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RNAi as a Next Generation Drug

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Abstract: RNA therapeutics are emerging as a revolutionary approach in treating a wide spectrum of diseases by directly targeting underlying genetic and molecular mechanisms. RNA molecules are currently exhibiting unique therapeutic opportunities over small molecule and peptide/protein-based drugs. The versatile RNA molecules can be specifically directed to target disease-causing transcripts which are otherwise difficult to be targeted by small molecules or peptides/proteins. A variety of RNA therapeutics have been approved for medical use by the FDA, they include aptamers, antisense oligonucleotides, interfering RNAs, mRNA therapeutics, and RNA-guided CRISPR-Cas genome editing. Thus, RNA might be the next generation drug to treat a variety of diseases. In this mini-review, we are discussing more on interfering RNAs which have garnered increased attention and significance in recent times.

Introduction

RNA-based therapy has attracted a lot of attention nowadays due to the research advancement in the field of RNA therapeutics and the simplicity in RNA therapeutic's design and development over traditional drug development methods. Small molecule drugs play a pivotal role in treating various diseases and are considered as first-generation drugs while peptides and proteins-based drugs emerged as second-generation drugs due to their high efficacy in treating diseases over small molecule drugs. However, RNA-based drugs are emerging as third-generation drugs as their mode of action is dependent on more specific Watson-Crick base pairing. Thus, the RNA molecules can be screened against a wide range of diseases to treat a variety of diseases such as cancer, hereditary diseases, viral infections, etc[1]. Among different RNA therapeutics such as aptamers, antisense oligonucleotides, interfering RNAs, and mRNA therapeutics, a double stranded synthetic RNAi molecule has garnered significance due to its ability to knock down a particular gene (mRNA). RNAi mechanism is a post-transcriptional silencing (PTGS) mechanism that downregulates gene expression[2]. Therefore, targeting a dysregulated gene before its translation into a disease-causing protein would be smart in mitigating a target disease[3]. It is widely proven that RNAi-based therapy is highly advantageous in treating dysregulated genes, particularly, untreatable diseases by traditional methods like hereditary diseases and cancer. For example, in hereditary diseases, RNAi-based drugs have been used to downregulate the disease-causing genes thereby advancing in the rare diseases management. Therefore, the future of the emerging field of RNAi therapeutics is promising and many clinical trials are undergoing and will be conducted to evaluate their potency and efficacy in the treatment of different diseases[4]. This mini-review mainly focuses on the RNAi mechanism, RNAi-based drugs, current use of RNAi-based drugs in cancer and the future potential of RNAi-based drugs as next-generation drugs.

A. RNAi History and Mechanism

The discovery of the RNAi mechanism won a Nobel Prize in Physiology by Andrew Fire and Craig Mello. RNAi mechanism is a type of defence mechanism of eukaryotic cells from different viral attacks. The RNAi molecules such as siRNA and miRNA are small non-coding double-stranded RNAs with a typical length of 21-27 nucleotides. siRNA is a perfect double-stranded while miRNA is having a bulge in-between the double strand and

they are endogenously present in a cell produced from shRNA. Dicer is an important enzyme, responsible for the processing of shRNA into miRNA[5]. The AGO2 is an enzyme that cleaves the passenger strand from double-stranded siRNA/miRNA and handovers the single-stranded guide strand from either siRNA/miRNA to RNA-induced silencing complex (RISC) as shown in **Figure 1**. The guide strand carried by the RISC complex is called as an activated RISC which is used to silence the target mRNA through mRNA degradation and inhibiting translation[1].

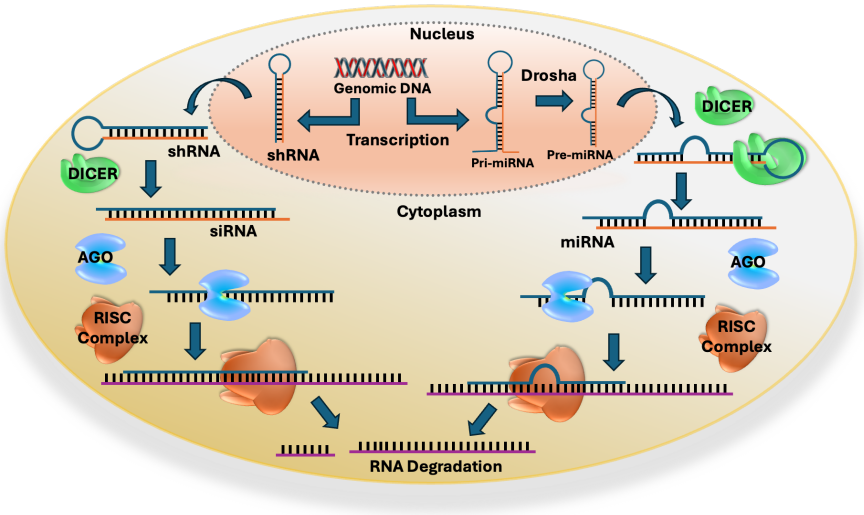

Figure 1. Schematic diagram of RNA interference Mechanism.

