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CONCEPT OF RNA SILENCING AND APPLICATIONS OF SIRNA IN HEALTHCARE

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ABSTRACT

After the Human Genome Project (HGP), scientists are able to decode the genetic roots of diseases such as cancer and rheumatoid arthritis; using new generation gene-sequencing technology. Now it is possible to reach the potential root of genetic diseases and gain more insight about the genetic component of the disease and its underlying causes. By using this information, it is possible to devise drugs which have the potential to act on these underlying disease causing genes; it paved the way for discovery of new technologies based on RNA and its potential versatile forms. RNA based therapeutics are categorized according to their mechanism of action, which involves and catalytically active molecules that bind to other proteins RNAi (RNA interference) and mRNA translation inhibitors (antisense). Researchers are working on developing vaccines that use RNA encoded proteins from pathogens such as influenza, rabies, or Zika virus. The new mRNA will translate the sequenced strands into protein, stimulating the immune system to produce a specific immune response to the exact strain of the pathogen. RNAi was the Nobel Prize-winning discovery in 2006, opening the door to a plethora of new and exciting therapies. The discovery of Mello and colleagues, which states the potential use of dsRNA (double stranded RNA) in degradation of target mRNA sequences in C.elegans and mammalian cells, has brought RNAi into the spotlight. RNA silencing can be mediated by siRNA (small interfering RNA) or miRNA (micro RNA); they are handled by the cellular enzyme Dicer for processing before being integrated into the RISC (RNA-induced silencing complex complex). The primary distinction between miRNA and siRNA is that miRNA can act on multiple mRNA targets whereas siRNA can only act on one mRNA target, which is very specific in targeting a mRNA molecule where the gene expression is to be silenced.

Key words: RNA Silencing, Gene knockdown, RNA interference, RNAi therapeutics, siRNA, RISC, dsRNA, miRNA, mRNA molecule target, RNA splicing.

INTRODUCTION

New insights into DNA and RNA are enabling a wide range of emerging new technologies,

allowing us to comprehend the diversity of these dynamic structures and functions. The most abundant nucleic acids in nature are ribonucleic acids, which can be found as oligonucleotides or polynucleotides. RNAs are involved in all levels of life regulation and are very versatile molecules that can store genetic information as well as exhibit enzymatic activities [1]. A variety of naturally occurring chemical modifications enable the diverse activities of RNAs: such as conjugation of other small molecules to RNA, methylation of the 2'-hydroxy ribose backbone or the nucleotide bases, formation of alternative bases via isomerization, reduction, and sulphur substitution for oxygen. These modifications broaden the biological role of RNA, including stem cell differentiation and developmental processes. These modifications are carried out enzymatically and removed at the specific site [2].

Sharp [3] discovered that the longer RNA sequences were made shorter by splicing mechanism (RNA splicing; split genes), resulting in a shorter version that encodes only proteins, introns, or intervening sequences in cellular genes. Then, in 1998, Fire and Mello discovered something revolutionary: introducing double-stranded RNA molecules into *Caenorhabditis elegans* cells; possibly blocks gene expression [4]. This discovery paved the way for future therapeutic approaches because it was a pivotal point of basic research in studying gene expression and how RNAs could play an active role in regulating gene expression. It also increased the possibility of understanding gene function by inhibiting gene expression and studying the effects and functions of the gene in question [5]. RNA therapeutics is the use of oligonucleotides to directly target RNA for therapeutic purposes and gene function analysis. The two primary mechanisms for targeting RNA are antisense oligonucleotides (ASO) and double-stranded RNA-mediated interference (RNAi) [6]. Antisense oligonucleotides bind to their target sequence via Watson-Crick base pairing hybridization, to a specific mRNA, and inhibit its expression, or alter gene expression via steric hindrance, splicing alterations, or target degradation. As a result, it prevents the genetic transfer from DNA to protein [7]. RNA interference works by activating ribonucleases and other enzymes and complexes on specific sequences post-transcriptionally. These complexes bind in coordinately and aid in the degradation of RNA after the original RNA target has been split into pieces [8].

The RNAi mechanism used by small interfering RNA (siRNA) to selectively silence target genes can affect gene expression and regulate gene activity by cleaving or inhibiting the translation of mRNA. . The discovery that siRNAs (21–23 nucleotide RNA duplexes) can mediate RNAi in mammalian cells paved the way for siRNAs to be used therapeutically [9]. SiRNA-based therapeutics have finally entered the healthcare market two decades after the discovery of RNAi.

