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Nucleic acid therapeutics in Viruses: Basic concepts and recent developments

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Introduction

Several nucleic acid-based technologies such as short interfering RNAs (siRNA), antisense oligonucleotides, aptamers, deoxyribozymes (DNAzymes), and ribozymes have been studied and used against management of respiratory viruses. These therapeutic nucleic acids can be efficiently delivered through the airways. Studies have also shown efficacy of gene therapy in clinical trials against respiratory syncytial virus (RSV) as well as models of respiratory diseases including severe acute respiratory syndrome (SARS), measles and influenza.

Nucleic acid-based therapeutics

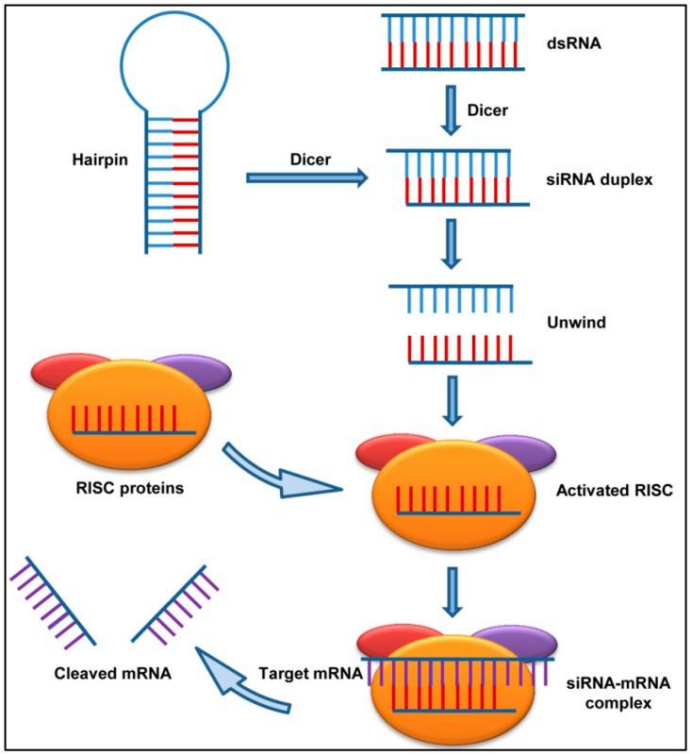
Nucleic acid-based therapeutics is broadly comprised of the DNA-based therapeutics and the RNA-based therapeutics. These molecules offer sequence-specific cleavage of transcripts and have gained popularity in recent years due to their ability to downregulate disease-causing genes either through RNA interference or catalytic cleavage of the transcripts.

RNA Therapeutics against Respiratory Viruses

RNA molecules were long believed to only serve as messengers having information from genomic DNA. However, since the last two decades, small RNA molecules (~20–30 nucleotides) have been evaluated as critical regulators of the expression and function of eukaryotic genomes. These small RNAs can be primarily categorized as short interfering RNAs (siRNAs) and microRNAs (miRNAs), both of which work by degrading the mRNA of specific genes with complementary nucleotide sequences, thus inhibiting their expression and preventing translation.

Short Interfering RNAs (siRNAs)

siRNAs and their role in the post-transcriptional gene silencing (PTGS) were discovered in plants by Balcombe's group in 1999 and in the roundworm Caenorhabditis elegans by Fire et al. in 1998. Years later, another group showed that the synthetic siRNAs could induce RNA interference (RNAi) in mammalian cells. This discovery was one of the most significant advances in biology that led to potential usage of RNAi in biomedical research and drug development. The siRNAs are typically 21–23 nucleotide long double-stranded RNA segments that interfere with the expression of specific target genes with complementary nucleotide sequences by degrading their mRNA post transcription.



Mechanism of action of short interfering RNAs (siRNA): The presence of a double stranded RNA as a consequence of viral infection triggers RNA interference (RNAi). The host enzyme Dicer binds to double-stranded RNA (dsRNA) and cleaves it into short pieces of ~20 nt called siRNA. One

Ribozymes (Rz):

Ribozymes are RNA molecules that have catalytic activity just like the DNAzymes. Since their discovery, the role of RNA catalysis has been shown in several biological processes such as the RNA splicing, RNA processing and the replication of RNA genomes. The Rz occur in nature and mainly cleaves the phosphodiester bonds of nucleic acids. There are several classes of ribozymes, of which only the hammerhead and hairpin ribozymes have received a great deal of attention because of their smaller size. The hammerhead Rz has a 22-nt-long conserved catalytic core, that target RNA with NUX (N-any

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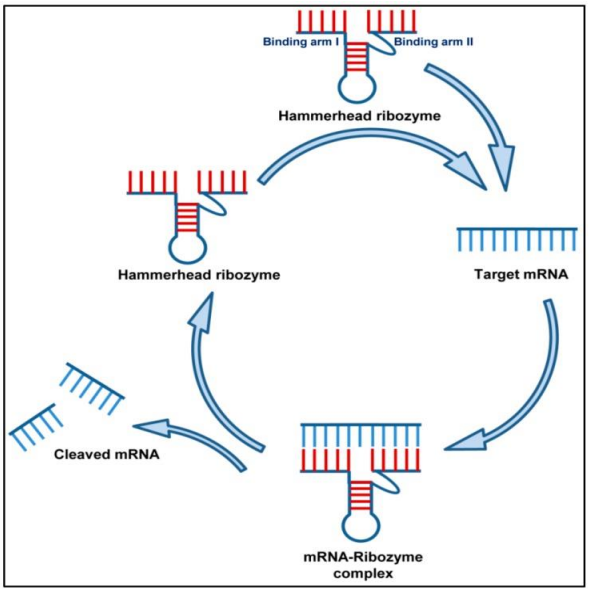
nucleotide and X-any nucleotide except guanosine) sequence, along with two flanked hybridizing arms (complimentary to target RNA). Several studies have utilized the hammerhead Rz for catalytically cleaving the target RNA due to its high catalytic activity. The hammerhead and hairpin ribozymes in different studies have been used to significantly disrupt and reduce viral replication hence effectively inhibiting pre-genomic RNA levels of infecting viruses.