B. RNAi as a Next-Generation Drug to Treat a Spectrum of Diseases

Over the past two decades, RNAi has emerged as a powerful biological phenomenon that has revolutionized medicine. By silencing the disease-causing genes, RNAi based therapy have been used in treating various diseases, they include hypercholesterolemia, hereditary diseases, neurodegenerative diseases, cardiovascular diseases, and viral diseases. Various pharmaceutical companies investing billions of dollars in RNAi research due to the ease of the synthesis of the siRNAs/miRNAs chemically. The FDA already approved many RNAi-based-gene drugs namely, Patisirna, Givosiran, Lumasiran, Inclisiran, Nedosiran, and Vutrisiran for the treatment of hereditary transthyretin-mediated amyloidosis, acute hepatic porphyria, primary hyperoxaluria type 1, atherosclerotic cardiovascular disease, primary hyperoxaluria, and hereditary transthyretin-mediated amyloidosis respectively[6]. And many more RNAi drugs under clinical trials at different phases. For example, Alnylam Pharmaceuticals is developing a siRNA-based drug ALN-RSV01 in the treatment of Respiratory syncytial virus (RSV) infection by targeting viral replication, RSV infection is observed in patients with lung transplant are strongly associated with bronchiolitis obliterans syndrome (BOS)[7]**.** Olpasiran is another siRNA-based drug under development against atherosclerotic cardiovascular disease, which reduces the abundance of lipoprotein(a) levels synthesized in the liver[8]. Leachman et al worked on the treatment of an autosomal dominant syndrome pachyonychia congenita (PC) and completed phase Ib clinical trial utilizing siRNA-based drug called TD101 which targets keratin 6a (K6a) N171K mutant mRNA[9]. The neurodegenerative disease hereditary transthyretin amyloidosis can be treated with the RNAi-based drug HELIOS-A by targeting transthyretin (TTR) which is under Phase III clinical trials[10].

Both the siRNA and miRNA have different mechanisms of action on the target gene. For example, the siRNA binds to the intended gene and downregulate the target gene only, whereas miRNA can bind to multiple genes and can influence their gene regulation (*viz.,* gene downregulation or upregulation)[11]. The miRNAs generally control various cellular activities, therefore, the miRNAs expression in a cell is crucial and any dysregulation in the miRNAs expression eventually leads to disease[12, 13]. However, the dysregulation in miRNA expression can be regulated by delivering miRNA mimics exogenously. For example, miRNA delivery is employed in cases when

essential miRNA expression is low and anti-miRNA is delivered to downregulate miRNA expressed more in a cell. Therefore, the complexity in understanding the miRNA expression and its use in therapeutics is still undergoing process and miRNA-based drugs are still in pre-clinical and clinical trials only[14].

C. RNAi in Cancer Treatment

Cancer is a multifactorial complex disease and is challenging to treat. It is characterized by alterations in cell physiological pathways and uncontrollable cell growth. RNAi-based therapy has emerged as a promising therapy for cancer by specifically targeting oncogenes, tumor suppressor genes, and drug-resistant genes. Overexpressed oncogenes and/or drug-resistant genes can be downregulated, and disease can be brought under control using the RNAi mechanism. One such example is the KRAS mutation, causing pancreatic cancer. The pancreatic cancer can be treated using a siRNA (siG12D-LODER) that targets the KRAS G12D gene and the siRNA (siG12D-LODER) is currently under phase II clinical trials. As discussed earlier, the exogenous anti-miRNA delivery can bring down overexpressed miRNA levels which cause cancer. For example, elevated levels of miRNA-21 cause breast cancer, and the miRNA-21 levels can be brought down using anti-miR-21 thus breast cancer can be mitigated[15]. On the other hand, miRNA can influence the expression of many oncogenes, therefore, a combination of both siRNA and miRNA would be a potential approach in treating untreatable cancer. Indeed, the combinational approach has been under study to treat different cancer types. A recent example of the combination therapy of siRNA and miRNA is studied for chronic myeloid leukaemia where the PTPRF interacting protein alpha 1 (PPFIA 1) gene is targeted by both siRNA and miRNA (miR-181a)[16].

