

THE POSSIBLE FUTURE APPLICATIONS OF RNAi AND THE
ASSOCIATED ETHICAL ISSUES

BY

Beatrice Rose Elliott

RESEARCH PAPER
BASED ON
PATHOLOGY LECTURES
AT VET-MEDLINK 2009

ABSTRACT

RNA interference (RNAi) is a relatively new technology that is revolutionizing the way that researchers study mammalian gene expression. RNAi has had a significant impact on the ease, speed, and specificity with which gene function analysis can be done in mammalian cells. This paper discusses its future medical applications, as well as discussing practicality and associated ethical issues.

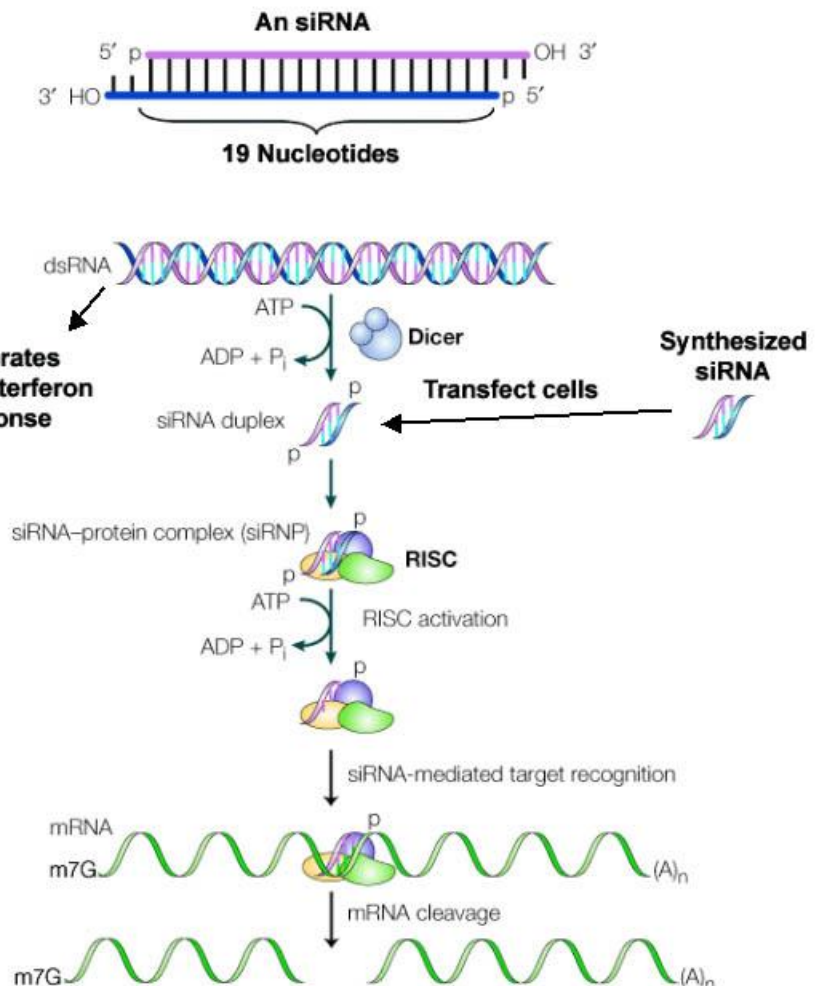
INTRODUCTION

Discovery of RNAi

The discovery of RNAi began with reports of unexpected outcomes in experiments of scientists in the U.S. and The Netherlands in the 1990s, whilst trying to alter flower colours in petunias. Additional copies of a gene encoding chalcone synthase, (a key enzyme for flower pigmentation) were introduced into petunia plants, which usually have a violet flower colour. The over-expressed gene was predicted to result in darker flowers, but instead produced less pigmented, with fully or partially white flowers. This suggested the activity of chalcone synthase had been decreased. Further investigation suggested that the reduction was due to post-transcriptional inhibition of gene expression via an increased rate of mRNA degradation. This phenomenon was called co-suppression of gene expression, although the molecular mechanism were not known at the time.