Mechanism of action of Ribozyme. A hammerhead ribozyme has two binding arms designed to bind to the complimentary RNA targets in a Watson–Crick pairing. The ribozyme binds to its target mRNA and makes an mRNA-ribozyme complex. The catalytic motif of the ribozyme then cleaves its target RNA into pieces, thus inhibiting gene expression. After cleaving the RNA target, the ribozyme becomes free again to enter into the next cycle.

DNA Therapeutics against Respiratory Viruses

Deoxyribozymes (Dz)

Deoxyribozymes, also known as DNAzymes or DNA enzymes, are synthetic catalytic single-stranded deoxyribonucleic acid molecules that display precise substrate recognition and have the ability to cleave sequence-specific mRNA molecules with greater biological stability. A Dz molecule has one central catalytic motif flanked by two arms. Both the arms (I and II) are designed complimentary to the target RNA molecule so that the designed Dz binds to it on a Watson–Crick basis. Of the several types of DNAzymes known to catalyze functions like RNA ligation, carbon-carbon bond formation and the hydrolytic cleavage of DNA, the best characterized one is still the RNA-cleaving DNAzymes. DNAzymes have not been reported to occur naturally as DNA molecules are predominantly double stranded; however, Breaker and Joyce generated a DNAzyme de novo by an in vitro selection process in the year 1994. Further in vitro selection experiments generated two prototypes denoted as the "10–23" and the "8–17" RNA-cleaving DNA enzymes. The 10–23 Dz studied by several researchers, has been shown to cleave the target RNA between a purine and pyrimidine both under the in vivo and in vitro conditions. The 8–17 Dz also cleaves the RNA, however it is less popular because of its less established efficacy. The 8–17 Dz cleaves between A and G nucleotides and require a rG–dT wobble pair in the enzyme–substrate complex,

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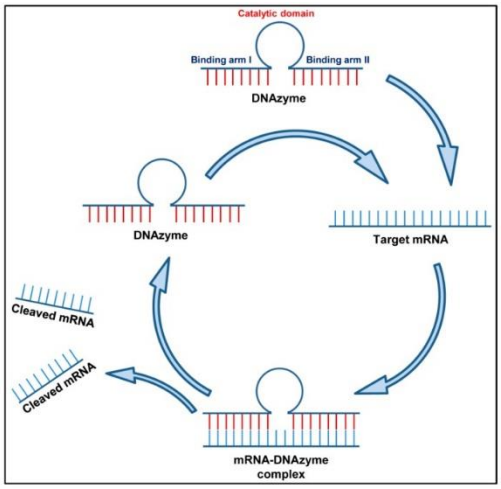
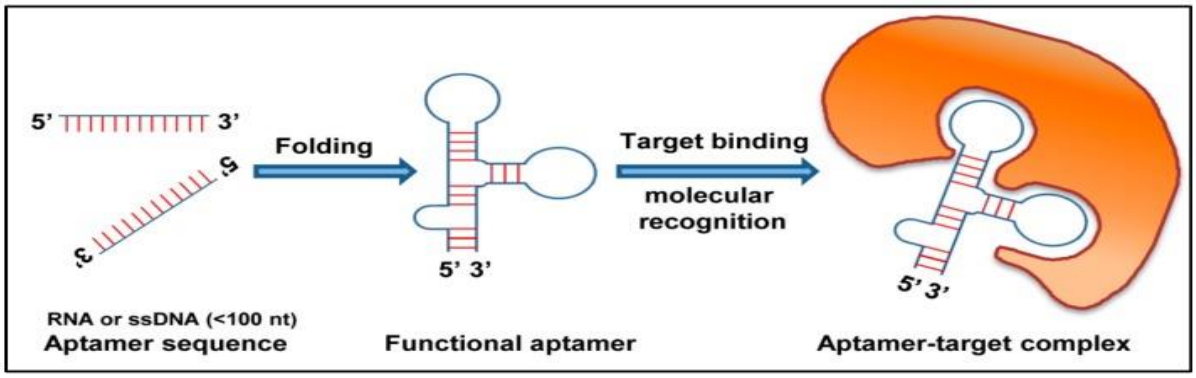
located immediately after the cleavage site for its cleavage activity.



Mechanism of action of DNAzyme: A DNAzyme has two binding arms designed to bind to the complimentary RNA targets in a Watson–Crick pairing. The DNAzyme binds to its target mRNA and makes an mRNA–DNAzyme complex. The catalytic motif of the DNAzyme then cleaves its target RNA into pieces, thus inhibiting gene expression. After cleaving the RNA target, the DNAzyme becomes free again to enter into the next cycle.

Aptamers

Aptamers are 20–90 nucleotides long, synthetic single strand nucleic acid molecules (DNA or RNA). They are designed to bind to various organic or nonorganic molecules—ranging from single atoms to a wide range of proteins. Aptamers are highly specific to target molecule and are generated by the SELEX (systematic evolution of ligands by exponential enrichment) method. For the last 20 years, aptamers have been used as a diagnostic tool for the treatment of viral diseases.



Schematic representation of action of aptamer: An aptamer folds into a three-dimensional structure. The functional aptamer then recognizes and binds to its target molecule resulting in a stable aptamer-target complex.

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