the major drawbacks of the current research is that because it is all relatively new, it is expensive, so only a limited number of laboratories can carry out sufficient experiments on the RNAi mechanism, which means that the rate at which new information on the subjects is obtained is very slow, and so the field as a whole cannot progress as quickly and efficiently as many would like. Nevertheless I believe that once enough government funding is put into research, it will become much easier and more affordable for many more facilities to start research on RNAi, and this can only be a good thing as the more scientists there are studying the subject, the less grey areas there within the topic, and so more experimental trials and medications can be made.

Cancer cells present the best opportunity for cure as normal procedures for cancer treatment involve damaging and destroying healthy tissues around the cancerous cells. With the RNAi this problem would be eradicated and it would hopefully remove the need for surgery to remove masses and lumps.

Whilst the majority of people would want to use RNAi to defeat terminal illnesses, it is certain that a number of people would want to use this complex procedure for aesthetic and

prosthetic purposes. This is ethically a very grey area as I am sure many people would be against using this procedure for anything other than life threatening diseases. I believe that this type of complex, possible dangerous technique should only be used once sufficient research has been done, and once the technique has been made as risk free as possible. Only once these conditions have been met should this technique be used on animals and people on a large scale, and I think that it should be made illegal to perform this procedure on people simply for visual benefit, as the time and money spent on that procedure would be much better spent on someone who needed it to save their life.

CONCLUSION

In conclusion, I must once again reiterate the importance of RNA interference research to scientific community as a whole. The discovery of DNA was one of the largest discoveries of all time; however I believe that the discovery of RNAi is equally as significant, as it may prove vital in combating disease across the world. If enough time and money is given to the research into being able to create a medication using RNAi, I believe that cancer, HIV and AIDS will be a thing of the past and that the discovery of RNAi will become the most important scientific finding in history. As the previous paragraphs have mentioned, there are countless diseases and illnesses that could be cured by perfecting a technique involving RNAi, is enough research is conducted.

I believe that if a drug is made that uses RNAi, it will primarily be used to treat HIV, AIDS and most of all, cancer, as these diseases all affect the DNA within a cell, and kill millions of people every year. However the drugs will not be limited to human use only, as they will be crucial in treating diseases such as Foot and Mouth which affect animals, as well as zoonotic diseases such as avian flu.

As the example in China shows, even with little research done into the subject of RNAi, there have been successful results when it is used, which is a very promising sign for the future. By creating a drug that utilises such basic mammalian biology, millions of lives will be changed for the better. However if this type of procedure becomes successful, which I am sure it will be, measures must be taken so that people do not abuse this technique for their own personal gain.

References:

1. Cogoni C, and Macino G. (2000) Post-transcriptional gene silencing across kingdoms.
Information on RNA functions and mechanism
2. http://www.odec.ca/projects/2004/mcgo4s0/public_html/t3/rna.html
3. Dirk Haussecker (2007) RNAi Therapeutics, May 21
4. New Scientist, 'Interfering' RNA helps monkeys recover from SARS, 27 August, 2005
William Marshall, Applications of RNAi, December 15, 2004
5. http://www.bio-itworld.com/archive/121504/rnai_applications.html
6. Gottesman, S (2004). The small RNA regulators of *Escherichia coli*: Roles and mechanisms