THE FUTURE OF RNA INTERFERENCE TREATMENTS.

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Abstract

During the course of my research paper, I am going to explore the possibilities that we may now have in stopping cancer growth; this will apply to both human and veterinary medicine. During the replication of new cells DNA separates into RNA and copies its base instructions to mRNA. The mRNA is then decoded to produce a specific polypeptide according to the specified genetic code. The genetic code uses messenger RNA as a template to synthesise a chain of amino acids that form a protein. However if the mRNA never arrives at the site of protein synthesis then no protein is made by the ribosomes. This means that any gene can be switched off at any time. I feel that with the development of the use of mRNA we will be able to cure cancerous tumours which do not respond as well to current treatments like chemotherapy and radiotherapy and greatly increase survival rates.

Introduction

RNA interference (RNAi) is the process in which foreign, double-stranded RNA is recognised and broken down by specialized protein complexes which are found in a large number of Eukaryotic cells. RNAi is believed to be an evolutionary defence mechanism against RNA viruses. When RNAi recognises the presence of foreign, double-stranded RNA, it causes a process known as Translation in which some of the cells messenger RNA's sequences are prevented, due to the presence of matching double-strands of RNA. It is known as Interference as it the RNAi interrupts the translation of the cells' own mRNA. RNA interference is also frequently known as Posttranscriptional gene silencing, as the effect of gene silencing occurs after the creation of mRNA during transcription. Thus far, RNA interference has been found in fungi, plants and animals, including; roundworm, fruit fly, zebrafish and mice.

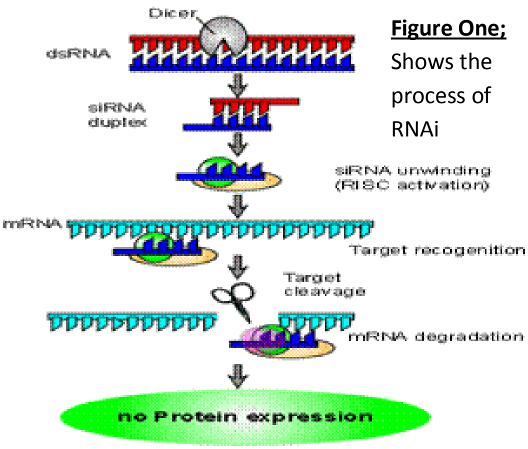


Figure One; Shows the process of RNAi

A small downside to RNA interference is that all messenger RNA activity is suppressed but only mRNA with complementary sequences are targeted. The most important feature of RNA interference is that it is able to turn off the expression of an individual gene; it is also versatile across a large number of species.

Recently in Cancer research, it has become increasingly common to use RNAi as a tool in which we are able to study a particular gene and its function. We can also use RNAi to screen genes and look at the interaction between genes and specific drugs.

RNA interference is responsible for turning off genes and, if we can learn how to successfully turn off genes, we can halt the Transcription process and stop cancer cells from developing. Therefore, by developing RNA and using it in cancer patients, we should be able to stop cancer growth if we catch it early enough and, by doing so, we should be able to save the lives of both animals and humans. RNA interference plays a key part in viral immunity but, at present, the role of RNA interference in mammalian viral immunity is not fully understood but eventually research from this area could help to reduce the effects of viruses on both animals and humans.

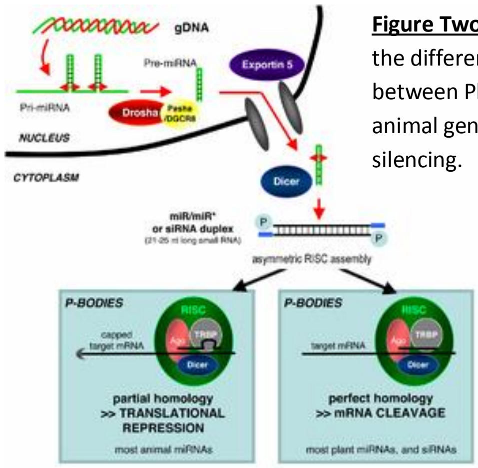
In 1998, Andrew Fire and Craig Mello discovered that by injecting RNA into roundworms had a dramatic effect in how genes is this small worm worked. They found that the injection of double-stranded RNA that had the same base sequence as the DNA of certain genes caused certain genes to be turned off.

