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RNA Interference: A Potential Approach For Silencing Splice Isoforms Associated With Human Diseases

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Abstract

Double-stranded RNA (dsRNA) initiates the post-transcriptional gene silencing process known as RNA interference. Small interfering RNA (siRNA), short hairpin RNA (shRNA), and bi-functional shRNA are techniques for mediating the RNAi effect. For some medical conditions (such viral injections), the ease of generating siRNA and the brief duration of each dosage make them ideal candidates. Optimized shRNA constructs, particularly those contained in a miRNA scaffold, provide high potency and long-lasting effects with low copy numbers and fewer off-target effects by leveraging the endogenous processing machinery. RNAi-based therapies' efficacy and safety might be further improved by bi-functional design. There is a great deal of interest in employing RNAi as a technique in many different contexts because of its specificity and resilience. Recent developments in the use of RNAi, a method for regulating normal gene expression, as a possible therapeutic agent for a variety of illnesses and disorders, including cancer, infectious diseases, and metabolic disorders. Clinical trials for RNAi have started, but significant challenges still need to be addressed before RNAi can be thought of as a conventional medicine, including off-target effects, toxicity, and dangerous delivery systems. In this article, we initially examined the RNAi mechanism before concentrating on some of its biological research uses, including the therapy of HIV, viral hepatitis, and a number of other disorders.

Keywords: dsRNA, siRNA, hairpin RNA, gene silencing, bi-functional shRNA

Introduction:

Most cells successfully control gene expression by an evolutionary regulatory mechanism called RNA interference (RNAi). After binding with certain nucleotide sequences, RNAi may properly inhibit or degrade mRNA transcripts, eventually impeding translation into proteins. The biological process that regulates gene expression and silences genes is widely recognized. Additionally, by affecting translation, RNAi can increase gene expression. There are two types of small ribonucleic acid (RNA) molecules that are employed in RNA interference: micro-RNA (miRNA) and small interfering RNA (siRNA) (Bernstein et al., 2001; Hammond et al., 2000).

Mechanism of RNA interference:

Double-stranded RNA (dsRNA) encourages the destruction of a target mRNA through a process known as RNA interference (RNAi) [Refer Figure 1]. Endonuclease Dicer, which is unique to dsRNA, breaks lengthy dsRNA into smaller interfering RNAs (siRNAs). A crucial stage in the RNA interference (RNAi) process is the creation of the RNA-induced silencing complex, or RISC. RISC first detects a double-stranded siRNA, but in the final functional ribonucleoprotein complex, only one strand is retained. After being removed during assembly, the non-incorporated or "passenger" strand is probably going to deteriorate. Messenger RNA (mRNA) transcripts that are complementary to one another are recognized by RISC using the single strand of RNA as a template (Matranga et al., 2005; Hannon et al., 2002; Pratt et al., 2009).