The first siRNA-based medication, ONPATTRO (patisiran), was approved by the European Commission (EC) and the US Food and Drug Administration (FDA) in 2018 for the treatment of adults with hereditary amyloidogenic transthyretin (hATTR) amyloidosis with polyneuropathy [10].

MATERIAL AND METHODS

RNA Silencing - An overview

RNA silencing is an evolutionary mechanism that targets and degrades exogenous or endogenous RNA molecules in almost all eukaryotic organisms [11]. The first instance of RNA silencing in plants occurred when the addition of copies of the flower pigmentation gene *Chalcone Synthase-A* (CHS) associated with anthocyanin synthesis in petunias was expected to result in coloration but instead resulted in the suppression of the transgene and the endogenous RNA [12]. Plants exposed to chalcone synthase were either completely white or pale, whereas transgenic plants did not exhibit

either of these phenotypes. This was later discovered to be the result of gene suppression, and the phenomenon was dubbed "co-suprression" by Jorgensen et al. [13]. Exogenously inserted genes in the genus *Neurospora crassa* inhibited the expression of endogenous genes with homologous sequences, Romano and Macino discovered in 1992. The researchers dubbed this phenomenon "gene suppression" [14]. In a 1998 study of gene silencing in C. elegans, Fire and Mello [4] administered sense, antisense, and dsRNA into C. elegans, respectively. They discovered that dsRNA was far more effective at gene silencing than either strand alone. This mechanism, termed as "RNA interference" (also known as RNAi), won the 2006 Nobel Prize in Physiology or Medicine. In 2000, using Drosophila extracts, two groups of biochemists independently found that 21-23 nucleotide long RNA was co-purified with RNAi constituents [15]. Tuschl et al. developed short interfering ribonucleic acids (siRNAs) with 2-3 nucleotide overhangs on their 3' ends and 21–22 nucleotide double-stranded RNAs. [16]. The ability of these constructs to silence endogenous genes in mammalian cells guided the way of siRNA-mediated therapeutics.

RNA Silencing - Mechanism of Action

The RNA silencing has been studied most thoroughly in Drosophila embryo extracts, and it was defined as a two-step reaction with initiation and effector phases. The initiation stage is defined by the endonucleolytic cleavage of longer dsRNA molecules into siRNA or miRNA species. These small RNAs are 21-26 bp in length and have 2 nucleotide 3'- overhangs and 5'- phosphorylated termini, which are typical of RNase-III-type enzymes from the Drosha/Dicer protein family [17]. MiRNA molecules contain mismatches, bulges, or G:U wobble base pairs, whereas siRNAs are formed from perfectly complementary dsRNA duplexes [18]. One strand of the siRNA or miRNA duplex is loaded onto the RNA induced silencing complex (RISC) in the effector stage of the RNA silencing pathway for sequence-specific recognition of target RNAs. [19]. The Argonaute (AGO) protein family's enzymatic activity causes the preassembled RISC to cut complementary mRNAs or halt their translation [20].

