

How to deliver the SiRNA complexes to all cells requiring them?

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PASS WITH MERIT

RESEARCH PAPER
BASED ON
PATHOLOGY LECTURES
AT VET-MEDLINK 2009

In this paper I am going to write about siRNA with a specific view to how to deliver the siRNA complexes to specific cells. I will also discuss ethical issues, gene silencing and how siRNA can help to possibly cure certain diseases. There are many advantages to siRNA and RNAi such as the ability to turn off any gene with little side effects, however there is still a lot of work and research that needs to be done before this may be a method of treatment available to all.

In living organism's characteristics and processes need for growth and repair as well as reproduction are controlled by deoxyribonucleic acid or DNA. DNA is made up of many monomers called nucleotides. Each nucleotide is made up of a base a phosphate and a sugar called deoxyribose. Each phosphate will join to a sugar creating a long strand of alternating sugar and phosphates with the bases all lined up on one side. When two of these strands form the bases will join by hydrogen bonds to one other base creating a spiral or double helix containing the bases in the inside of the spiral. There are four different bases Adenine, Guanine, Thymine and Cytosine of which only Adenine and Thymine will bond and only Cytosine will bond to Guanine. The order of these bases will create different genes and so code for different products. Genes can code for many proteins such as Enzymes which help in many reactions such as digestion.

DNA codes for proteins in a process called protein synthesis which partly takes place outside of the nucleus using Ribosomes. In protein synthesis the double stranded DNA unwinds using the Enzyme Helicase which breaks the hydrogen bonds between bases, free nucleotides then come and bond to their specific bases however RNA the base Uracil is used instead of Thymine. RNA Polymerase then joins the chain of nucleotides together creating Messenger RNA or mRNA. This process is called transcription the mRNA then leaves the nucleus through nucleus pores and enters the cytoplasm. In the cytoplasm the mRNA then undergoes a process called translation, this occurs when the mRNA meets a ribosome and the start codon bonds to the ribosome. This starts the process of translation in a stage called initiation. On the chain of mRNA each three nucleotide bases code for an amino acid, the three bases on the mRNA are known as a codon. When the ribosome starts the process of translation tRNA containing an amino acid and anti-codons, which are the complementary bases to the three on the codon, come and join in the ribosome to the codon. These anti-codons have an amino acid attached to them so as the ribosome moves along the mRNA it creates a chain of amino acids known as a protein. When the small base unit of the ribosome attaches to the mRNA it creates a process called initiation, as the tRNA bonds the amino acid chain becomes longer as more are added this process is known as elongation. The fourth and final stage is termination this happens when the mRNA has a stop codon meaning no more tRNA can bond to it. Translation can be stopped at any one of the four stages.

RNAi or RNA interference is a natural system in living cells which can stop protein synthesis this is useful as any gene can be switched off stopping it being produced. RNA interference happens when double stranded RNA is introduced into cells. RNA interference is a form of gene silencing which relies on RNA and is controlled by RNA-induced silencing complex or RISC. The process is initiated by short double stranded strands of RNA in the cell's cytoplasm. The double stranded RNA which may come from a virus is recognized and cut into short strands by the Enzyme Dicer.

The Enzyme Dicer cuts the dsRNA into shorter strands between 21-25 base pairs long which also have a few unpaired bases overhanging on each end. When cut the dsRNA is still double stranded. The short double stranded RNA are known as small interfering RNA or siRNA. These siRNA strands are then opened into single strands by the RISC and the more

stable strand is bound to the RISC, the other strand is degraded. The RISC and siRNA complex recognizes complementary RNA strands and activates RNase which cleaves the mRNA preventing it from being used as a template for protein synthesis.

This method of gene silencing can be used to stop a gene or virus from replicating, however when dsRNA complex of over 23 base pairings are introduced into a cell it activates the interferon response. Which means interferon's stop protein synthesis and activate natural immunity such as killer cells and macrophages, this effectively shuts down the whole cell and can cause other cells to release interferon's as well. To use RNAi as a method of treatment in mammals dsRNA must be below 23 bases pairings when in cells.

To cure diseases the siRNA must be introduced into the cells that require it for example cancer cells in a specific part of the body. As cancer cells have no Hayflick limit they can divide more than other cells this means there is more chemical activity and a higher metabolic rate. In PET or positron emitting tomography these areas can be found as when radioactive tracers which emit positrons are placed in the body, they encounter an electron which is the opposite of the positron and annihilate each other. When the positron and electron annihilate they produce a burst of energy such as a gamma ray, using PET scanning these areas of higher metabolic activity can be found and so the cancer cells can be found. SiRNA could then be put into the cell which would create a RNAi response killing the cancer cells and hopefully curing the patient.