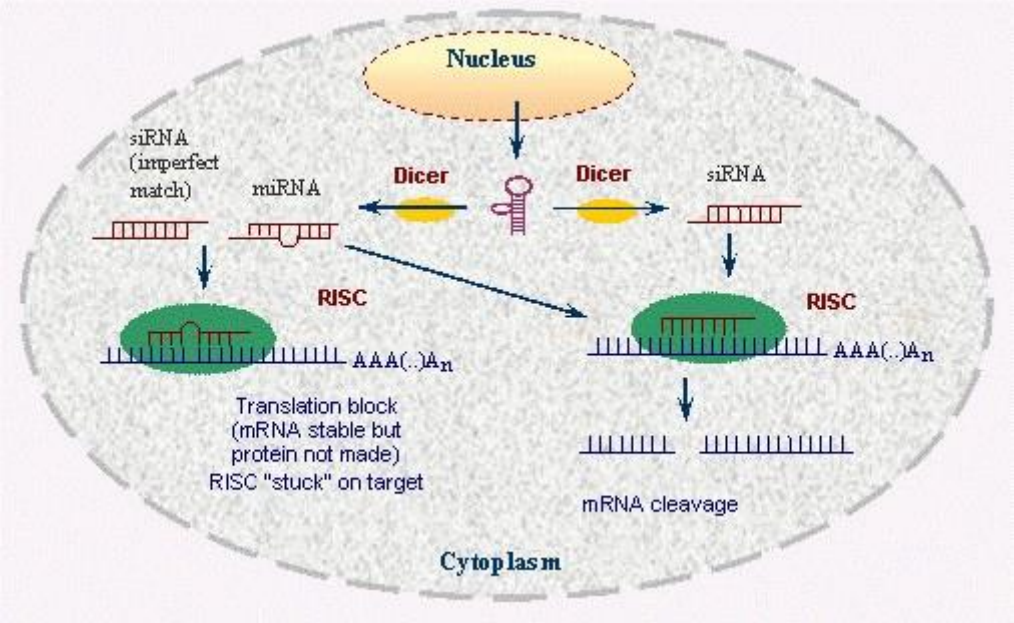

Figure 1 Schematic representation of RNA interference (As represented in https://www.ncbi.nlm.nih.gov/probe/docs/techrnai/)

One of the RISC proteins, Argonaute, activates and cleaves the mRNA upon its discovery. Ten nucleotides upstream of the 5-most site of the siRNA-target mRNA duplex is where the target mRNA cleavage starts. Members of the Argonaute family have been linked to post-transcriptional silencing mechanisms, while the exact composition of RISC remains unknown. The RNAi machinery relies heavily on argonaute proteins, which bind with a variety of short RNAs to perform effector tasks. Argonaute proteins attach to microRNAs (miRNAs) or small interfering RNAs (siRNAs) to inhibit the expression of genes via siRNA.MiRNA-mediated post-transcriptional repression, which encompasses translational inhibition and/or mRNA destruction, or directed cleavage of the target mRNA transcript (Matranga et al., 2005; Hannon et al., 2002; Pratt et al., 2009).

Role of RNAi in prevention of viral infection:

When it comes to controlling gene expression and innate antiviral immune responses, RNA interference is essential. It is thought to be a naturally occurring defensive system against exogenous viral invasion and mobile endogenous transposons. The first siRNA with shown efficacy in humans was ALN-RSV01, a 19 bp RNA duplex with two (2'-deoxy) thymidine overhangs on both 3 ′ ends to prevent nuclease breakdown. ALN-RSV01 focuses on a highly conserved region found in the mRNA of the RSV nucleocapsid protein. Additionally, in Phase 2 randomized, double-blind, placebo-controlled studies, ALN-RSV01 was demonstrated to lower the incidence of bronchiolitis obliterans syndrome in lung transplant patients infected with RSV (Levanova et al., 2018; DeVincenzo et al., 2010; Gottlieb et al., 2016). Additionally, eight siRNA formulations that combat the hepatitis B virus (HBV) have been given consideration for human studies. Two synthetic siRNAs that target the common area at the 3′-end of all HBV transcripts from episomal HBV DNA made up the first-generation anti-HBV siRNA pool, ARC-520. Because the siRNAs were coupled to cholesterol, it is easier for cells to absorb them and prevents serum RNAses from breaking them down. These conjugates were co-injected intravenously with a polymer-based system consisting of N-acetylgalactoseamine, which is responsible for

hepatocyte-specific delivery via the highly expressed asialoglycoprotein receptor on the surface of hepatocytes, and amphipathic membrane active peptide, which is necessary for endosome escape (Levanova et al., 2018; Nair et al., 2014; Wooddell et al., 2013). Anti siRNA formulations have been developed and using moderately and he same has been given in Table 1.

