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Abstract
The purpose of this paper is to better understand the methods problems and some solutions for siRNA treatments. The benefits of this novel medical treatment are explored and its benefits are expounded on by comparing it to other more complex and futuristic treatments. The exact process of siRNA silencing and down regulation is unknown. Some hypotheses of how it may work are discussed giving precedence to the most widely accepted hypothesis. Although siRNA treatments are not yet used on a major scale for many diseases, different possible treatment options are compared and explained. Particular care was taken to give a broad range of illnesses in order to show the vast possibilities of siRNA therapy. The common problems and some methods to overcome them are arranged in an orderly manner starting from the time the siRNA treatment is administered, through its delivery and subsequent potency, followed finally by its degradation. SiRNAs viability as a treatment is then analyzed primarily on its large interest in many fields, as well as the enormous progress it has made since its discovery just a short while ago.

Viability of SiRNA as a Clinical Treatment

The future of medicine is heading closer and closer to perfecting gene therapy. While research is bringing the option to alter genes closer to fruition, there is still a long way until it will be able to be implemented. Simpler therapies albeit with their own set of problems, seem to be a more realistic option for the foreseeable future. One in particular that has seen some progress is the use of small interfering RNA (siRNA). "Since its discovery by Fire and Mellow in 1998, siRNA has proven to be a powerful tool for modulating the expression of almost any gene in various species." (Nechaev, et al, 2013) During Göran Hansson's speech after receiving the noble prize for the discovery of a form of RNA silencing, he said, referring to RNAi (more general term for siRNA), that it "has added a new dimension to our understanding of life and provided new tools for medicine." (Eggleston, 2009).

It can have many applications and doesn't necessarily require the alteration of DNA. It allows the DNA to remain in its state of disarray however it won't allow the problematic gene to be translated. This allows for control of the gene without having to alter the DNA. The applications for this are countless. SiRNA can treat many otherwise untreatable diseases ranging from viral infections to complex genetic disorders (Endo-Takahashi. et al. 2012). Although the concept of siRNA is not a new one, there are some kinks in how to use it as a treatment. One major setback is that although it is now possible to create this aforementioned silencing RNA (siRNA), its method of delivery is problematic. The siRNA, if delivered indiscriminately, can wreak havoc in cells it wasn't intended for. It can stop crucial growth and functional elements of important life sustaining cells. It is therefore important to deliver these potentially harmful substances only to their intended targets. This is just one of a few issues that can be a major hindrance in siRNAs future as a treatment. It is imperative to understand how siRNA works before an approach can be found to tackle the issues that can arise in using siRNA as a treatment.

SiRNA silences the expression of specific genes allowing for particular cell functions to seize. SiRNA begins its life as a double stranded RNA molecule (dsRNA). The dsRNA is cleaved into shorter siRNAs. This process requires dicer to cleave the large dsRNA. The siRNA is incorporated into a protein complex referred to as RNA inducing silencing complex (RISC). The still double stranded siRNA is unwound by the protein complex via multiple steps. The now single strand of siRNA leads the RISC to the target RNA destroying it (Schwarz, et al, 2014). The destroyed mRNA is no longer able to perform the function it was coded for. This allows for a cell to continue with its other functions as long as the specified protein isn't crucial for the cells normal function. The siRNA does not interact with the genome directly allowing for less risk of creating mutant cells.

Possible Applications of siRNA

SiRNA has been studied largely for its application as a cancer suppressor. It has however, many applications beyond that. whether in its ability to lower circulating cholesterol (Tep, et al, 2012) by inhibiting the translation of a specific protein, or as a method of controlling HIV-1 (Zhou, et al, 2008) as well as possibly reducing any other potentially harmful protein synthesis. SiRNA can have other non-clinical applications as well. Although the same basic ideas and methods are used for all potential treatments associated with siRNA the broad range of diseases it can potentially treat allows for some unique applications.

