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RNA-Based therapeutic oligonucleotide strategies

İsmail Korkmaz [*1], **Gamze Taşkan** [1], **Serdal Arslan** [1]

[1]*Mersin University Department of Medical Biology, Türkiye, ismailkrkmazz@gmail.com, gamzecaakirr@gmail.com, arserdal@yahoo.com*

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Abstract

Oligonucleotides, which have RNA-based therapeutic effects, constitute therapeutic strategies with very high potential with the elucidation of the mechanism of action of RNA molecules in cellular processes and with the developing technology. These strategies offer new alternatives for the mechanisms, pathophysiological processes, diagnosis, treatment, and prevention of many diseases. It also opens new doors to many pathophysiological pathways previously known as "non-targetable". RNA-based oligonucleotides are promising new therapeutic modalities thanks to the diverse reservoir of molecular tools they provide.

Introduction

Oligonucleotides are single- or double-stranded nucleic acid molecules produced in vitro that can silence, block or suppress a target by direct interaction [1]. The disadvantages associated with the therapeutic potential, stability, distribution, rapid degradation, and immunogenicity of oligonucleotides began to decline with advancing technology [2]. However, the addition of chemical modifications to the backbone structures of oligonucleotides highly improved the efficacy of oligonucleotide therapeutics [3, 4].

Antisense oligonucleotide (ASO) therapeutics: ASO; are short, single-stranded, typically 8-50 nucleotides long, synthetic RNA (or DNA) oligonucleotides. These oligonucleotides bind to target mRNA, pre-mRNA, or ncRNAs via complementary Watson-Crick base pairing. After binding to their targets, they mostly function by causing endonuclease-mediated transcript degradation and consequent reduction in transcript levels [1, 5]. The mechanisms of action of these therapeutics in the cell are diverse and these mechanisms are classified into five categories. (I) DNA-based ASOs bind to an RNA target to form a DNA: RNA hybrid. This hybrid structure acts as a substrate by RNase H and enables this hybrid to be degraded. (II) ASOs stop the translation of mRNA targets that bind to the AUG start site of the Open Reading Frame (ORF), which blocks the assembly of ribosomal subunits. (III) regulates translation by sterically interfering with RNA-binding proteins. (IV) Regulates pre-mRNA splicing by binding to exonic or intronic sequences in the splice regions of pre-mRNA leading to the omission, omission, or inclusion of a particular exon. By binding to the Upstream Open Reading Frame (uORF) located in the (V) 5'UTR region, it can enhance translation by supporting the efficiency of the main ORF located downstream of this uORF. [1, 4, 6].

Small interfering RNA (siRNA) therapeutics: siRNA is short, synthetic, 19-22 nucleotide duplexes that typically contain asymmetric nucleotide overhangs at their 3' ends and are inserted directly into the cytoplasm of cells. siRNAs exploit the RNA interference (RNAi) pathway, which is a cellular defense mechanism. These RNAs are produced from endogenous long, double-stranded RNAs (dsRNA) by cleavage by an endoribonuclease Dicer

enzyme [7]. Composed of two single strands, dsRNA combines with the endonuclease Argonaute (AGO) to form the siRNA-induced silencing complex (siRISC). The sense strand is then released and the antisense strand is loaded into the RISC. Under the guidance of the antisense helix, RISC is activated and binds to the target mRNA with a completely base-complementary pairing, leading to degradation, thereby inhibiting gene expression. The binding of siRNA to its target is highly selective and can distinguish even between sequences that differ by a single nucleotide [8]. This specificity in binding has made siRNA suitable therapeutic tools. Moreover, RISC and guide siRNA are recyclable and therefore one siRNA molecule can degrade a large number of mRNA molecules, resulting in high-efficiency gene silencing [4, 9, 10].

MicroRNA (miRNA) therapeutics: miRNAs are small, non-coding RNAs that exist endogenously throughout the genome where complementary regions lead to the formation of a defective RNA hairpin. When processed by the RNAi mechanism, the result is a 19-25 nucleotide RNA duplex containing regions of mismatch. Endogenously formed miRNAs mostly bind the 3' untranslated region (3' UTR) of mRNAs and prevent them from being translated into protein. miRNAs can have many targets in the cell, as the miRNA needs to complement the target mRNA to suppress the translation of only a few nucleotides. The mechanism of gene repression by miRNA depends on the degree of complementarity of an RNA target; [11, 12] miRNA mimics or synthetic miRNAs can be used therapeutically to reduce the expression of mutant proteins [7]. A miRNA can regulate hundreds or thousands of genes, and a gene can be regulated by many different miRNAs. Therefore, identifying the miRNA to regulate a particular gene is difficult and may cause unexpected side effects [13]. The use of miRNA-based therapeutics has dual advantages. First, miRNAs are molecules that occur naturally in human cells as opposed to synthetic ASOs and therefore have all the mechanisms for their processing and downstream target selection. Second, miRNAs act by targeting multiple genes in a pathway, thereby causing a broader but specific response. The use or targeting of naturally occurring miRNAs could represent a promising alternative to existing RNA-based therapies and potentially enhance therapeutic effects compared to synthetic siRNAs or ASOs that only affect a single target gene [14].