Table1 Antiviral siRNAs in clinical trials (Levanova et al., 2018; Wooddell et al., 2017; Symonds et al., 2016)

Target virus	**Drug details**
Human immuno-deficiency virus (HIV-1)	A combination of the shRNA for downregulation of CCR5 and the HIV-1 fusion inhibitor, C46
	rHIV7-shi-TAR-CCR5RZ is a concoction of anti-CCR5 ribozyme, trans-activation response RNA (TAR) decoy, and shRNAs directed against the tat/rev common exon.
Hepatitis C virus (HCV)	TT-034, three shRNAs targeted to HCV genome
Zaire ebolavirus (ZEBOV)	TKM-100802 (TKM-EBOV-002). two siRNAs to target regionsof the viral polymerase Land VP35
	TKM-130803 is a combination of siRNA, but in order to guarantee specificity to the West African Makona strain of EBOV, anti-L siRNA has one nucleotide alteration and anti-VP35 siRNA has two.
Respiratory syncytial virus (RSV)	ALN-RSV01, a single site siRNA targeted to nucleocapsid gene
Hepatitis B virus (HBV)	NUC B1000 has four shRNAs: three are complementary to the gene encoding the hepatitis B surface antigen (HBsAg), and one is directed towards the polymerase gene.
	ARC-520, 2 synthetic siRNAs mapping to the common regionat the 3' end of all HBV transcripts from episomal DNA

However, the integration of the HBV genome into the host DNA resulted in the loss of the ARC-520 target sites, which is why some patients responded to ARC-520 therapy very somewhat, according to results from phase II clinical trials. Thus, the subsequent formulation (ARC521) contained siRNA targeting viral mRNA produced from the integrated HBV genome in addition to previously verified siRNA sequences (Levanova et al., 2018; Wooddell et al., 2017; Wooddell et al., 2018). Viral infections caused by the human immunodeficiency virus (HIV) and the hepatitis C virus (HCV) may be treated by RNA interference. HCV replicon activity was suppressed in cell culture by short inhibitory RNAs that targeted the internal ribosome entry site (IRES) and non-structural proteins NS3 and NS5b that expressed mRNAs. Anti-HCV siRNAs have also been demonstrated to "cure" Huh-7.5 cells that regularly replicate HCV replicons (Eastman et al., 2002; Wilson et al., 2003). Perhaps because the HIV life cycle and gene expression pattern are well known, HIV was the first infectious agent to be targeted using RNA interference (RNAi). Synthetic and expressed siRNAs have been used to target a range of early and late HIV-encoded RNAs, including as the TAR element, tat, rev, gag, env, vif, nef, and reverse transcriptase. RNA interference has also been shown to efficiently down-regulate cellular cofactors such as NFjb, the HIV receptor CD4, and co-receptors CXCR4 and CCR5, which has led to the inhibition of HIV propagation. Moreover, a range of human cell lines and primary cells, such as T lymphocytes and macrophages generated from hematopoietic stem cells, have shown inhibition of HIV replication (Ambesajir et al., 2012; Jacque et al., 2002).
However, challenges include delivery difficulties and potential toxicity. Despite these advances, there are still significant hurdles to overcome for widespread therapeutic application of siRNAs. HIV was the first infectious agent targeted by RNAi, and siRNAs have been used to target early and late HIV- encoded RNAs, cellular cofactors, and co-receptors.

Implementation of RNAi therapy in cardiovascular disease treatment:

For the first time, the high efficacy of an RNAi therapeutic strategy in a cardiac disease has been demonstrated by the combination of a highly efficient cardiotropic RNAi vector to silence the cardiac-expressed regulatory protein phospholamban and a cardiotropic vector system based on adeno-associated virus (AAV). While C-C chemokine receptor type 2 is silenced by a very efficient cardiotropic RNAi vector, C-C chemokine receptor type 2 is silenced by synthetic siRNA encapsulated in nanoparticles, which lowers Ly-6C monocyte

recruitment, inflammation, and left ventricular remodeling during myocardial infarction (Suckau et al., 2009; Majmudar et al., 2013).