How does the injection of double-stranded RNA in the experiments of Fire and Mello turn off a gene? The double-stranded RNA that is introduced into any animal cell is immediately recognised and cut into smaller pieces of double-stranded RNA by a protein enzyme called a Dicer and consists of roughly twenty-one nucleotides. These new double-strands of RNAs are called small interfering RNAs or siRNAs. It is these small interfering RNAs that are responsible for turning off a gene. The siRNA is recognised by a protein complex called RNA induced silencing complex or RISC. It is the RISC that is able to use the siRNA to turn off genes.

Recently in 2007, scientists at Abbots Laboratory used RNAi to evaluate the genes that play a role in maintaining human tumour cell survival. They discovered that Ran and TPX2 preferentially reduced the survival of cells that had been transformed by K-Ras, a known oncogene, and therefore these two genes are possible potential therapeutic cure for cancer cells.

In 2008, a group of scientists from Ohio State University showed that there was a global increase in the transcription of miRNA genes has occurred in cirrhotic and hepatitis-positive livers and they showed that miRNA expression may also be a useful tool for determining the behaviour and outcomes in hepatocellular carcinomas. Another study in 2008 showed that there was a link between certain strands of miRNA and the development of bladder cancer. And specifically GEMIN4 was linked to a 25% increase in bladder cancer.

RNAi was initially discovered in plants but it is now also believed to be conserved in animal species. We are now able to trigger RNAi by introducing small interfering RNA into living cells to prevent the expression of specific genes and this holds the possibility to prevent the expression of certain disease-associated genes in humans and animals.



<u>Figure Two;</u> Shows the differences between Plant and animal gene silencing.

It has been recognised that siRNA is sufficient enough to turn off a gene. Therefore there are a large number of diseases that could be treated with siRNA therapy, these include; Cancer, autoimmune diseases, dominant genetic disorders and viral infections. Currently these diseases alter and end a large number of people's lives each year, with 155,484 deaths from cancer in the UK alone in 2007, but with further research into RNA interference we could look to significantly reduce the number of lives lost to these diseases.

<u>Discussion</u>

In the future it may be possible to cure a variety of genetic diseases and prevent predispositions to certain diseases showing. It may also be possible to use RNA interference to choose and alter the characteristics of unborn children. For example, if a child has an allele for blue eyes and an allele for brown, we may be able to select which eye colour is shown regardless of which allele is dominant and which is recessive, this will present many moral and ethical issues regarding "designer babies".

It has also been found that RNA interference plays a key role in viral immunity and therefore it may be an invaluable resource when looking to find vaccinations and cures for viral infections. This has been seen in both plants and animals, but, however, in mammals, the results have not shown same results for RNA interference playing such a key role in viral immunity.

There is also a large amount of evidence for the fact that it may be used as a treatment for malignant, cancerous tumours. It has been found that microRNAs have a link in formation of tumours and the dysregulation of the cell cycle; however they have also been found to function as both oncogenes and tumour suppressors.

In 2009 a group of scientists under the command of Sunderland, also managed to create a ribonucleotide by recreating some of the chemicals and conditions which were proposed by Charles Darwin, for example the "warm little pond", which was evaporated, heated, rained on and then the sun shone. They managed to form a ribonucleotide by carrying out these conditions on the chemicals, a sugar, a phosphate and one of four different nitrogenous molecules. Having been able to successfully manufacture a ribonucleotide in the laboratory, hopefully over time, we will be able to complete the next step for RNA synthesis, and eventually, we will be able to manufacture RNA interference molecules for treatment uses in both the medical and the veterinary world.