One study on the effects that siRNA can play in treating ovarian cancer used a pretty classical method of using siRNA. The surface protein cd44 is necessary in activating signal pathways necessary for cancer, specifically metastasized, to continue its growth and destruction. These pathways instruct the cell to continue transcribing proteins necessary for cell growth, as well as other factors that allow the cancer cells to live and multiply. The cd44 protein is also only found to be expressed by metastasized cancer cells, thus negating many of the issues associated with siRNA delivery that will be discussed later. The study used siRNA as well as an anticancer drug to see the combined effects they could have on metastasized ovarian cancer. The siRNA was specific to mRNA coding for cd44. It was carried out in vitro as well as in vivo with similarly successful outcomes; the cancer cells growth was slowed dramatically in both tests. Compared to just the use

of the cancer drug and other chemotherapies, it was deemed more efficient and aggressive. This is a truly great step forward in finding a new cure with this rarely used method of treatment. (Shah, et al, 2013).

SiRNAs use as a treatment for HIV is also being considered. "HIV-1 gene expression, during productive and chronic infection, is essentially dependent upon the early regulatory genes tat and rav." (Caputo, et al, 1997) If a method to stop these regulatory genes from being expressed can be found it can be used to virtually control HIV. Although there have been many improvements in controlling and preventing new outbreaks of HIV and the associated life altering conditions, HIV is still a still prolific disease(Centers for Disease Control, 2013) .

Working on this premise, researches created a siRNA delivery method that is specific to the HIV strain they were interested in controlling. The siRNA was manufactured to be specific to the genes that coded for tat and rav. In stopping the expression of these genes that are crucial for the viability of HIV-1 they were in essence stopping the disease. Through methods including quantitative real time pcr, they were able to confirm that the genes associated with rav and tat were significantly down regulated (Zhou, et al, 2008).

The extensive range of treatments that can be possible with a treatment involving siRNA doesn't just apply to cases of cancer and the HIV virus. It is being evaluated for its potential as a method of treating conditions involving an increased level of low density lipoprotein (LDL) cholesterol. "In individuals with 5-year risk of major vascular events lower than 10%, each 1 mmol/L reduction in LDL cholesterol produced an absolute reduction in major vascular events of about 11 per 1000 over 5 years" (Cholesterol Treatment Trialists' (CTT) Collaborators. 2012). A reduction in ones LDL levels, specifically in the amount found in one's blood stream, has proven to reduce the risk of cardiovascular disease (Manninen, et al, 1992), and might be a factor in lowering the risk of those who have had a stroke previously from having another (Sacco, et al, 2006) .

SiRNA can be used indirectly as a way to lower LDL cholesterol levels. " A novel therapeutic approach to lower LDL-c that is currently in clinical development involves blocking VLDL assembly and secretion by inhibiting the microsomal triglyceride transfer protein (Mtp)" (Tep, et al, 2012). By using siRNA to inhibit translation of the gene responsible for Mtp, they were able to reduce the levels of LDL cholesterol found in the blood stream. This is especially useful for those who don't react well to the current protocol set to reduce LDL levels by prescribing statins.

The broad range of application in a clinical sense for the use of siRNAs is apparent from the few studies mentioned. There are many other applications that can be utilized, but those mentioned here show how broad its ability as a drug can be. Most of these studies however, are very theoretical. There are many hurdles that must be overcome before being able to use it as a treatment. The main hurdles being its delivery to a specific cell and stopping a specific protein without affecting others.

Problems and Their Potential Answers

Delivery of SiRNA to cells has been a major problem for researchers. There are quite a few reasons that it is difficult to deliver molecules of siRNA, or more specifically its precursor dsRNA, to cells. The first is its really short lifespan. Even if the dsRNA won't degrade before reaching its desired location, the trouble of getting it into the cytoplasm of the cell is a hurdle in itself. However the biggest issue seems to be how to get these crucial siRNAs to a specific cell. Once in the correct cell, problems may still arise. SiRNA can be finicky at times and down regulate proteins that it wasn't intended for. Basically the siRNA has major issues with its specificity on the cellular level as well as intercellular.

"Unmodified, naked siRNAs are relatively unstable in blood and serum, as they are rapidly degraded by endo- and exonucleases, meaning that they have short half-lives in vivo." (Akhtar, Benter, 2007). This limits the ability to administer siRNAs in their "naked" already modified form. However, chemically modifying the siRNAs seems to prevent their untimely degradation. Studies were done comparing siRNA in its naked, already modified form, to siRNA that was chemically modified either by way of caging, nanoparticles, or PEGylation. When this comparison was done under conditions that simulated living in the blood stream, the siRNA that had been modified showed a significant increase in life span. Chemically modifying siRNA seems to be the way to allow for the siRNA to survive its journey to the cell. (Shah, et al 2013)

Surviving its journey to the cell is just the first of many hurdles for this important new treatment. The next major stumbling block to overcome is how to get it to the cell of choice. Although siRNA is selective for specific proteins it can cause havoc in a cell it wasn't intended for. For example, even if a cell doesn't have the specific protein that this siRNA codes for it can cause unwanted mRNA degradation due to off targeting (B. Scaggiante, et al, 2011), a complication that will be discussed in a more general sense in the coming paragraphs.

