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Brief Review

Therapeutic Use of RNA Antisense Oligonucleotides

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Abstract

Antisense oligonucleotides (ASO) have the potential to serve as therapeutic agents for various diseases in living organisms by selectively targeting specific genes and altering their expression. By halting the production of defective proteins, ASOs can help to control the source of diseases. This field is gaining increasing importance in drug development and provides a positive alternative to downstream targeting processes. However, to translate ASO-based therapies into clinical success, it is crucial to address challenges such as off-target side effects and insufficient biological activity. This review paper aims to provide a comprehensive summary of ASOs, including their preparation, mode of action, and biological activities.

Key Words: Human gene therapy, DNA vaccines, ASOs, ncRNA, miRNA, siRNA, RNAi pathway.

Introduction

In 1990, the first human gene therapy treatment was approved for adenosine deaminase deficiency. Gene therapy has shown success in curing genetic diseases, as demonstrated in patients with severe combined immunodeficiency in 2002. Research is currently underway to develop gene therapies for more complex disorders like Alzheimer's disease and polygenic cancers.

DNA-based therapeutics, on the other hand, have a wider range of applications beyond gene replacement. DNA vaccines are currently used primarily in veterinary clinics, but trials are underway to explore their potential for protecting against diseases such as malaria, tuberculosis, Ebola, HIV, and influenza.

Antisense oligonucleotides (ASOs) are synthetic RNA or DNA sequences designed to selectively bind to RNA that encodes the target gene. ASOs have been tested for a variety of disorders, and when they bind to their target, they can alter mRNA, prevent its attachment to ribosomes, or recruit RNase H to degrade it.

Non-coding RNA (ncRNA)-based gene regulation often depends on small, stable RNA molecules like miRNA. These molecules can be isolated from endosomes and micro-vesicles and used as biomarkers or therapeutics for many diseases. miRNA is a small, single-stranded ribonucleotide that regulates gene expression at the primary stages. They load onto an Argonauto (Ago) protein to form RNA-induced silencing complex (RISC), which represses mRNA translation by binding to complementary sites in the target transcript's 3' UTR. Human miRNAs have specific target sets and expression patterns, making them vital for controlling biological processes and developing drugs for clinical trials.

Short interfering RNA (siRNA), by contrast, are small, double-stranded complexes that trigger the RNAi pathway. While this pathway occurs naturally in some organisms, it does not occur naturally in humans and other mammals. Synthetic siRNAs can be utilized to bind and cleave RNA.

Method for antisense oligonucleotides

One of the main techniques used to prepare oligonucleotides is called phosphoramidite synthesis (SPS). This method involves attaching the sequence to a solid support group or resin using a long chain linker. The synthesis cycle then begins with detritylation, which removes the DMT protection group.

In the second step, a nucleotide phosphoramidite monomer is activated with a catalyst, and the base functional group of the monomer corresponds to the next appropriate monomer. In the third step, the newly activated phosphoramidite monomer or base quickly reacts by joining the 5' end of the hydroxyl group of the previous base, which extends the growing sequence. The sequence is immobilized on a solid support or bound to the core structure used for liquid-phase synthesis, and this step is known as coupling.

After each coupling, each hydroxyl group at the end of the molecule is capped via acetylation to prevent deletion mutation, degradation, or the formation of unwanted side products. This process is called capping.

In the fourth step, oxidation occurs between the two nucleotides, forming a phosphotriester linkage. This cycle is repeated until the oligonucleotide reaches the desired length. This step is known as oxidation. Finally, the oligonucleotide is cleaved from its solid support using ammonolysis or ester hydrolysis, and protection groups are removed with caustic reagents.

Mode of action

The action of antisense oligonucleotides (ASOs) can occur in two ways: either by causing RNA cleavage or by RNA blockage. RNA cleavage can happen through RNase H1 mediated cleavage or RNA interference (RNAi), where siRNA associated with RISC degrades mRNA. On the other hand, RNA blockage can happen through steric hindrance, where the ASO-mRNA complex blocks the interaction of mRNA with ribosomes, or splice modulation, which produces the correct form of protein by skipping mutated exons. These ASOs are designed with a unique genetic code that binds to specific sequences of nucleotides in the target mRNA, interfering with the production of abnormal proteins and ultimately stopping the disease progression. Numerous studies have confirmed that synthetic ASOs can work through RNA cleavages or RNA blockages.

Applications

1. SARS-CoV-2 antibody response

Various vaccines have been developed worldwide against SARS-CoV-2, using messenger ribonucleic acid (mRNA) that was prepared by inactivating the virus particle. The antibodies were created by collecting serum samples from MS clinics, aliquoting them into 1000 μL polypropylene tubes, storing them at -80°C, and then using Chemiluminescent microparticle immunoassay (CMIA) to quantify IgG antibodies against the receptor-binding domain (RBD) of the S1 subunit of the spike protein of SARS-CoV-2.