Short activating RNA (saRNA) therapeutics: saRNA is a 21-nucleotide, double-stranded, non-coding RNA with two nucleotide overhangs at the ends. Inside the cell, saRNAs are first loaded into the AGO protein, into which the antisense helix is split. The saRNA-AGO complex then enters the nucleus and binds to the promoter regions of genes to increase transcription [15]. saRNAs targeting specific gene promoters induce transcriptional gene activation and induce RNA activation (RNA-a). RNAi and RNA-a pathways show similarities in terms of molecular mechanism. In RNA-a, the RNA-induced transcriptional activation (RITA) complex is formed by the saRNA-AGO complex. The RITA complex consists of RNA helicase A and RNA polymerase-associated protein CTR9, which interacts with RNA Polymerase II to trigger transcription initiation and elongation. saRNA plays a role as a new therapeutic modality for upregulating gene expression in diseases with suppressed transcriptional or translational activity. There are several advantages to developing saRNAs therapeutically, including low immunogenicity and locus-specific gene transcription activation; however, disadvantages such as RNase degradation sensitivity and off-target effects are also critical challenges [13].

RNA Aptamers: Aptamers can form complex structures, binding proteins, and disrupt multiple protein complexes or inhibit their function. They are short, 25-80 nucleotides, single-stranded oligonucleotides that can consist of both DNA and RNA. Thanks to its three-dimensional structure, RNA aptamers show activity by binding to their targets through an appropriate interaction [16, 17]. Because of the similarity of the way aptamers bind to their targets to the immune system, they are considered “chemical antibodies”. This situation; Thanks to the special chemical synthesis, provides advantages such as scalability, low intergroup variability, ease of post-synthesis modification, and low immunogenicity [4, 15].

Synthesis and Modification of Oligonucleotide Therapeutics: Oligonucleotide synthesis is accomplished by a chemical process using nucleoside phosphoramidides, which are analogous nucleotides with conserved reactive groups. RNA oligonucleotides can be synthesized via solid-phase synthesis. The synthesis is carried out in a flow column reactor with a pump-driven system. The first nucleoside is attached to the solid support and then loaded into the column reactor. Typically, there are four steps involved in adding the second nucleoside on top of the first: (I) Detritylation: The 5'-dimethoxytrityl protecting group is removed from the support-bound nucleoside. (II) Binding: The appropriate phosphoramidite monomer (A, G, U, C) is coupled with the aid of an activator. (III) Thiolation/oxidation: The newly formed phosphite triester internucleotide bond is converted to phosphorothioate or phosphodiester by thiolation or oxidation agents. (IV) Capping: unreacted 5'hydroxyl groups are capped with capping agents. After these four steps, a cycle is completed and a new cycle is started [4]. In addition, siRNAs can also be synthesized enzymatically by in vitro transcription with a subsequent DNase treatment and colon purification [18, 19]. If RNA aptamers are to be produced, a prior design is required. A widely used methodology for designing aptamers is the systematic evolution of ligands by exponential enrichment (SELEX) [4, 20].

RNA molecules are inherently highly unstable as they have a 2'-OH group. Chemically modifying the base, sugar, or backbone of a synthesized RNA molecule aids stability, increases their resistance to nucleases, improves efficiency and target specificity, or aids delivery into a cell [21]. However, excessively modified RNA can have toxic effects or make the molecule less efficient [22]. Synthetic RNA-based oligonucleotides are usually modified with phosphorothioate, 2'OMe, 2'-fluoro, 2'-O-methoxyethyl (2'MOE), or 2'4'-methylene [23]. These modified RNA-based oligos have an increased affinity to bind to and inhibit their targets in vivo, are more resistant to degradation and have increased bioavailability [24].

Results

As a result, these oligonucleotide therapeutics using the RNAi pathway constitute therapeutic strategies with very high potential with the developing technology. These strategies offer new alternatives for the mechanisms, pathophysiological processes, diagnosis, treatment, and prevention of many diseases. Today, 16 FDA-approved RNA therapeutic drugs are used in the clinic, and many RNA therapeutics are under development. This will open the door to new treatment methods for many diseases shortly.

Discussion and Conclusion

Increasing knowledge of the versatile roles of RNAs has spurred the development of new classes of RNA-based drugs. Drugs under development are used with mechanisms that show activity from many pathways, such as inhibiting gene expression, producing functional proteins, and changing splices. Although it is difficult to use these mechanisms and turn RNAs into drugs, we are on the verge of a revolution in drug development. Successful application of RNA-based therapeutics requires an unprecedented interdisciplinary approach, including technical advances in molecular biology, immunology, pharmacology, chemistry, and nanotechnology. The development of RNA drugs is rapidly increasing. In the near future, RNA-based drugs could become an increasing component of the pharmacopeia, greatly expanding the universe of drug-applicable targets to provide treatment for previously incurable diseases and potentially ameliorate genetic diseases.

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