A schematic outline of these RNA silencing pathways is shown-

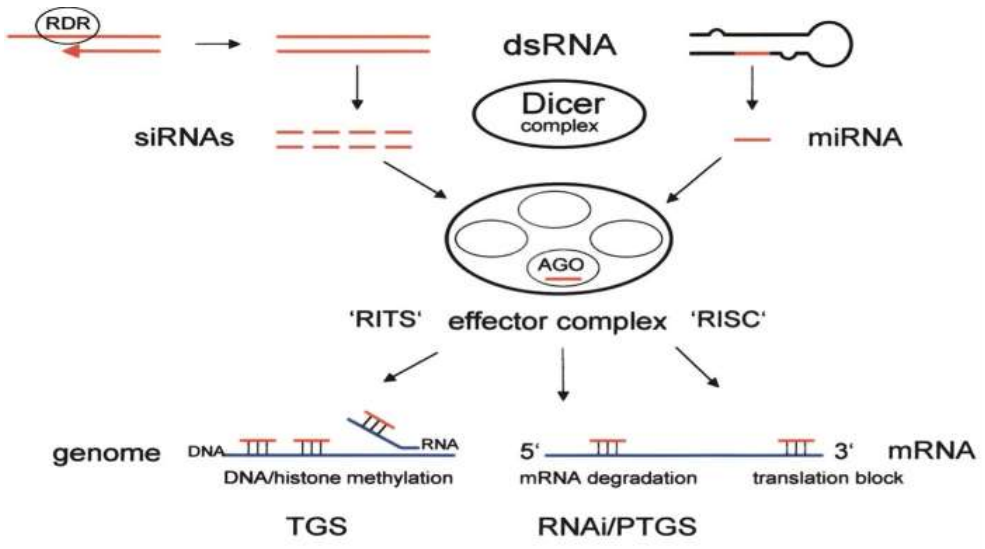

Figure 1: RNA silencing pathways- ssRNA double-stranded by RDR, nature dsRNA, or pre-miRNA could serve as trigger RNA. DICERs convert trigger RNAs into small RNAs. Small RNAs are loaded into effector complexes (RISC) that perform various functions. RISCs play a role in

transcriptional silencing [21]

Pathway for siRNA silencing mechanism -

In this pathway of siRNA induced RNA silencing the DICER cleaves long, double-stranded RNA molecules to produce siRNAs. The RISC complex grabs siRNA and selectively adds the antisense strand while discarding the sense strand. Argonaute 2 cleaves the mRNA transcript after the antisense strand binds to it. The active RISC complex is then subjected to several rounds of mRNA silencing.

The RISC loading complex consists of three parts which are proteins in nature:

(1) Dicer,

(2) trans-activation response RNA binding protein (TRBP), and

(3) Argonaute 2. (Ago2).

For siRNA silencing mechanism DICER and TRBP are the two proteins required for the initiation process [22]. The RNAi process is initiated when the RNase III enzyme Dicer recognises foreign double-stranded RNA and catalytically fragments it. Dicer is made up of a number of domains, including two nearby RNase III domains, a dsRNA-binding domain (dsRBD), a DExD/H ATPase domain, a DUF283 domain, and a PAZ domain [23]. The PAZ domain interacts with the active sites of the RNase III domains by binding to the terminals of the RNA substrate. RNase III domains break the duplex as the substrate gets close to the processing core, leaving 2-nt 3'-overhangs that can be used to create siRNAs. The C4 domain, dsRBD1, and dsRBD2 are the three structural domains of TRBP. The C4 domain is responsible for protein-protein interactions, whereas the dsRBD domains bind to an RNA substrate. The C4 domain of the RISC complex binds to Dicer's ATPase/helicase domain, strengthening TRBP-Dicer interactions [24]. Dicer is left to bind to the opposite end of the siRNA after the TRBP protein has bound to one end. As a result, one strand of the duplex is designated as the antisense strand and the other as the sense strand [25]. When RISC selects the antisense strand, a third protein called Argonaute cleaves the sense strand between positions 9 and 10, counting from the 5' end, to form the active RISC complex [26]. The PAZ domain, the PIWI domain, the N domain, and the Mid domain are the four aspects that distinguish Argonaute proteins [27]. Active RISC is thought to process mRNA in the same manner that it processes siRNA's sense strand. The phosphodiester bond within mRNA is cleaved between the nucleotides that are base-paired to the 10th and 11th positions of the antisense siRNA strand, counting from the 5'-end, after the antisense strand within the active RISC complex binds to its complementary target [23].