Future developments in RNA interference are such that eventually we will be able to suppress the growth of malignant tumours before the need arises for more invasive treatments such as chemotherapy and radiotherapy. However, there may be a large number of ethical issues involved in the future developments of the uses of RNA interference in the treatment of Cancer. For example, there will be issues with clinical trials carried out on both humans and animals. There may also be side effects of using RNA interference as a treatment, we will need to know in detail about the possible side effects before it can be used in human or animal patients. There are also some medical concerns with the introduction of RNA into the body. It may weaken the immune system in the same way that any foreign object that enters the body does. This may be a potential issue as it could reduce the effects of gene therapy on the body.

Before we are able to use RNA interference as a treatment for cancer, many clinical trials and the four phases of human trials will need to be completed, which normally takes place over many years. If the new RNA drug passes the first three stages it is then passed onto the national regulatory authority for use in the human population. However, the drug still needs to pass stage four; which has been designed to look for any long term side effects over a longer period of time than was possible in stages one to three of trials. During these trials, scientists will also evaluate the possible risks and benefits of potential treatment methods.

There have been resent research projects that show the ability to stop cancer mastitis in animal models and, hence, have suggested a novel perspective on the treatment of cancer. The small interfering RNA were injected into the animal model and stopped the tumour from continuing to grow. From this research, we have the ability to deduce that once further research has been conducted and all four stages of the clinical trials have been completed we could have developed drugs that will suppress cancer cells. We will not necessarily be able to just manufacture a uniform drug for all types of cancer, due to various different compounds in different types of cancer, for example, lung and breast cancers.

Although now the use of RNA interference is only being done at a cellular level, in time, it will hopefully pass clinical trials, so that we are able to create a form of RNA that will suppress cancer cell division that can be given in the form with fewer side effects than current treatments like chemotherapy and radiotherapy.

The main focus of my paper is that over time we will be able to induce cell mutations in different types of tissue in the laboratory in order to then find and formalise the small interfering RNA that will suppress cancer cell division. Due to there currently being about two hundred different types of cancer, it is possible that each type may need a slightly different modification of RNA.

It may lead to a considerable advantage in the treatment of patients if we are able to isolate a specific form of small interfering RNA for each type of cancer. Although this will be very time consuming and expensive, in the long term, it will enable us to treat patients more efficiently. If we can isolate and use the specific small interfering RNA molecules along with identifying the form of cancer of each patient and then, if we can deliver the isolated form of small interfering RNA, the small interfering RNA would then only suppress the specific cancerous growth without weakening the immune system too seriously. The small interfering RNA triggers the process of RNA interference and would suppress cancer growth. There are a few medical issues with this type of treatment. If the patient's cancer has spread and this is not identified at the time when the first RNA interference treatment is given, the second tumour may not be caught early enough and instead treatments like chemotherapy or radiotherapy may be required. Therefore, during the use of RNA interference treatment, the patients would need to be kept under close observation, for example weekly appointments with their veterinarian or their doctor, to make sure the cancer has not spread to a secondary site of growth. But hopefully, the use of RNA will not significantly weaken the patients' immune system and they should be able to live a relatively normal life and show no obvious signs of having or being treated for cancer.

In order to treat cancer patients using RNA, we first need to find a way of extracting and isolating the RNA from a cell. This can be done in a laboratory by carrying out several stages of centrifugation. This will extract all the types of RNA from a cell but we then need to isolate small interfering RNA. This can be done via the means of repeated centrifugation of the pellets, until the pellet contains siRNA. The stage of RNA isolation is also a series of centrifugation which is used to separate the different components, of the cell in layers within the suspension. The yellow colour precipitate is mainly fat and can be discarded. The layer of interest is tinted red and contains the total RNA. Phenol chloroform extraction will then take place, followed by a series of alcohol washes, and then the RNA is ready for mRNA and siRNA isolation. This process is currently being conducted to isolate mRNA and therefore we should be able to develop a similar process to isolate and extract siRNA. Although it may be that we need considerably large amounts of RNA to extract a large enough quantity of siRNA to treat a large number of patients. But once we have developed and tested a method for the use of siRNA in cancer treatment, the method of preparing the treatment will become more and more efficient and, if the treatment proves successful, we will be able to treat a large number of patients both animals and humans around the world.