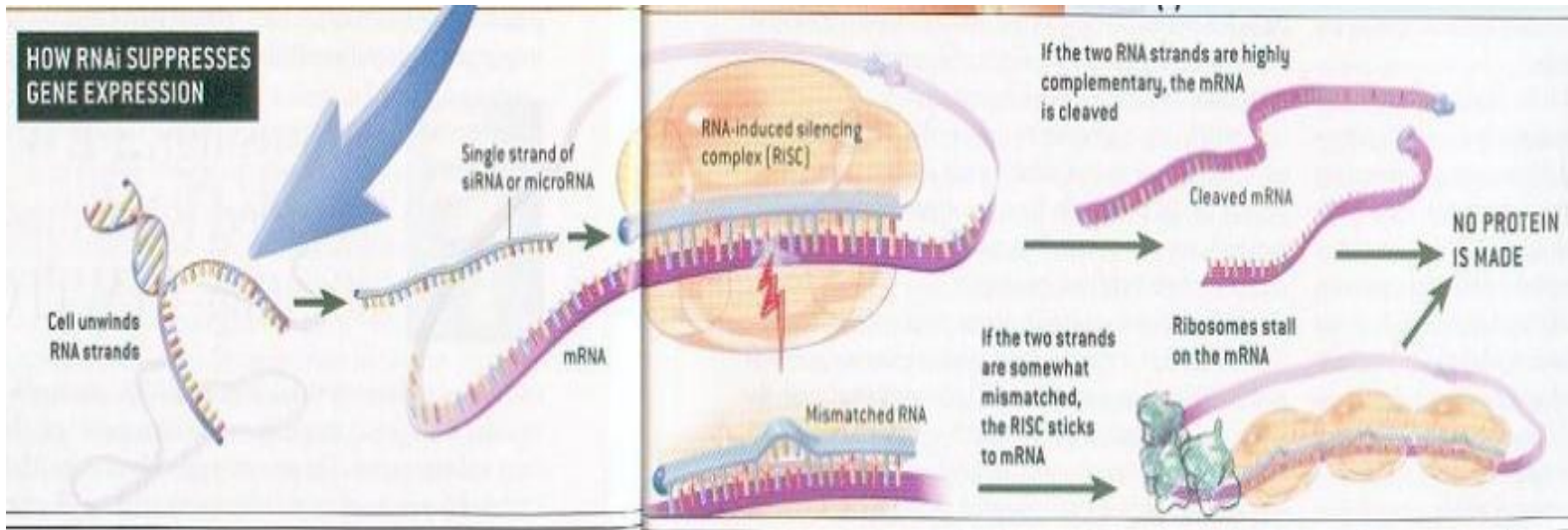
How does RNAi work?

Simply, RNAi works by destroying the molecular messengers that carry information coded in genes to the cell's protein production sites (ribosomes). The messenger RNAs (mRNAs) carry out an essential function, without which a gene is effectively 'silenced'. Upon entering a cell, the double-stranded RNA molecules that trigger RNAi are cut into small fragments by an enzyme known as Dicer. The small fragments then guide the cell's RNAi apparatus to mRNAs that match the genetic sequence of the fragments. The apparatus then slices the cellular mRNAs and in doing so destroys their messages, shutting off the relevant gene.



In more detail, long double-stranded RNAs are used to silence target genes. On entry, the long dsRNAs enter a cellular pathway known as the RNA interference (RNAi) pathway. First, the dsRNAs get processed into 20-25 nucleotide small interfering RNAs (siRNAs) by a RNase III-like enzyme called Dicer . Then, the siRNAs assemble into endoribonuclease, which contains RNA-induced silencing complexes (RISCs). The siRNA strands then guide the RISCs to the corresponding RNA molecules, where they cleave and destroy the associated RNA . Cleavage of related RNA takes place near the middle of the region bound by the siRNA strand.

The process is different in mammalian cells. When dsRNA are introduced, it causes a strong antiviral response. This response can, however, be avoided by introducing siRNAs.



What does this mean for medicine?

RNA interference systems could be used clinically to suppress gene expression as a therapeutic strategy in many diseases caused by elevated gene function.

This ability to silence a gene means that we may be able to cure a number of diseases or even prevent them from developing. We may be able to ‘turn off’ the mutated gene responsible for causing cystic fibrosis, identify certain genes which predispose a person to suffering from a condition, or even ‘turn off’ cancer cells by inhibiting the oncogenes responsible for giving cancer cells their unique properties.

Current applications of RNAi in medicine:

RNAi, although only in its early stages of development, is proving useful in the control of a large array of diseases as a therapeutic tool. Many tests have been carried out in cell cultures and a few in lab animals, yet remain untested in humans for a range of safety reasons. The main use for RNAi at present is for treating cytomegalovirus infections in AIDS patients by blocking the replication of the cytomegalovirus.

