

Shoot the Messenger!

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PASS WITH MERIT

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This article shall examine the possible uses and potential of RNA interference in humans and animals in order to cure cancer and other diseases, and to furthermore look at the uses of changing the genetic structure to improve general wellbeing. It will also look into the ways that SiRNA (which activates RNA interference) could be distributed around the body into every cell's structure. However, one of the main points of this discussion is on the possibility of using RNA interference in order to make humans and animals immune to ageing.

RNA interference is used mainly by plant organisms as a defence mechanism to destroy any pathogens threatening the plant. It is initiated when long double strands of RNA (consisting of up to 5500 nucleotides) enter the cytoplasm of a cell and is recognized as a foreign compound (RNA of a pathogen), where it is processed into shorter strands of about 20-25 nucleotides long by an RNase III-like enzyme called a 'Dicer'. The small, double strand (known as SiRNA) is then unzipped to form two short, single strands of SiRNA that then can either associate with RISC (RNA induced silencing complexes), or will reattach to another strand in a particular sequence. Then the RdRp (RNA dependent RNA polymerase) copies the sequence along the RNA strand to form more double stranded RNA. This is then cut up again by Dicer enzymes, forming more SiRNA, that is then unzipped to form more single stranded SiRNA and so on, enabling more RISC to associate with the SiRNA. The assisted SiRNA then guides the RISC to the (complementary) target RNA molecule which will then cleave the target RNA (pathogen). Following this, the target DNA is broken into singular nucleotides causing it to become inactive due to the fact that it has become singular nucleotides, so doesn't code for any task within the cell. The singular nucleotides are engulfed by lysosomes that contain powerful digestive enzymes, which will break down the singular nucleotides into its organic compounds (Phosphate, 5 carbon sugar and organic base) That can be recycled to make more RNA in the nucleolus. This would stop the translation of the RNA from the nucleus of the cell to the ribosomes, that take the RNA and use its genetic code to create proteins. So, if RNA interference takes place, it stops the RNA of the pathogen from creating protein to replicate itself, which will cause the pathogen to die out (as it is unable to reproduce). The idea of RNA interference in medicine and veterinary medicine is a very important concept in the development in anti-cancerous drugs.

Mutations are genetic defects. Hereditary mutations are gene defects that are passed on from a parent to child, so some people are more likely to develop cancer simply because they have a genetic predisposition to mutations. Acquired mutations are when cancers are caused by DNA changes that are acquired during a person's life, but can be called acquired, sporadic or somatic mutations. It is, however, important to remember that mutations in cells happen all the time. If the mutation is not repaired, the cell will get a signal to 'commit cell suicide' and undergo apoptosis. Most oncogenes are mutations of normal cells called proto-oncogenes. These genes normally control what kind of cell it is and how often it divides. If a proto-oncogene mutates into an oncogene, it grows rapidly out of control which can lead to cancer. In understanding oncogenes and tumour suppressor genes, new kinds of cancer therapies can be developed. Cancer may develop when tumours become malignant and spread to other parts of the body through the lymphatic system or blood stream. A tumour is a mass of abnormal cells which have grown despite there being no need for them due to the mitotic cell cycle going out of control, which causes rapid reproduction of abnormal cells.

The tumour starts with a gene going faulty inside a cell, which then causes the cell to mutate into some cancerous cells that clump together to form a tumour. If RNA interference takes place you could turn off the abnormal gene before it causes the cell to mutate and become cancerous. RNA interference could also be used to turn off the gene which codes for the cell reproduction, which would stop the cancerous cells reproducing in the mitotic cycle. The problem is that when a cell becomes cancerous, the cell has an inflammatory response which deactivates all reactions in the cell against itself (including apoptosis) so RNA interference can't occur.

Turning off the gene with RNA interference could also be used for other more advanced treatments as well as curing diseases, halting the ageing of cells, so that the body doesn't deteriorate over time, thereby initiating the possibility of living forever by However there are lot of ethical issues regarding a technically immortal population with regards to providing this possibility in the absence of diseases and physical harm.

Luk Van Parijs, the Ivan R. Cottrell Career Development Assistant Professor in Immunology, is investigating how cell growth and cell death signals control immune function. This could lead on to the extending of life forever if the absence of disease and physical harm is controlled. It is probable that the gene that controls aging and decay of cells could be uncovered, and that a complementary SiRNA complex could be created. This would stop the Messenger RNA from the nucleus reaching the ribosome and prevent the transcription process that produces the protein which ages the cell normally, by deactivating and destroying the messenger RNA and effectively turn off the ageing gene, with the possibility of immortality. "Survival of the fittest", as per Darwin's theory of evolution, will no longer rely on natural selection but would fall on genetic engineering through our ability to 'shoot the messenger'.