Once we have efficiently found a way to isolate and prepare the siRNA we will need to develop a safe way of administering the new treatment to patients. I suggest the methods of administration to be via a liquid solution or a gel in the form of injection. This would minimise the effects of treatment as no surgery or invasive treatment would be needed.

The means of administration must economically and ethically viable. Due to the fact that the treatment using siRNA will be ongoing and to be economically viable we need to be looking at a similar cost to that of the current cancer treatments for the drug and treatment to be accepted.

There will also be a large amount of testing involved before the new treatment would be released for the use on patients and this will take a significant amount of time and money. The form of siRNA drug will be required to go through a large number of clinical trial phases, the phase of testing will be a series of pre-clinical tests both in-vitro (test tube) and in-vivo (in animal or cell tissues). These tests will obtain information on the toxicity and efficacy by using a wide range on doses, and studying the resulting effects. During this stage, the pharmaceutical company will decide whether or not the drug has enough merit to continue to the further stages of clinical trials. If the drug is considered worthy it will continue through the further stages such as Phase 0 in which the first in human model trials are conducted, but no safety of the drug is established as the doses given are too small. The next testing stage is Phase 1 and this is where the drug is evaluated for safety and the first in-human trials take place using a relatively small sample and, in our case, animal testing would need to be carried out also as we intend to use the drug in both the world of

Medicine and Veterinary Medicine; this test is used to establish safety and tolerability of the drug. These trials also tend to contain a dose-ranging test, to establish what doses should be given to different weights and sizes of different people and animals. Once the initial safety of the drug has been proved it progresses to phase 2, where it is tested on a larger sample between two and three hundred test subjects. Phase 3 trials are the most expensive and time consuming and include test on a large sample once a drug has passed this stage with satisfactory results a large document is published containing all the details of the methods and results of the testing stages. Finally there is phase 4 which monitors the safety and ongoing support after the drug has received the permission to be sold.

Once we have obtained the ability to isolate and administer the siRNA we should be able to safely administer it to patients. The siRNA will need to be injected as near to the area or areas where malignant tumours are situated, the small interfering RNA should stop the cancerous cell division. It may be that the patient will still require surgery to remove the mass but hopefully the siRNA should prevent the continuous growth and the spreading of the cancer, which is also known as metastasis. The patient whilst undergoing the treatment will need to be monitored closely to ensure that, if the cancer should spread to a secondary site, then we begin treatment on the second site as soon as that site is found.

Hopefully, by using RNA, we will be able to save a large number of lives, and make treatment more pleasant for patients with cancer and other genetic diseases that may eventually be treated by using RNA interference.

Conclusion

Using RNA interference to treat cancer is beginning to look like a possible reliable treatment route, which may lead to an improvement in the quality of life in all cancer patients, although currently there is fairly limited amount of published research on the functions and possible applications of RNA interference. Currently the main sources of research are focus on studies in plants and animals, from this we can establish the fairly likely possibility of RNAi having a positive effect on tumours in animals, but, due to limited research in humans, it is impossible to say whether or not RNAi will be effective, due to variations in the anatomy of humans and animals. There remains to be a lot of practical research that must be carried out before we can decipher whether or not the use of RNAi will have a positive effect on tumours but there is still the possibility of negative side effects that may be seen in early stages of the clinical trials. However, it is clear that investigation and development into RNAi will be expensive, but, if it proves to save millions of lives, it may be a risk we have to take.

It seems that it is fairly likely that RNAi will have a positive impact on both medicine and veterinary medicine but it is difficult to explore the scale of these possibilities without carrying out any practical research. From reading resources and studies that have been conducted around the subject of RNA and RNA interference, it seems likely that RNAi will have an impact on cancer but to develop a successful method of development and administration may take years. There is some evidence to suggest that RNAi may lead us into a new world of treatments for a wide variety of diseases, including genetics, viruses and cancers.

Overall I feel it is just a matter of time before we see a change to veterinary medicine and medicine, via the use of RNAi or similar chemicals. It is difficult to say how long it will take to develop a secure and viable product alongside a treatment program, but it is evident that at some point we will see a positive change in the treatment of genetic diseases and cancer through the use of RNA interference.

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