Table 2 Future Directions in Cardiovascular RNA Interference (Suckau et al., 2009; Poller et al., 2013)

Cardiac-targeted RNAi	Cardiotropic AAV9-based phospholamban-based Ca^{2+} cycle regulator silencing for intravenous therapy of severe heart failure
Monocyte-targeted RNAi	Myocardial infarction-related suppression of the monocytic chemokine receptor CCR2 using synthetic siRNA encapsulated in nanoparticles
RNAi imaging in vivo	A promoter comprising a hypoxia response element and a PHD2-shRNA sequence are what drive the firefly luciferase reporter gene.
Allele-specific RNAi	Treatment of human-induced pluripotency stem cell cardiomyocytes using allele-specific RNA interference
Plaque stabilization by RNAi	Lentivirus-based RNAi to silence chymase increased plaque stability in atherosclerosis in vivo

Moreover, RNA interference has been applied in tandem with cardiovascular stem cell treatment. Poor stem cell survival in the sick milieu is one of this approach's drawbacks. However, transplanted adipose-derived stem cells in the infarcted myocardium showed improved survival and paracrine activity after RNAi-based prolyl hydroxylase domain protein 2 suppression. In human-induced pluripotency stem cell cardiomyocytes, allele-specific RNA interference (RNAi) restored the long-QT syndrome phenotype, offering a potential therapeutic option for the treatment of several autosomal-dominant-negative illnesses. RNA interference (RNAi) has been utilized in heart transplantation to establish alloimmune tolerance by squelching MyD88, a toll-like receptor adaptor, and TIR-domain-containing adapter-inducing interferon-β. The utilization of Adeno-associated virus (AAV) technology has demonstrated great efficacy in RNAi therapeutic options for cardiac diseases. Phospholamban is silenced by a highly efficient cardiotropic RNAi vector, whereas C-C chemokine receptor type 2 is silenced by synthetic siRNA encapsulated in nanoparticles. This reduces Ly-6C monocyte recruitment, inflammation, and left ventricular remodelling during myocardial infarction (Poller et al., 2013; Wang et al., 2013)

Conclusion and Future prospects:

Although RNAi is presently undergoing clinical trials, significant obstacles including toxicity, off-target effects, and risky delivery methods need to be overcome before RNAi can be considered a conventional treatment. RNA interference (RNAi) treatment is often judged by its in vitro and in vivo efficacy, specificity of silencing effects, and absence of toxicity. A risk-benefit analysis should be carried out if RNA interference is to be used therapeutically. The possible hazards and advantages should be compared. Additionally, as was already said, clinical trials and delivery methods are still in the early phases of study and are far from finished. It is impossible to foretell what RNAi therapies may include in the future given the rapid advancements in creativity and technology.

Reference

1. Bernstein, E., Caudy, A. A., Hammond, S. M., & Hannon, G. J. (2001). Role for a bidentate ribonuclease in the initiation step of RNA interference. *_Nature, 409_*(6818), 363-366.
2. Hammond, S. M., Bernstein, E., Beach, D., & Hannon, G. J. (2000). An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. *_Nature, 404_*(6775), 293-296.
3. Matranga, C., Tomari, Y., Shin, C., Bartel, D. P., & Zamore, P. D. (2005). Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes. *_Cell, 123_*(4), 607-620.
4. Hannon, G. J. (2002). RNA interference. *_Nature, 418_*(6894), 244-251.
5. Pratt, A. J., & MacRae, I. J. (2009). The RNA-induced silencing complex: a versatile gene-silencing machine. *_Journal of Biological Chemistry, 284_*(27), 17897-17901.
6. Levanova, A., & Poranen, M. M. (2018). RNA interference as a prospective tool for the control of human viral infections. *_Frontiers in Microbiology, 9_*, 2151.