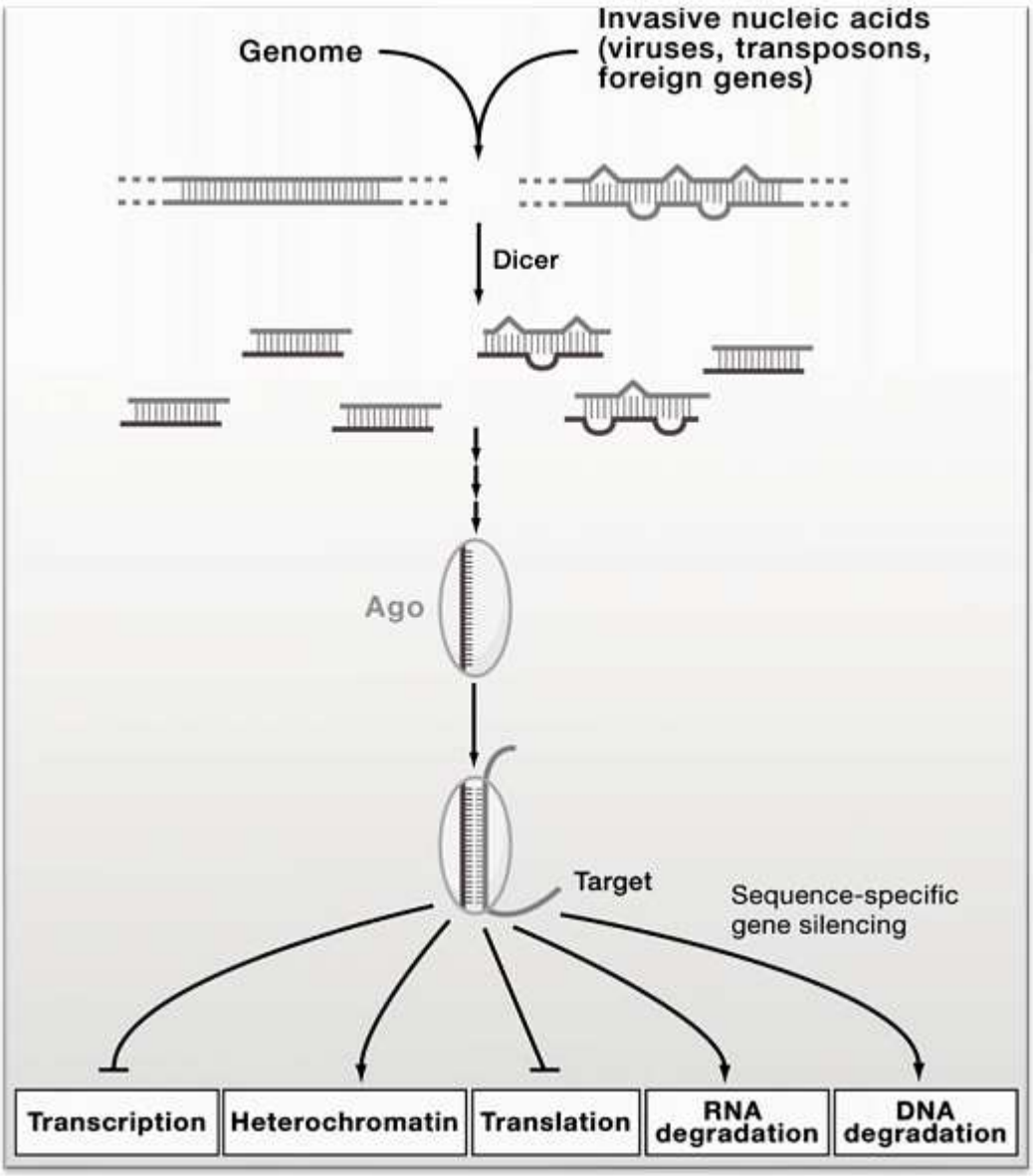

Figure - 2 - siRNA pathway. Dicer is a protein that degrades double-stranded RNA precursors into short fragments (20–30 nt). One strand of the processed duplex is loaded into an Argonaute protein, which recognises target RNA using Watson-Crick base pairing. Once the target has been identified, one of several mechanisms modulates its expression [23]

Applications of siRNA -

siRNA-based therapeutics are not only an intriguing new tool in molecular biology; they are also the next frontier in molecular medicine. The future of personalized medicine is siRNA-based therapeutics, where the amalgamation of biotechnology and the pharmaceutical industry has paved the way to try to manufacture drugs that can subsequently curb genetic or heritable illness caused by mutations.

Cancers frequently have upregulated or misappropriated genes, resulting in uncontrolled cell proliferation. Because of their specificity, siRNAs can be used as specific chemotherapeutic agents

to fight cancer. The following are some of the siRNA targets used in chemotherapeutic treatments [28-29]

Cancer	*siRNA target*
CML	BCR/ABL fusion protein
Leukemia	c-raf, bcl-2
Cervical carcinoma	E6, E7 (HPV)
Pancreatic carcinoma	$K\text{-}RAS^{V12}$
Melanoma	ATF2
	$BRAF^{V599E}$
Ovarian carcinoma	H-Ras
	mVEGF
	COX-2
Prostate cancer	P110α, p110B of PI 3 kinase
Wilms' tumor	Wt1, Pax2, Wnt4

Nanosized particles are being developed by researchers to improve the delivery of siRNA-based therapeutics. These nanoparticles are designed to avoid one or more of the obstacles that siRNA encounters on its way to the cytosol. When dealing with dose-dependent toxicity, siRNA distribution via tissue engineering matrices may increase the number of cells that exhibit mRNA knockdown while decreasing the amount of vector used [30].