DISCUSSION:

What are the possible future applications of RNAi and the associated ethical issues?

The first challenge that we face when applying the RNAi mechanism is how to administer the siRNA complexes to all cells requiring them. There are two main ways in which we can do so; somatic cell gene therapy and germline cell gene therapy.

Somatic cell gene therapy:

As an organism grows, cells become specialised to function. Within these specialised cells, certain genes are switched on and others are switched off. This means that although the cell still contains a full genome, relatively few of them are active in producing proteins. We can take advantage of this when using RNAi. We can silence specific genes, causing dangerous cells to die off.

In somatic cell gene therapy, the siRNA could be introduced into target cells either in the body or the target cells are removed, treated and then replaced (ex vivo therapy). Introduction to somatic cells however, means that any treatment is short-lived and has to be repeated regularly. This is because the specialised cells containing the siRNA will not divide to pass them on.

There are also many difficulties in getting the siRNA into the cell in a functioning state. It is possible to use genetically modified viruses, however hosts could become immune to them and so the cells would not accept the virus vector on second or subsequent treatments.

Another method of getting the siRNA into target cell could be using liposomes. These are artificial vesicles in which siRNA could be enclosed. Once inside, the liposome acts as a vector, allowing the siRNA to pass through the plasma membrane of the target cell. However this method may prove to be inefficient.

Other possible ways of getting siRNA into target could be to insert them into or disguise them as molecules which are readily taken up by the target cells. For example, it is known that cancer cells duplicate rapidly and so have a high metabolic rate, and so take up much more glucose than healthy cells. We take advantage of this in current day medicine in Positron emission tomography (PET scanning). By inserting the siRNA into/ disguising it as glucose, large quantities of it will be delivered to the target cells which will then break down the molecule, releasing the siRNA into its cytoplasm.

The main disadvantage of somatic cell gene therapy is that the manipulations are restricted to the actual patient and so would not help in protecting future generation from inheriting certain disorders.

Germline gene therapy:

All embryos begin when a sperm fertilises an egg, forming a zygote that undergoes cell division. Each cell of an early embryo is a stem cell, which can divide and specialise to become any cell type within the body. Each could potentially also become a new being (hence these are called germline cells). Engineering siRNA into a sperm, egg or zygote or into all of the cells in an early embryo means that as the organism grows, every cell contains a copy of the siRNA. Methods for introduction into germline cells are more straightforward than for somatic cells.

Although widely employed in experimental animals, germline gene therapy in humans is illegal and seen as ethically unacceptable. They argue that an inadvertent modification of DNA into the germline could create a new human disease or interfere with human evolution in

an unexpected way. Permanent modifications to the human genome such as this raise difficult moral, ethical and social issues which will be debated later in this paper.

Possible future applications of RNAi to real world situations:

Influenza:

RNAi could be used to fight influenza by disrupting the way the virus binds to cells and releases its contents via channel proteins.

Unlike bacteria, viruses are not strictly living things. Instead they are a kind of 'molecular machine' that forces its way into living cells to reproduce and spread itself. RNA is the core of the influenza virus' 'machinery' and once inside a cell it uses the chemicals within to clone itself. Surrounding the RNA is a protective envelope composed of glycoproteins, which are also found in the body.

The virus has two main types of glycoproteins – haemagglutinin (HA) and neuraminidase (NA). The HA helps the virus attach to and get inside the cell, whilst intrinsic proteins within the membrane allow protons to pass into the centre of the virus. This triggers a breakdown which releases the RNA 'machinery', which then inserts itself into the cell's own system and begins copying itself. The NA then releases the copies by cutting off the sugars which anchor the copies to the cell.

In this process there are many possibilities to use RNAi to halt the invasion and replication of the virus. The first option being shutting down the ion channels which act as the pathway for the protons. By isolating and silencing the gene responsible for the production of the relevant intrinsic protein we could stop the virus' RNA from being released into the cell.

However, there are issues with this method. The ion channels are also important to the functioning of the human cell, allowing essential substances to pass into and out of it. By destroying these channels, the functioning of the cell may be severely impaired and could ultimately lead to cell death.

Another possible way to target the virus invasion is to remove the 'glue-like' component from the process. Sialic acid binds the newly made virus to the surface of the host cell. We could potentially use RNAi to identify and silence the genes responsible for producing sialic acid. This would mean that the new virus would not be able to get onto the surface of the host cell and so could not go on to be released by the NA and spread further.