7. DeVincenzo, J., Lambkin-Williams, R., Wilkinson, T., Cehelsky, J., Nochur, S., Walsh, E., ... & Vaishnaw, A. (2010). A randomized, double-blind, placebo-controlled study of an RNAi-based therapy directed against respiratory syncytial virus. **Proceedings of the National Academy of Sciences, 107**(19), 8800-8805.
8. Gottlieb, J., Zamora, M. R., Hodges, T., Musk, A., Sommerwerk, U., Dilling, D., ... & Vaishnaw, A. (2016). ALN-RSV01 for prevention of bronchiolitis obliterans syndrome after respiratory syncytial virus infection in lung transplant recipients. **The Journal of Heart and Lung Transplantation, 35**(2), 213-221.
9. Nair, J. K., Willoughby, J. L., Chan, A., Charisse, K., Alam, M. R., Wang, Q., ... & Milstein, S. (2014). Multivalent N-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing. **Journal of the American Chemical Society, 136**(49), 16958-16961.
10. Wooddell, C. I., Rozema, D. B., Hossbach, M., John, M., Hamilton, H. L., Chu, Q., ... & Oropeza, C. E. (2013). Hepatocyte-targeted RNAi therapeutics for the treatment of chronic hepatitis B virus infection. **Molecular Therapy, 21**(5), 973-985.
11. Wooddell, C. I., Yuen, M. F., Chan, H. L. Y., Gish, R. G., Locarnini, S. A., Chavez, D., ... & Kanner, S. B. (2017). RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg. **Science Translational Medicine, 9**(409), eaan0241.
12. Symonds, G., Bartlett, J. S., Kiem, H. P., Tsie, M., & Breton, L. (2016). Cell-delivered entry inhibitors for HIV-1: CCR5 downregulation and blocking virus/membrane fusion in defending the host cell population. **AIDS Patient Care and STDs, 30**(12), 545-550.
13. Wooddell, C., Zhu, R., Hamilton, H., Chu, Q., Sternard, H., Schumacher, J., ... & Given, B. (2018). Development of subcutaneously administered RNAi therapeutic ARO-HBV for chronic hepatitis B virus infection. **Journal of Hepatology, 68**(1), S18-S19.
14. Eastman, S. J., Baskin, K. M., Hodges, B. L., Chu, Q., Gates, A., Dreusicke, R., ... & Scheule, R. K. (2002). Development of catheter-based procedures for transducing the isolated rabbit liver with plasmid DNA. **Human Gene Therapy, 13**(17), 2065-2077.
15. Wilson, J. A., Jayasena, S., Khvorova, A., Sabatinos, S., Rodrigue-Gervais, I. G., Arya, S., ... & Richardson, C. D. (2003). RNA interference blocks gene expression and RNA synthesis from hepatitis C replicons propagated in human liver cells. **Proceedings of the National Academy of Sciences, 100**(5), 2783-2788.
16. Ambesajir, A., Kaushik, A., Kaushik, J. J., & Petros, S. T. (2012). RNA interference: A futuristic tool and its therapeutic applications. **Saudi Journal of Biological Sciences, 19**(4), 395-403.
17. Jacque, J.-M., Triques, K., & Stevenson, M. (2002). Modulation of HIV-1 replication by RNA interference. **Nature, 418**(6896), 435-438.
18. Suckau, L., Fechner, H., Chemaly, E., Krohn, S., Hadri, L., Kockskämper, J., ... & Wang, X. (2009). Long-term cardiac-targeted RNA interference for the treatment of heart failure restores cardiac function and reduces pathological hypertrophy. **Circulation, 119**(9), 1241-1252.
19. Majmudar, M. D., Keliher, E. J., Heidt, T., Leuschner, F., Truelove, J., Sena, B. F., ... & Wojtkiewicz, G. (2013). Monocyte-directed RNAi targeting CCR2 improves infarct healing in atherosclerosis-prone mice. **Circulation, 127**(20), 2038-2046.
20. Poller, W., Tank, J., & Skurk, C. (2013). Cardiovascular RNA interference therapy: the broadening tool and target spectrum. **Circulation Research, 113**(5), 588-602.
21. Wang, W. E., Yang, D., Li, L., Wang, W., Peng, Y., Chen, C., ... & Jiang, J. (2013). Prolyl hydroxylase domain protein 2 silencing enhances the survival and paracrine function of transplanted adipose-derived stem cells in infarcted myocardium. **Circulation Research, 113**(3), 288-300.