RNA interference is a new therapeutic approach and, as such, the ethical issue of the method has not been discussed in detail. Some major obstacles such as unsafe delivery methods, toxicity and off-target effects have to be dealt with before RNAi can be used as a conventional drug. Many problems face scientists and researchers in the developments of using RNA interference to cure such diseases like cancer in humans. Phil Zamore, a biochemist at the University of Massachusetts Medical School in Worcester, showed that small interfering RNAs could stop HIV infection in cells grown in the lab. Sharp, former MIT biology professor Paul Schimmel, Zamore and others have co-founded Alnylam Pharmaceuticals in Cambridge to develop RNAi-based drugs. Lots of research is now on going using RNA interference to develop a therapy for cancers, most of which are aggressive and respond poorly to standard therapies. The proto-oncogene KRAS is mutated in a wide array of human cancers. In spite of the fact that there has been progress on identifying specific oncogenes which respond to clinically effective therapies, the KRAS has remained resistant. A strategy currently under investigation for targeting KRAS is to identify the genetic code that, when inhibited, result in cell death only in the presence of an oncogenic allele using systematic RNA interference to detect synthetic lethal partners of oncogenic KRAS and deactivate and destroy it. It was found that suppression of TBK1 (SiRNA) induced apoptosis specifically in human cancer cells

that depend on oncogenic KRAS expression.(information taken from Nature 462, 108-112 published online 21 October 2009)

One of the main problems is when long double strands of foreign RNA (e.g. cancer) are introduced into the cells cytoplasm, it causes an inflammatory response which shuts down all the cells defence mechanisms, including RNA inference. So the cell will be open to mutations and then replicating to form a tumour. However it has been discovered that if SiRNA (shorts double strands of RNA, smaller than 23 nucleotides long) is added to a cell, it doesn't cause a inflammatory response, allowing RNA interference to take place inside a human cell which normally does not occur. The SiRNA initiates the RNA interference reaction within cell. This RNA interference is very specific, and therefore should you want to use it for a cure to cancer, you would need a specific SiRNA with the same genetic pattern as the cancerous (mutated) gene. If the RISC can attach to the unzipped single stranded SiRNA introduced in the cell, it can keep copies of itself in order to destroy cancerous gene that may occur later and threaten the cell. This would bring immunity as it would the stop cancerous gene (destroyed via RNA interference) before it can cause an inflammatory response. As the RISC dependant RNA is already there to cut up the cancerous gene, lysosomes are able to digest them. This is only in the initial stage of research and there are many problems ahead before a cure can become a reality.

For example, if SiRNA does work as a cure for cancer and other diseases, you would require various SiRNA all with different genetic codes (one different genetic code for each different virus.) This would result in a lot of RISC with different coded SiRNA attached. This may cause confusion in the cell and slow down the response rate of the RISC in destroying the foreign or mutated gene giving the foreign or mutated gene more time to react to the RISC presence. It may be advisable that RNA interference is only used to cure viruses, such as AIDS, HIV and Hepatitis for which there is no known cure rather than bacteria that can be controlled/destroyed by antibodies outside the cell through other known vaccinations and antibiotics.

Furthermore, there would be difficulty in delivering the SiRNA complex into every required cell in the body. Reaching all these individual cells poses fresh obstacles as using a microscopic needle to inject the different SiRNA complexes is unrealistic and impractical and it is time consuming. Treatment would have to protect the integrity of the cell by avoiding damage the cell membrane and the cell itself (we would be using a nanometre scale). In short, a needle will be ineffective as a tool for delivery of SiRNA complexes to cells due to the time it would take, the inaccuracy, unreliability to reach every cell and the inability of a needle to penetrate bone and the strong outer mesh of collagen surrounding bone marrow.

Further consideration for a method of treatment is to inject the SiRNA complexes into the single cell of a human embryo before it can multiply, thereby allowing the cells to multiply containing the SiRNA complex. The newborn baby will consequently grow up with the SiRNA complex contained in every cell. A problem with this method is if the SiRNA needs to re-administered (more detail further on in the paper) or a new virus comes along resulting in the necessity of a new SiRNA complex to be cooperated into the cells, no solution is available for delivery at this stage. Future generations may benefit from this procedure but not the current population. The same applies for direct administration of the SiRNA complex into stem cell. Due

to the delay it would take to spread through the new cells, various organs such as the heart, brain, liver, kidney and lungs would not benefit as it would take too long to be replicated where needed to replace old cells.