Some methods are being studied as ways to allow the delivery to be more specific. The use of aptamers has been relatively successful in allowing the siRNA to reach specific cells, although it has its own unique set of issues (Liu, Gao, 2013). Another method

that has been used is connecting the siRNA enclosed in nanoparticles to an existing delivery system. This has shown promising results especially when the intended drug is also used (Shah, et al, 2013). Other methods are showing promising results as well. This leads to the next problem of the siRNA being delivered across the cell membrane.

SiRNA can be especially difficult to deliver through the cell membrane due to its charge. The cell membrane has an affinity to the siRNAs inherent charge causing it to be repelled. There are several methods being studied to find the most efficient way of allowing molecules of siRNA to penetrate the cell membrane. The first is the physical or mechanical method. Using electroporation or ultrasound, for example, to allow for cellular uptake. Another method being looked at is the use of chemicals either to neutralize the negative charge of siRNA or deliver it through other methods. (Zhou, et al, 2008)

With passage of siRNA through the cell now possible, off targeting, the last issue of delivery needs to be resolved. Although siRNA is coded uniquely to suppress the desired protein, it isn't always so specific. If a similar protein is also produced in the cell that the siRNA is delivered to, it can occasionally down regulate that protein as well. A specific protein is one that has a similar genetic code even though it can serve a very different and sometimes crucial role in the viability of the specified cell.

A few methods have been used to negate this off target silencing. The methods mainly use modifications to the siRNA or dsRNA starting material. By modifying the backbone of the strand, improved specificity was able to be reached. Although useful the modifications tend to make the siRNA less efficient against the target mRNA as well. There is one particularly successful option that doesn't affect the down regulation of the target mRNA as much as many of the previous methods tried. This process requires adding a one nucleotide bulge on the antisense strand specifically at the second positon. The modified siRNA was found to be more specific to the intended mRNA producing less mistaken down regulation while still properly down regulating its intended target. (Dua, et al, 2011)

With its intended target found, the siRNA can now efficiently down regulate it. However, the stable dsRNA that the RISC now unwound to use as a mRNA inhibitor, is no longer as stable. Due to its less stable state when joining the RISC, "Transfected synthetic siRNA works for only a few days in mammalian cells." (Sioud 2004) While multiple treatments may reduce the issue of short term effectiveness, a better solution may be available. Using retroviral drugs the siRNA can be integrated into the genome more specifically as hairpin RNA. This is like the naturally occurring non synthetic siRNA. Using siRNA the cell can regulate itself post transcriptionally. By integrating the codons to produce siRNA the time frame for siRNA longevity becomes indefinite. This is not by it increasing in stability rather it allows for a constant dose without further intervention. (Stewart, et al, 2003)

Conclusion

The concept of using siRNA as a treatment for humans is one that excites and can open many previously unavailable options to those suffering many debilitating diseases. It is so promising that any stumbling block that has arisen along the way hasn't deterred researchers from their final goal of using siRNA in the clinical setting. They have attacked it from every angle and found solutions for many problems and ideas for others. The main issue of delivery primarily affects reaching specific cells or tissues with specificity. There are clinical trials mainly focused on using siRNA as a topical treatment. Although this method of treatment has been proven successful, by eliminating many of the issues associated with delivery, it only works on areas that are able to react to topical treatments. Topical siRNA treatments have seen much progress and continue to pass multiple phases of their testing; some have even been approved for clinical use. These treatments bypass many of the issues discussed, and show the ability of siRNA as a potential treatment for other diseases that need a more complex delivery system.