Cystic fibrosis:

RNAi could be used to identify and silence the faulty gene responsible for causing Cystic fibrosis.

Cystic fibrosis (CF) is a common hereditary disease. It affects the entire body and can cause progressive disability and early death. Difficulty breathing is the most serious symptom and results from frequent lung infections. Other symptoms include sinus infections, poor growth, diarrhoea, and infertility.

CF is caused by a mutation in a gene called the cystic fibrosis transmembrane conductance regulator (CFTR) found on the chromosome 7. This gene helps create sweat, digestive juices, and mucus. Although most people without CF have two working copies of the CFTR gene, only one is needed to prevent cystic fibrosis. CF develops when neither gene works normally.

Individuals with cystic fibrosis can be diagnosed prior to birth by genetic testing or in early childhood by a sweat test. The fact that CF can be diagnosed prior to birth gives us the option to treat it with germline therapy. If there is only one mutated CFTR gene, it could be

isolated and silenced as the cells remain in their early stages of development, meaning that eventually all of the embryo's cells will contain copies of the siRNA.

Alternatively, CF could be treated after birth by somatic gene therapy, although obviously the treatment would not last for very long and thus would have to be repeated regularly. It is relatively simple to deliver RNAi drugs to the respiratory system as patients can inhale them. The siRNA complexes could be put inside liposomes and a nasal spray could be used to deliver them to the lung cells. This would cause the lungs to make healthy mucus, thus improving breathing, making physiotherapy easier and reducing the risk of chest infections.

Cancer:

RNAi could also be used to treat cancer by silencing the genes responsible for cancer cells' unique properties or in combination with chemotherapy.

Cancers are caused by abnormalities in the genetic material of the transformed cells. This can be due to carcinogens, such as tobacco smoke, radiation and chemicals. Other cancer-causing genetic abnormalities can randomly occur through errors in DNA replication, or are inherited.

Oncogenes are usually activated in cancer cells. These lead to hyperactive growth and division, protection from controlled death as a result of abnormalities in the proteins which mediate apoptosis and the ability to become established in diverse tissue environments. Tumour suppressor genes are then inactivated in cancer cells. This causes the loss of normal functions in these cells, such as accurate DNA replication, control of the cell cycle, orientation and adhesion within tissues, and interaction with protective cells of the immune system.

With the use of RNAi, we may be able to silence the expression of a gene responsible for cancer cell cycles or the anti-apoptotic gene (e.g. Bcl-2), stopping tumour growth and eventually killing the cancer cells. To eliminate only cancer cells and not damage normal cells, RNAi must target a gene specifically involved in the growth/ survival of cancer cells.

Another way we can use RNAi to tackle cancer is to 'starve' the cancer cells of essential substances. Breast cancers require the hormones oestrogen and progesterone to grow, and have receptors for those hormones. In this case RNAi could be used to turn off the gene responsible for the production of the proteins which make up the hormone receptors. This would mean that the cancer cells would not be able to take up these essential hormones, limiting their growth and possibly causing death. Alternatively, RNAi could also be used to target the genes responsible for oestrogen and progesterone production.

RNAi could also be used in combination with chemotherapy to help combat cancer. The main drawback to chemotherapy is drug resistance, which causes between 20% and 50% of all treatments to fail. The gene responsible for this resistance has already been identified as 'P-glycoprotein'. The protein removes the drug from the diseased cells, meaning that they can continue to uncontrollably grow and divide as if they had not been treated at all.

Using RNAi, we could isolate the gene responsible for the production of the P-glycoprotein and silence it. This would restore the sensitivity of the diseased cells to the drugs, vastly improving the efficiency and effectiveness of the chemotherapy treatment.

Yet another way in which RNAi could also be used is in preventing the development of cancer. It has been found that certain genes predispose some individuals to developing cancer (in particular breast cancer).

Screening programmes could use genetic tests to identify women at the greatest risk of developing the disease. They can then be treated through somatic therapy where the siRNAs could be delivered to the cells by transfection. In the future, further medical developments may make it possible to screen for these predisposing genes prior to birth, thus enabling us to decide whether or not to treat earlier with germline therapy or in some cases terminate the pregnancy.

Neurodegenerative disorders:

Another way in which we could use RNAi is to treat neurodegenerative disorders by reducing neuron death and genetic mutations.