Hereditary variation transthyretin amyloidosis (ATTRv) is a rare genetic abnormality that generates amyloidosis as a result of misfolding of mutant transthyretin (TTR) protein fibrils. Patisiran is a one-of-a-kind medicine that is a liposomal siRNA against TTR that targets this protein selectively, lowering TTR accumulation in tissues and improving neuropathy and heart function. Patisiran (ONPATTRO; Alnylam Pharmaceuticals Inc.) is a siRNA-based oligonucleotide that was approved in August 2018 by the US Food and Drug Administration (FDA) and the European Commission (EC) for the treatment of ATTRv polyneuropathy in adults [31]. It is a double-stranded siRNA that inhibits TTR synthesis in ATTRv, causing it to misfold and aggregate as insoluble amyloid fibrils. The drug is formulated as a lipid nanoparticle for delivery to hepatocytes, which are the primary site of TTR production [32].

Alnylam Pharmaceuticals is developing givosiran for the treatment of acute hepatic porphyria (AHP). Givosiran (GivlaariTM) is a covalently coupled small interfering RNA (siRNA) directed at aminolevulinate synthase 1 (ALAS1) that is delivered to hepatocytes. This results in the downregulation of ALAS1 mRNA, which prevents the buildup of neurotoxic -aminolevulinic acid and porphobilinogen levels, which are linked to acute porphyria attacks. Based on the positive results of the international, phase III ENVISION trial, givosiran was approved for the treatment of AHP in the United States in November 2019 [33].

Alnylam Pharmaceuticals developed lumasiran (OxlumoTM), a subcutaneously administered small interfering RNA (siRNA) that targets the mRNA for the hydroxyacid oxidase 1 gene (HAO1; encodes glycolate oxidase), for the treatment of primary hyperoxaluria type 1. (PH1). Lumasiran depletes glycolate oxidase by suppressing the glycolate oxidase gene, reducing the production of oxalate, the toxic molecule directly linked to the clinical symptoms of PH1. Lumasiran was approved in the United States for the treatment of PH1 in adults and children on November 23,

2020 [34].

CONCLUSION

RNAi therapeutic research is a rapidly evolving field, and much knowledge on RNAi has been acquired since the mechanisms of RNAi were discovered in 1998. Silence is golden, as the saying goes, and siRNA-based medications have provided a ray of hope for treating hereditary disease. Before these treatments are considered standard medicines, it is critical that the ethical implications of RNAi be investigated. The main issue with such RNAi-based drugs right now is the cost, which will be reduced by mass manufacturing of components. The side effects of such siRNA-based drugs could be easily monitored and help to treat the patient, no such life-threatening alterations are so recorded in any of the clinical trials. With rapid advancement in the clinical phases one can consider more such RNAi mediated drugs for very specific genetic disorders. Keeping in mind about the general population and the advancement of global health one should consider the allocation of such drug costs and the measurable ways to make it available for the healthcare market at affordable prices.

REFERENCE

[1] Alberts B, J. A., Lewis J, et al., The RNA World and the Origins of Life. In Molecular Biology of the Cell. 4th edition., Garland Science: New York, **2002**.

[2] Ontiveros RJ, Stoute J, Liu KF. The chemical diversity of RNA modifications. *Biochemical Journal*. **2019** Apr 30;476(8):1227-45.

[3] Sharp PA. Split genes and RNA splicing. Cell. 1994 Jun 17;77(6):805-15.

[4] Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. nature. **1998** Feb;391(6669):806-11.

[5] Corey DR. Chemical modification: the key to clinical application of RNA interference? The *Journal of clinical investigation*. **2007** Dec 3;117(12):3615-22.

[6] Chery J. RNA therapeutics: RNAi and antisense mechanisms and clinical applications. *Postdoc journal: a journal of postdoctoral research and postdoctoral affairs*. **2016** Jul;4(7):35.

[7] Dias N, Stein CA. Antisense oligonucleotides: basic concepts and mechanisms. Molecular cancer therapeutics. **2002** Mar 1;1(5):347-55.

[8] Agrawal N, Dasaradhi PV, Mohmmed A, Malhotra P, Bhatnagar RK, Mukherjee SK. RNA interference: biology, mechanism, and applications. Microbiology and molecular biology reviews. **2003** Dec 1;67(4):657-85

[9] Ryther RC, Flynt AS, Phillips J3, Patton JG. siRNA therapeutics: big potential from small RNAs. Gene therapy. **2005** Jan;12(1):5-11.