The extensive opportunities for siRNA use are driving many to discover new and improved methods. The way in which siRNA has turned from a discovery into clinical trials in a mere fifteen years attests to its great potential and likely future as a viable treatment. There are many areas that are still unclear especially regarding the methods in which siRNA works. With more discovery and new technology the methods of delivery and treatments will likely improve and start the important work siRNA was intended for.

References

Akhtar, S., I. F. Benter, Nonviral delivery of synthetic siRNAs in vivo

The Journal of Clinical Investigation 2007; 117,12, 3623-36322

Caputo, A, C Rossi , R Bozzini , M Betti , MP Grossi , G Barbanti-Brodano and PG Balboni, Studies on the effect of the combined expression of anti-tat and anti-rev genes on HIV-1 replication, Gene Therapy (1997) 4, 288–295

Castanotto, D., & Rossi, J. J. (2009). The promises and pitfalls of RNA-interference-based therapeutics. Nature, 457(7228), 426–433

Centers for Disease Control and Prevention. HIV Surveillance Report, 2013; vol. 25. http://www.cdc.gov/hiv/library/reports/surveillance/. Published February 2015. Accessed febuary 24, 2015)

Cholesterol Treatment Trialists' (CTT) Collaborators. (2012). The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. Lancet, 380(9841), 581–590. doi:10.1016/S0140-6736(12)60367-5

Dua, P., J. W. Yoo, S. Kim, D. Lee. Modified siRNA Structure With a Single Nucleotide Bulge Overcomes Conventional siRNA-mediated Off-target Silencing. Molecular Therapy, (2011). 19(9), 1676–1687

Eggleston, AK.. Editorial: RNA silencing, Nature 2009; 457, 395

Endo-Takahashi Y, Negishi Y, et al. Efficient siRNA delivery using novel siRNA-loaded Bubble liposomes and ultrasound. Int J Pharm. 2012;422(1-2):504-9

Liu, H. Y., X Gao, A Universal Protein Tag for Delivery of SiRNA-Aptamer Chimeras, Scientific Reports 3, 2013;3129

Manninen, V, L Tenkanen, P Koskinen, et al, Joint Effects of Serum Triglyceride and LDL Cholesterol and HDL Cholesterol Concentrations on Coronary Heart Disease Risk in the Helsinki Heart Circulation, 1992;85:37-45

Nechaev S, Gao C, Moreira D, Swiderski P, Intracellular processing of immunostimulatory CpG-siRNA: Toll-like receptor 9 facilitates siRNA dicing and endosomal escape. J Control Release. 2013;170(3):307-315

Sacco, R. L., R Adams, G Albers, et al, Stroke. Guidelines for Prevention of Stroke in Patients With Ischemic Stroke or Transient Ischemic Attack 2006; 37: 577-617

Scaggiante, B., B Dapas, R Farra, et al,Drug Metabolism (1st ed., Vol. 12). (2001). Hilversum, Netherlands: Bentham Science. 11-23

Schwarz DS, Hutvágner G, Haley B, Zamore PD. Evidence that siRNAs function as guides, not primers, in the Drosophila and human RNAi pathways. Mol Cell. 2002;10(3):537-548

Shah V, Taratula O, Garbuzenko OB, Taratula OR, Rodriguez-Rodriguez L, Minko T. Targeted Nanomedicine for Suppression of CD44 and Simultaneous Cell Death Induction in Ovarian Cancer: an Optimal Delivery of siRNA and Anticancer Drug. Clin Cancer Res. 2013;19(22):6193-6204

Sioud, M. (2004). Ribozymes and siRNA protocols (2nd ed.). Totowa, N.J.: Humana Press. 501

Stewart, S. A., D. M. Dykxhoorn, D Palliser, et al Lentivirus-delivered stable gene silencing by RNAi in primary cells RNA, Vol. 9, No. 4 pg 493-501 2003

Tep S, Mihaila R, Freeman A, et al. Rescue of Mtp siRNA-induced hepatic steatosis by DGAT2 siRNA silencing. Journal of Lipid Research. 2012;53(5):859-867

Whitehead, K., Langer, R., & Anderson, D. (2009). Knocking down barriers: Advances in siRNA delivery. Nature Reviews Drug Discovery, 412-41

Zhou J, Li H, Li S, Zaia J, Rossi JJ. Novel dual inhibitory function aptamer-siRNA delivery system for HIV-1 therapy. Mol Ther. 2008;16(8):1481-1489