Another form of possible treatment would be to use vectors these are viruses that have had their DNA removed and replaced with siRNA. These vectors work as when a virus binds to a cell they introduce their genetic material into the cell. However there are many problems with this such as how to make sure the viruses go to all the cells requiring them and also making sure the viruses cell aren't destroyed by any of the immune system.

There are many problems with using SiRNA and delivering it to the cells requiring it an example of the research to try and combat this problem is from the Massachusetts Institute of Technology (MIT) that have developed a nanoparticle drug delivery system that maximises the amount of SiRNA that can enter a cell. Sangeeta Bhatia, M.D., Ph.D., and Phillip A. Sharp, Ph.D., of the MIT-Harvard Center of Cancer Nanotechnology Excellence, and Alain Charest, Ph.D., M.Sc., Tufts University School of Medicine, led the study, whose results appear in the journal *ACS Nano*.

Their method of SiRNA delivery uses nanoworms that the investigators call "dendriworms." Which are synthetic polymers that can be used to carry a large range of molecules such as SiRNA they are made up of magnetic nanoparticles as well as a fluorescent nanoparticle which allow the nanoworm to be traced as to where it is. As the nanoworm is so small it is able to penetrate into the cell membrane and go into the cytoplasm.

To test whether this dendriworm would work in a living animal, the researchers used a strain of mice that were genetically engineered to develop glioblastoma tumors spontaneously in the brain. The investigators found that the dendriworms were able to penetrate the tumors, deliver their therapeutic siRNA cargo into tumor cells, and silence the targeted gene in those cells. As SiRNA have a negative charge the nanoworms were coated with positively charged lipids. This made them bond strongly to the SiRNA molecules.

The work with dendriworms, which is detailed in the paper "Functional delivery of siRNA in mice using dendriworms," was supported by the NCI Alliance for Nanotechnology in Cancer,

a comprehensive initiative designed to accelerate the application of nanotechnology to the prevention, diagnosis, and treatment of cancer. Investigators from Brigham and Women's Hospital also participated in this study. The work on magnetically guided siRNA therapy is detailed in the paper "A novel magnetic crystal-lipid nanostructure for magnetically guided in vivo gene delivery."

Future developments mean that diseases such as foot and mouth can be cured and other diseases could be cured by delivering SiRNA to stop the protein synthesis of the genes. However this will only work if both the gene is identified and the SiRNA is delivered quickly and to all areas of the infected mammal.

Foot and mouth is caused by a virus entering an animal and releasing its RNA into a cell, where the RNA is replicated and proteins are made, when a large quantity are created the cell bursts causing the RNA to enter other cells and repeat the process. Due to foot and mouth being made from a single strand of RNA it does not create the RNAi effect however if SiRNA was made it could be used to treat foot and mouth. However due to the fact that foot and mouth virus constantly mutates and changes it means that a long on going treatment may not work and the animal must be kept in complete isolation.

As with radioactive tracers already in use these could be used to find the infected area and a possibility of a injection into the blood stream where nanoparticles could diffuse into the infected cell would be useful as they would allow easy curing of multiple animals, for example on large farms to treat infected herds with foot and mouth.

There are many ethical issues that may result from SiRNA and RNAi such as that if two genes share similar DNA then some genes that are not harmful may be switched off causing the person or animal to become ill or die.

There is also the problem that as the cells divide and as the gene therapy is not permanent it will mean that many rounds of treatment will be required which will also cause the problem that many farmers will not be able to afford treatment for their animals. When the DsRNA is split into two strands there may be a possibility that the strand not used could cause the disease to become present.

As well as this there is a contraceptive using RNAi which prevents the formation of a protein that allows sperm to stick to eggs, there is a possibility that if this SiRNA stays in the cells certain people may never be able to have children.

Although successive rounds of treatment may be required the immune system may prevent the vector from being able to transmit the SiRNA to the cells, as virus vectors are used there is a possibility of the viruses not being entirely safe and causing disease. When SiRNA is tested as in mice a tumor is deliberately induced in the animal this creates many ethical issues as many people believe that this is wrong as if the animal does not get better then it has gone through a long and unnecessary suffering.

Throughout my research I have found that RNAi and SiRNA is a very effective way to combat viruses as they allow any gene to be switched off; however there is a lot of research needed on how to deliver and maintain SiRNA in cells that require them. There are also a lot of ethical issues surrounding the creating of disease in healthy animals and the possibilities of problems resulting from SiRNA targeting the wrong gene.

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