Target Specificity and Delivery Considerations In Creating RNAi Therapeutics

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Abstract:

RNAi is a naturally occurring process with the ability to destroy foreign RNA. Recently, RNAi is being used to create therapeutics for diseases and foreign viruses. Although it seems rather simple to create a therapeutic through natural processes there are concerns regarding using RNAi as a therapeutic. In this review I address challenges with this form of therapy such as target specificity and delivery. In regards to target specificity I include methods which directly alter the siRNA to increase the specificity of the therapies impact. I also discuss various delivery methods that were used in the past along with methods that are most preferred today. Lastly, I propose a possible model for the therapeutic which combines different specificity and delivery methods.

Introduction:

RNAi, also known as RNA interference, is a process in which a cell can destroy foreign RNA that enters into the cell. Double stranded RNA (dsRNA) or short hairpin RNA (shRNA) often stimulate RNAi. The process begins with a complex called Dicer, which will cut up the foreign RNA and create micro RNAs (miRNA), which are essentially smaller pieces of the foreign RNA. The miRNAs are detected by the RISC complex and are separated from their double stranded structure to be single stranded. The single strand will then be used to find complementary sequences in the cell's genome. If a complementary sequence is found the RNAi will destroy the RNA, preventing it from creating a protein (Figure 1). The RNAi process is a natural function the human body possesses and does not require external influences. Although a fairly simple process, using RNAi in therapeutics continues to be challenging due to the various obstacles that need to be addressed such as target specificity, accurate delivery strategies, and longevity of the therapeutic in the human body. Scientists are trying to solve these barriers because RNAi could prove to be a very useful way to cure numerous diseases. (National Library of Medicine)

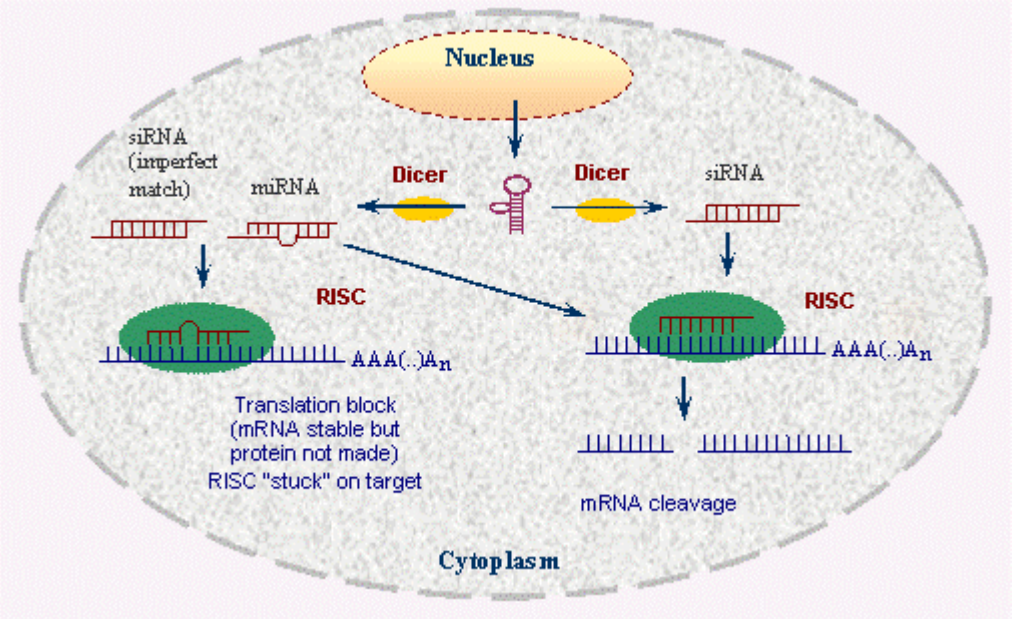

Figure 1 (National Library of Medicine)

Target specificity:

There are currently a multitude of challenges with the target specificity of the RNAi regarding issues such as silencing incorrect genes and over expressing the targeted gene. Fortunately, researchers have been testing various methods to avoid these inefficiencies regarding RNAi specificity.

The first issue regarding specificity lies within the structure of the RNAi itself. The seed region of the RNAi often tends to accidentally match with the 3'-UTRs of incorrect genes, which leads to the silencing of "off-target," sometimes necessary genes. RNAi is a process of reverse transcription and, much like regular transcription, primers called hexamers are used to guide polymerases across the RNA. A recent discovery uncovered that the frequency of off-target silencing is directly related to the frequency of hexamers in the 3'-UTRome. siRNAs with a low amount of hexamers had less occurrences of off-target silencing than those who had medium or high concentration of hexamers, and even the distribution of these hexamers plays a key role in the specificity of the siRNAs. In an experiment conducted looking for human Huntington's disease (HD), the siRNA coding sequences with a low frequency of hexamers would have to follow two criteria, one of them being a faithful loading of the wanted guide strand and the second a GC-content between 20-70. The overall results displayed that two siRNA models that followed the criteria were found to be the most efficient with the fewest amount of off-target silencing and a long-term effect.This method could be effective on humans except that 3'-UTR sequences are specific to every species so the method that works in mice may not work in humans so more care is to be taken when implementing these methods clinically. (Boudreau et al., 2011)