Another method is to insert the SiRNA directly into the blood stream, enabling the SiRNA to diffuse into the cell from the blood, however it would spread through every capillary to every cell equally due to diffusing to equilibrium causing it to be evenly spread out over the body. Even though you would need a lot of SiRNA injected into your blood stream, it would only need many injections in smaller quantities, which would be more feasible to administer to large populations, utilising current health facilities. This also applies in updating immunity of other diseases.

A particular issue concerning the use of SiRNA is that is very specific, reacting only with one specific virus, however being easily transmitted through out body via the blood stream, it would be plausible to distribute new SiRNA that code against multiple new viruses. Viruses adapt quickly, developing mutations resulting in resistance to cures, demonstrating the importance of urgency in the SiRNA attaching to RISC, thereby deactivating and destroying the virus before it can react to the present RISC dependant SiRNA. To prevent this from occurring, it may be suggested to maintain high levels of SiRNA in the cells and this could be achieved by the utilisation of a booster vaccine of new SiRNA complexes regularly.

RNA interference is a new therapeutic approach and as such the ethical issue of the method have not been discussed in detail. Some major obstacles such as unsafe delivery methods, toxicity and off-target effects have to be dealt with before RNAi can be used as a conventional drug. In order ascertain the possible harms against benefits a risk/benefit analysis will have to be performed. For instance, the theory that it is ethically correct to proceed if treatment achieves the greatest good for the majority, accepting that a few may fall victim on the way. This however could conflict with the Hippocratic Oath (“I will use treatment to help the sick according to my ability and judgement, but I will never use it to injure or wrong them”). Clinical trials utilising new drugs can be classified into 4 phases. Prior to approval for use in the general population animals are often used which opens the debate on cruelty to animals. Some may argue that the suffering animals may go through is needless as animal cells are genetically different to human cells and so success in one may not result in the other. Extensive pre-clinical studies are also conducted in test tubes and cell cultures bringing forward the religious aspects regarding “acting as God” in modifying human cells. The question arises as to whether we have the right to extend life or change one’s fate, in particular regarding the possibility of stopping age-related degeneration (this could bring up some economic concerns as this older generation will have to be supported and only the rich would be able to afford this opportunity). Participants in a clinical trial would receive additional medical attention, have access to a new treatment and hopefully a beneficial/successful outcome. This has to be compared with some possible risks. The new treatment may have bad side-effects or risks that were unexpected. The patients may be given a placebo rather than the desired treatment. Finally the procedure may not be a success. Considering these issues more consideration is given to the risk-factors rather than the benefit-factors in order to minimise harm done to patients, for instance only the targeted genes are affected otherwise essential genes may be incorrectly blocked.

Besides taking into account specific religious, social or political morals that may affect these principals we also have to remember the patients and their families. On this matter, care must be taken to insure future generations are not affected by genetic changes and consideration on the risk of generating infection-competent viruses that could infect non-consenting people. A further issue to be discussed is whether the same opportunity of health care should be available to all people or whether some should forfeit their right if they are personally responsible for the illness to be treated, eg. Smokers who develop lung cancer. This issue could become complicated however as many of these diseases result from various factors, not just one i.e. the smoker may also have a genetic predisposition and may live in an urban area where environmental conditions contribute. It is clear there are many aspects to be considered and it is essential to form a guideline before therapies are performed on human patients.

In conclusion, RNA interference is a very vital aspect of medicine and veterinary medicine if we are to make significant advances in healthcare, particularly in the fields of cancer and viral immunity. Research is in it's infancy stage and many issues require addressing before treatment can become a reality. A more efficient delivery platform is vital as well as a better understanding of it's components and mechanisms. Many aspirations such as the ability to turn off the ageing gene is still out of reach however with the financial backing that the large drug companies are providing it is only a matter of time before the impossible may be achieved bringing with it ethical issues. It could be said that we are taking matters too far acting as God and interfering with evolution. Further issues will be obvious in that third world countries would probably be the last to benefit and initially all treatment will be expensive limiting it to the rich. We are already expecting a food shortage as the Earth's population rapidly increases and these treatments will only exacerbate this problem. If ageing can be stopped, presumably RNAi could also be developed for use as a weapon, and certainly any development in this area will be vigorously supported by governments! Treatments may also be withheld as a form of sanctions in the political arena. The reality is success in utilising this treatment will affect every aspect of our lives politically, financially and socially.

## References

Background video's on RNA interference

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