Alzheimer's, Parkinson's and Huntington's disease are common age-related disorders which have been seen to be increasing with increases in life expectancy. Each of the conditions result from the dysfunction or death of neurons. In Alzheimer's disease it is the hippocampal and cortical neurons which are affected. These are responsible for the memory and learning. With Parkinson's disease, the dopamine-producing neurons which control body movements are affected. A small proportion of Alzheimer's and Parkinson's cases are caused by genetic mutations. However, all cases of Huntington's disease are caused by genetic mutations of the Huntington protein.

A number of biochemical processes leading to neuronal death have been identified, such as increased oxidative stress and dysregulation of cellular apoptosis. One way in which we could apply RNAi is to silence the genes associated with neuronal apoptosis such as the p75 neurotrophin receptor. Alternatively, we could try to directly silence the faulty Huntington gene itself. However, this may prove problematic. It is thought that only one allele is affected by mutations, but when we target the Huntington gene, we may accidentally turn off the normal allele. This could have a huge impact on the patient as the gene is responsible for a range of physiological functions.

Of course, we could try to develop allele specific siRNAs; however this may prove highly complicated as the point of mutation may vary.

Obesity:

Other possible applications of RNAi could be to silence genes which predispose people to obesity.

In some obesity cases there are mutations in various genes controlling appetite and metabolism. Obesity is a major feature in several syndromes, such as Prader-Willi syndrome, Bardet-Biedl syndrome, Cohen syndrome, and MOMO syndrome. In people with early-onset severe obesity it has been found that 7% stem from a single point DNA mutation.

This means that there is potential to apply RNAi in the treatment or prevention of obesity and obesity related syndromes. By identifying and silencing the genes which go on to mutate could help to relieve obesity sufferers of their metabolism and appetite problems.

There are several pathophysiological mechanisms involved in the development and maintenance of some obesity cases. Leptin (a 16 kDa protein hormone produced by adipose tissue) participates in the regulation of appetite and food intake, storage patterns of adipose tissue, and development of insulin resistance. Administration of leptin can be effective in a small proportion of obese individuals who are leptin deficient. However, most obese individuals are thought to be leptin resistant and have been found to have high levels of leptin. This resistance is thought to explain in part why administration of leptin has not been shown to be effective in suppressing appetite in most obese people. Another important hormone is Ghrelin, which is produced by the stomach and helps with modulating short-term appetitive control (i.e. to eat when the stomach is empty and to stop when the stomach is stretched).

Leptin and ghrelin control appetite by acting on the hypothalamus, a region of the brain central to the regulation of food intake and energy expenditure. There are several circuits inside this region of the brain such as the melanocortin pathway. The circuit begins with an area of the hypothalamus, the arcuate nucleus, which signals to the lateral hypothalamus (LH)(responsible for feeding) and ventromedial hypothalamus (VMH) (responsible for satiety).

The arcuate nucleus contains two main types of neurons. The first group have stimulatory inputs to the LH and inhibitory inputs to the VMH. The second group has stimulatory inputs to the VMH and inhibitory inputs to the LH. Both groups of arcuate nucleus neurons are partially regulated by leptin. Leptin inhibits the first group and stimulates the second, in doing so it decreases desire to eat and increases satiety.

Some obesity cases have been linked to a leptin deficiency or to leptin resistance, in which case the eating habits of the sufferer are significantly altered. Resistance arises from impaired leptin transport across the blood-brain barrier, now thought to be the result of increased triglyceride levels, and also by defects in leptin receptor signalling.

With the use of RNAi, we may be able to identify and silence the gene which causes or predisposes the leptin receptors to become ineffective. We may also be able to identify the gene responsible for the regulation of production of the proteins which make up the receptors. By finding the gene responsible for the down regulation of the protein production and silencing it, we could be able to increase the number of leptin receptors and in doing so increase levels of leptin intake, in turn reducing signals from the brain telling the sufferer to eat. We could also identify some of the genes responsible for producing the triglycerides thought to impede leptin transport and silence them.

Ethical Implications:

There are many ethical issues associated with the applications and testing discussed in this paper. For example, scientists we must consider where the line between treatment and enhancing desired traits lies.

One must also consider whether the treatment will have an effect on offspring, and if so, what are they? In this case sRNA addition to egg and sperm cells, would allow the sRNA to be passed on to future generations. This idea is controversial as while it could spare future family members suffering from having a genetic disorder; it may have an unknown impact on the development of the foetus or present long-term side effects which are currently unknown.