Currently, indirect methods such as adjusting siRNA concentrations and setting appropriate controls in in vitro experiments, often have various setbacks and often cannot be applied for human therapeutics, so direct and permanent methods related to modifying the siRNA could possibly be a better solution for off-targeting. In fact, methods such as modifying nucleotides like 2'-*0*-methyl nucleotides at position 2 have shown reduced off-target activity but also a reduction in on-target activity. This method focuses on nucleotide positions that affect the reverse transcription of the miRNA. The most important factors of the miRNA are within the 2-8 positions. These positions contain seed matches which contain information that could help with identifying miRNA-like off-target sites. The Ago miRNA-target complex, which was used in the experiment, uses position 2-6, which are fixed into an A-form helical structure, making it more susceptible to base pairing. A common issue in miRNA is the structural kink from positions 6-7 which cause a process called the initiation step in which only positions 2-5 are used for base pairing, which often is far too little information to allow accurate base pairing. In order to eliminate these kinks the translational nucleation model was applied to the Ago miRNA. This process allows the nucleation bulges to form consecutive base pairs, so that positions 2-8 can all be used for base pairing, increasing the information used for transcription leading to more target specificity. (Lee et al., 2015)

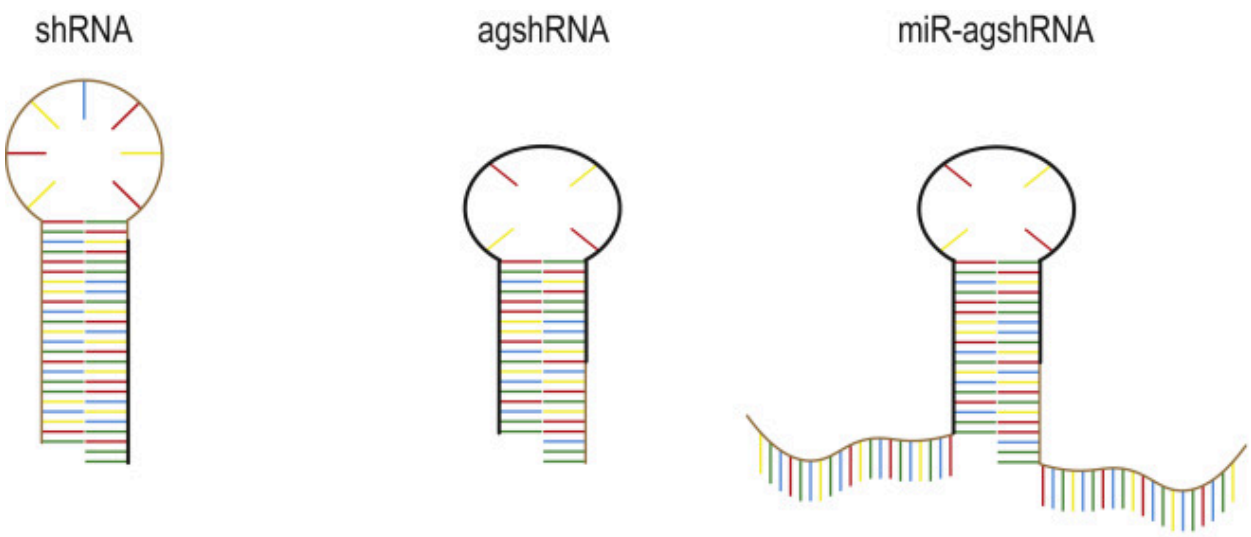

Figure 2 (Becker et al.)

The most recent development for the specificity of RNAi is a hybrid guide RNA that consists of the backbone of an miRNA with a Ago2-dependent shRNA. This hybrid is completely independent of the DICER complex and is processed by Ago2 in the cytoplasm, where the 3' hairpin stem is cleaved at positions 10/11. The hybrid was also found to be independent of Ago1, Ago3, and Ago4 reducing its competition with endogenous miRNA, which are beneficial for the body, and making it more specific. This new hybrid has virtually no passenger strand activity and reduced toxicity compared to a regular shRNA. More work needs to be done to understand how to create beneficial efficiency in vivo. (Becker et al., 2022)

Delivery:

Besides target specificity, delivering the RNAi to the correct location in the body and ensuring it is not destroyed in the process is also a problem yet to be solved. As of now the most successful RNAi therapeutics have been done in easily accessible areas of the body such as the liver or eyes, but there still remains a challenge when trying to reach areas through the bloodstream or through oral delivery because of the instability of the RNA.