[10] Weng Y, Xiao H, Zhang J, Liang XJ, Huang Y. RNAi therapeutic and its innovative biotechnological evolution. Biotechnology advances. **2019** Sep 1;37(5):801-25.

[11] Sontheimer EJ. Assembly and function of RNA silencing complexes. Nature Reviews Molecular Cell Biology. **2005** Feb;6(2):127-38.

[12] Van Der Krol AR, Mur LA, de Lange P, Mol JN, Stuitje AR. Inhibition of flower pigmentation by antisense CHS genes: promoter and minimal sequence requirements for the antisense effect. Plant molecular biology. **1990** Apr;14(4):457-66.

[13] Napoli C, Lemieux C, Jorgensen R. Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. The plant cell. **1990** Apr 1;2(4):279-89.

[14] Romano N, Macino G. Quelling: transient inactivation of gene expression in Neurospora crassa by transformation with homologous sequences. Molecular microbiology. **1992** Nov;6(22):3343-53.

[15] Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature. **2000** Mar;404(6775):293-6.

[16] Zamore PD, Tuschl T, Sharp PA, Bartel DP. RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. Cell. **2000** Mar 31;101(1):25-33.

[17] Hutvágner G, McLachlan J, Pasquinelli AE, Bálint É, Tuschl T, Zamore PD. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science. **2001** Aug 3;293(5531):834-8.

[18] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. cell. **2004** Jan 23;116(2):281-97.

[19] Tomari Y, Zamore PD. Perspective: machines for RNAi. Genes & development. **2005** Mar 1;19(5):517-29.

[20] Bernstein E, Caudy AA, Hammond SM, Hannon GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature. **2001** Jan;409(6818):363-6.

[21] A Matzke, Marjori; J. M Matzke, Antonius (**2015**): RNA-Mediated Silencing. PLOS Biology. Figure. https://doi.org/10.1371/journal.pbio.0020133.g001.

[22] Daniels SM, Gatignol A. The multiple functions of TRBP, at the hub of cell responses to viruses, stress, and cancer. Microbiology and Molecular Biology Reviews. **2012** Sep 1;76(3):652-66.

[23] Carthew, R. and Sontheimer, E. (**2009**). Origins and Mechanisms of miRNAs and siRNAs. Cell. 136: 642-655.

[24] Gredell JA, Dittmer MJ, Wu M, Chan C, Walton SP. Recognition of siRNA asymmetry by TAR RNA binding protein. Biochemistry. **2010** Apr 13;49(14):3148-55.

[25] Vukovic L, Koh HR, Myong S, Schulten K. Substrate recognition and specificity of double-stranded RNA binding proteins. Biochemistry. **2014** Jun 3;53(21):3457-66.

[26] Leuschner PJ, Ameres SL, Kueng S, Martinez J. Cleavage of the siRNA passenger strand during RISC assembly in human cells. EMBO reports. **2006** Mar;7(3):314-20.

[27] Wang Y, Juranek S, Li H, Sheng G, Tuschl T, Patel DJ. Structure of an argonaute silencing complex with a seed-containing guide DNA and target RNA duplex. nature. **2008** Dec;456(7224):921-6.

[28] Wilda M, Fuchs U, Wossmann W, Borkhardt A. Killing of leukemic cells with a BCR/ABL fusion gene by RNA interference (RNAi). Oncogene **2002**; 21: 5716–5724.

[29] Ryther RC, Flynt AS, Phillips J3, Patton JG. siRNA therapeutics: big potential from small RNAs. Gene therapy. **2005** Jan;12(1):5-11.

[30] Tokatlian T, Segura T. siRNA applications in nanomedicine. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology. **2010** May;2(3):305-15.

[31] Yang J. Patisiran for the treatment of hereditary transthyretin-mediated amyloidosis. Expert Rev Clin Pharmacol. **2019**;12(2):95–9.

[32] Al Shaer D, Al Musaimi O, Albericio F, de la Torre BG. 2018 FDA tides harvest. Pharmaceuticals (Basel) **2019**;12(2):52. https://doi.org/10.3390/ ph12020052.

[33] Caillaud M, El Madani M, Massaad-Massade L. Small interfering RNA from the lab discovery to patients' recovery. Journal of Controlled Release. **2020** May 10; 321:616-28

[34] Scott LJ, Keam SJ. Lumasiran: first approval. Drugs. **2021** Jan 6:1-6.