Another ethical issue is the right to chose treatment. The unborn foetus is unable to decide or express their wishes regarding the treatment and so we are left with the question, who has the right to make decisions for this unborn person, or a more challenging question being, when can we class a foetus as a living being, and at what point should it be granted its basic human rights? For those who wish to be followers of the Lord, we must be willing to love our unborn neighbour and the tiny human embryo as we do our selves. Even if you can't see them or witness their suffering, they are part of the human race; therefore they are our neighbours. "Love your neighbour as yourself," Matt 22:39.

Many opposers to genetic testing question whether we have any human conscience, or whether we purely wish to breed babies as written by Huxley in 'Brave New World', where rapid production of specialized children enhances the efficiency of society. They remind us of how Adolf Hitler would have relished this sort of technology in his mass extermination plans

and his quest to create the 'perfect' Aryan race. We must ask, what makes it acceptable to continue with such a similar program, when the last ultimately led to the death of around 6 million innocent Jews in the holocaust of World War II. When will we realise that the disabled and "genetically inferior" have a place in our society, and that nature has never intended for us to be a perfect race.

It is also questioned whether we have decided that we no longer leave the matter of life and death up to the 'universal creator'. Or have we in fact come to see ourselves as superior to God? The Bible shows us that God is the creator and giver of life. "So God created man in his own image, in the image of God he created them; male and female he created them," Gen 1:27. Believers feel that human life is not ours to treat as a commodity. We cannot decide which life is worthy to keep and which life should be destroyed.

It is also queried where we draw the line between acceptable and abnormal traits and when we choose to intervene. Those against testing claim that the search for 'defective' embryos is just the thin end of the wedge and that genetic testing has to stop, as none of us is or ever will be perfect. They state that genetic testing is not a way of preventing suffering or enhancing scientific knowledge, but merely a way of removing what we see as uncomfortable or undesirable in future human beings. It would seem that scientific developments have not entirely helped society move forward; in fact it may be making us less able to cope with the harsh realities of life.

On the contrary, many feel that the testing is a promising and important step forward for science and mankind. It is helping us to reduce suffering from hereditary diseases and that without this kind of testing; the rate at which science could develop would be severely impeded.

Conclusion:

Overall I believe that RNAi presents massive opportunity to treat or prevent a vast array of diseases which currently have no or ineffective treatments. It also gives scientists the opportunity to discover more about genetics and identify some functions of the many genes discovered through The Human Genome Project.

However, RNAi remains in its early stages of development, with many tests still only being done on cell cultures. It may be several years before we begin to apply these techniques to humans and even longer before we become aware of the long term effects it may have. Another main issue facing this treatment is the ethical debate over embryonic testing, without which, the future development of RNAi may be severely restricted. Scientist must try to present their argument to the public, helping them to become aware of how necessary the testing is to enable these much needed medical developments, which in turn could help to save millions of lives worldwide.

References:

Developing anti-viral drugs and combating Malaria in Africa
Catalyst – volume 20, number 3 (Feb 2010)

RNAi testing in mice and side-effects:

<http://www.sciencedaily.com/releases/2007/09/070926172235.htm>

General history and process of RNAi

http://en.wikipedia.org/wiki/RNA_interference

Importance of genetics in the aetiology of obesity

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1642700/>

General background information on Huntington's Disease and current treatments

http://hcd2.bupa.co.uk/fact_sheets/html/huntingtons_disease.html

Treatment, genetics and symptoms of Huntington's Disease and other neurodegenerative conditions

http://www.medicinenet.com/huntington_disease/article.htm

Treatment, genetics, testing and global impact of cystic fibrosis

http://www.ornl.gov/sci/techresources/Human_Genome/posters/chromosome/cf.shtml

Genetics behind cystic fibrosis and current gene therapy

<http://www.genetics.med.ed.ac.uk/cysfib/>

Video outlining RNAi mechanism

<http://www.youtube.com/watch?v=kCxQdXX0Dbk>

Video and general information about RNAi history, process and recent developments

<http://www.pbs.org/wgbh/nova/sciencenow/3210/02.html>

Gene Knock Down in vivo by RNAi

<http://www.taconic.com/wmspage.cfm?parm1=1799&qclid=CJ3cyo73rqACFSUulAodFCkXag>

Current treatments based on RNAi

http://www.nbsbio.co.uk/categories.asp?cID=285&_kk=rnai&_kt=e4f4c611-c521-458f-8951-238c8337475c&qclid=CPiK06P3rqACFRc9IAodQRzqaA&c=103914