Viral delivery

Viral delivery has been a commonly tried form of delivery in the early years of RNAi therapeutics. There are various approaches to viral delivery; some of them include retrovirus delivery, lentivirus delivery, and adenovirus delivery. The viral delivery methods were widely unsuccessful due to their adverse inefficiencies. (Li et al., 2006)

The retrovirus method proves an efficient method in in-vitro experiments; however, they can be extremely dangerous for humans as they have multiple side effects. The use of a retrovirus indicates that the introduction of the miRNA or shRNA is through insertion into the genome. This can often lead to the foreign material being inserted into the genome which is

known as insertional mutagenesis or carcinogenesis which can become the initiation of cancer. (Li et al., 2006)

Although slightly more successful than retrovirus therapy, lentivirus therapy still has a multitude of side effects making it dangerous to use for therapy. Unlike retrovirus delivery, lentivirus therapy avoids the insertion of foreign genetic material into the genome and is able to differentiate between non-dividing cells and primary cells. Lentiviral vectors also have the capability to carry more genetic information increasing the specificity of the therapy. Despite its benefits compared to retroviral therapy it is still very likely to induce an immune response causing unwanted side effects; an immune response will work to destroy the foreign RNA defeating the purpose of the therapeutic. (Li et al., 2006)

The most successful viral method tried is the adenovirus method. The adenovirus method is typically used for tumor targeting therapies and is able to take action in a short period of time. Unlike other viral therapies, adenovirus therapy disseminates its genetic information outside of the target cell's nucleus, avoiding the risk of interfering with the host cell's genome. One major setback to the adenovirus method is its weak tissue tropism, it relies on receptors and other markers on host cells in order to effectively find its target, but not all cells contain receptors and/or markers making it difficult for the virus to generalize this method as a therapeutic. (Li et al., 2006)

Though viral methods have shown possible successes, they all have major setbacks regarding efficient delivery regarding immune responses and infecting a multitude of cells. The methods also tend to be incredibly unstable and only show positive results when tried in vitro.

Direct and oral delivery

Direct and oral delivery are commonly preferred by patients for their accessibility and simple administration, but for these delivery methods to function as intended there are various obstacles such as the path in which the therapeutic will travel and the access to different organs and tissues.

Direct delivery can be administered through nasal sprays, eye drops, or any other non-invasive methods. Previously common methods included injection into the retina or central nervous system based on the disease that was being tested. The local administration of the RNAi poses less obstacles, but limits the organs and diseases able to be treated. So the use of nanoparticles was implemented in order to reach different organs through the circulatory system without degradation. When it comes to systematic delivery the size of the siRNA plays a key role. Typically with the size of 10 nm or less, siRNA often exits the body through renal clearance. This type of method is seen best fit for tumor tissue therapy, the macromolecules and nanoparticles with a diameter less than 400 nm generally gather in the vasculature of tumor tissue. In order for this to work an siRNA with around 100 nm has been used. The 100 nm particle will prevent renal clearance and allow the siRNA to extravasate the tumor tissue. It was also proposed that the nanoparticle change sizes as it entered different environments. For example, the 100 nm nanoparticle will shrink to 10 nm after the extravasation of the tumor tissue. Furthermore, to increase the chance of the RNA reaching the correct location receptor

based endocytosis could pose as a solution. The nanoparticle would have ligands that would bind to the receptors of the tissue or organ being targeted. (Zhou et al., 2013)

Being one of the most preferred types of treatment, oral delivery can be self administered and is easy to transport and use. However, oral therapy proves to be a challenge because of the harsh pH conditions of the stomach and possibility of degradation of RNA under nucleases. In order to avoid these issues, the use of inorganic nanoparticles has been proposed. Most challenges with oral delivery exist within the GI tract. Using nanoparticle-based coatings can possibly propose a solution to this issue. Polymeric nanoparticles tend to interact negatively with charged nucleic acids which essentially improves their cellular internalization. In one study Ballarín-González et al found that the siRNA filled with the nanoparticles can work in multiple organs where simple siRNA cannot. Lipid based nanoparticles have also been tried for oral delivery as they contain easy binding structures. Through the journey that an oral medication takes, the esophagus is not an ideal place of delivery due to the first transition time –time it takes to travel through the esophagus, normally one to two seconds in a healthy person– , the stomach also has barriers with its low pH and thick mucus layer which often degrade drugs. Since, the small intestine is the most likely place for delivery as it has a long retention time, large surface area, high permeability, and sufficient absorption more work needs to be done in order to consider oral delivery as a solution. (Afrin et al., 2023)

Conclusion:

Through the multitude of proposed models and methods in the paper thus far, a combination of the different methods for specificity and models for delivery could possibly create an efficient RNAi therapeutic. For the delivery of the therapy, oral delivery seems the most efficient for patient use and comfort, but specifically, the RNAi would be embedded in an inorganic nanoparticle to ensure its survival through the GI tract and intestines (Afrin et al., 2023). For the RNAi itself, out of all the proposed models the hybrid shRNA model shows the most promising results for accurate targeting (Becker et al., 2022). Lastly, for more specificity the translational nucleation model would be implemented before the RNAi is in the nanoparticle, removing the bulge between 6-7 and opening up more information to improve specificity (Lee et al., 2015). Although all these methods and models still need to be worked on and modified, the combination of various approaches could possibly result in a working therapeutic.

Sources

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