D. Conclusion and Future Outlook

The field of RNAi therapeutics is growing day by day, and emerging a revolutionary approach to treat diseases that are difficult to treat. Moreover, the FDA approval and clinical studies of many RNAi drugs reinforce the confidence in the development of new-generation RNAi drugs thus, RNA might become the next-generation drug to treat different types of diseases. However, it is very imperative to address the important questions to advance RNAi therapy. They include i. nuclease degradation; ii. immunogenic reactions of RNAi sequences iii. nucleoside modifications and their impact on mRNA targeting; iv. targeting the disease site *in vivo* and ensuring the intended

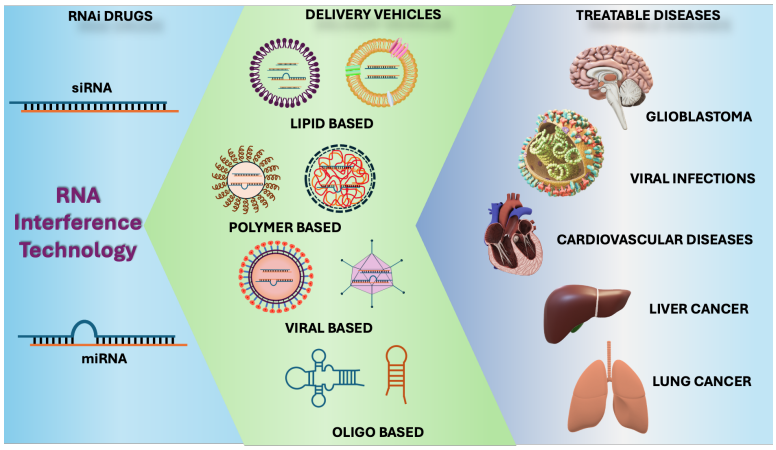

Figure 2. Schematic diagram of RNAi delivery vehicles and treatable diseases.

gene regulation; v. understating the gene expression patterns after RNAi delivery; vi. dosing and rate of cellular uptake *in vivo;* vii. renal clearance; viii. phagocyte uptake; and ix. off-target effects[17]. Though some of the above-mentioned obstacles are addressed to some extent such as nuclease degradation and immune responses by phosphate backbone and sugar modifications, however, the safe delivery of the RNAi to the disease site is still a challenge while avoiding the aforementioned problems[18]. Therefore, various viral vectors such as adeno associated virus (AAV) and retroviral vectors and non-viral carriers such as lipid nanoparticles, polymeric nanoparticles,

aptamers, and dendrimers have been studied for RNAi delivery (**Figure 2**). However, these carriers face a chain of obstacles *in vivo.* Among them, the biocompatibility and endosomal escape of the carriers are the two major concerns[19]. Therefore, the use of biocompatible carriers such as RNA nanoparticles and/or extracellular vehicles (EVs) might be ideal to overcome the challenges. The RNA nanoparticles or the extracellular vesicles can escape the endosomal entrapment. This is because, the RNA nanoparticles are negatively charged thus, can act as a proton sponge which in turn can lead to endosomal escape[20]. On the other hand, extracellular vesicles are biocompatible, nano-sized, and natural carriers in the cells and are known to release the payload into the cytosol through different mechanisms such as macropinocytosis, cell surface membrane fusion, cell-specific EV uptake, etc[21]. Thus, the RNA nanoparticles and exosomes might serve as carriers to deliver the RNAi therapeutics to treat various cancer types or diseases. The safe and accurate delivery of RNAi would promise the development of RNAi therapeutics as a third generation in the coming years for the treatment of a variety of diseases. When it comes to cancer treatment using RNAi, a combinational RNAi therapy approach might be more effective compared to a single drug regime. However, RNAi therapy concerning cancer treatment is at a very early phase and several years of research is required to rightfully judge the therapeutic potential. Overall, by developing the right RNAi designing method, optimizing novel administration routes, gaining a deeper understanding of RNAi mechanism, and consistent innovations in delivery systems would upbring the clinical efficacy and safety of RNAi therapeutics and would position them as cornerstone of next-generation therapeutics to treat many diseases.

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