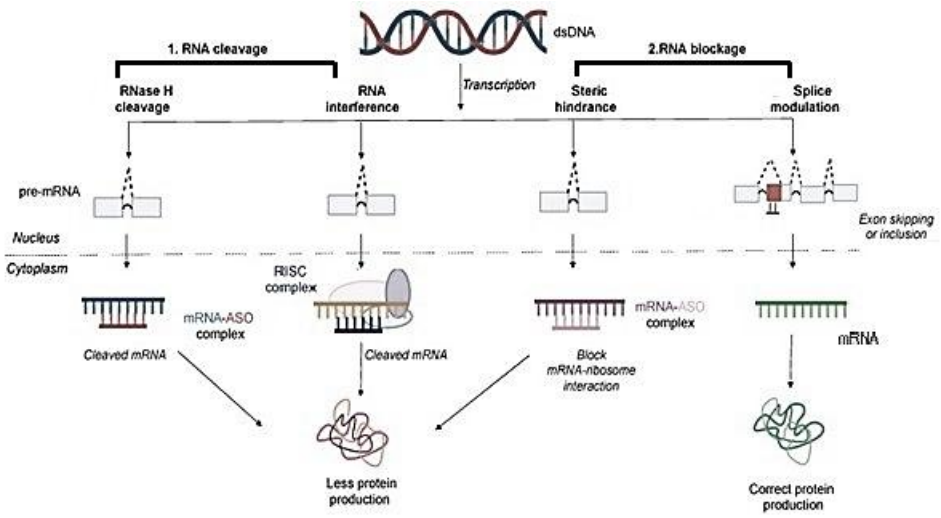

Fig - 1: RNA a1

2. Monogenic retinal degenerative disorders

Lipid-based nanoparticles (LNP) are nanostructures that contain various classes of lipids, such as cationic or ionizable lipids (CILs), PEG-conjugated lipids (PEG-lipids), and structural lipids like phospholipid or sterol. These can self-assemble under controlled microfluidic conditions when mixed in an aqueous solution containing nucleic acid. CILs, which are known for their amphiphilicity and positive charge, bind and encapsulate mRNA into organized LNPs. After delivering the mRNA to the back of the eye, the kinetics of gene expression show a rapid onset (within 4 hours) that persists for 96 hours. Gene delivery is cell-type specific, with most expression in the retinal pigmented epithelium (RPE) and limited expression in the Müller glia.

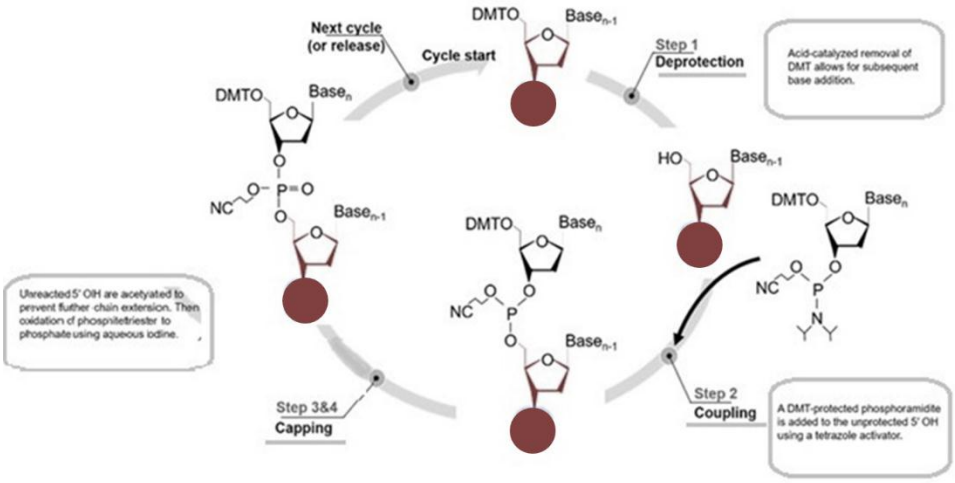

Fig - 2: RNA b1

3. mRNA vaccines for rabies

The first candidate for an mRNA rabies vaccine was CV7201, which consisted of lyophilized, temperature-stable mRNA made up of free and complexed mRNA that encoded rabies virus glycoprotein (RABV-G), along with a cationic protein protamine as stabilizer and adjuvant. The data showed a transient but significant increase in RABV-G-specific CD4+ T cells at day 42, which declined to baseline levels on day 91, consistent with the contraction and memory phase of the immune response, confirming the effectiveness of the mRNA vaccine.

4. Modified mRNA Vaccines against Zika Virus Infection

Modifying the preM-E RNA encoding mutation by destroying the conserved fusion loop epitope present in the E protein can produce a variant that can protect against ZIKV. LNP-encapsulated modified mRNA vaccines, which code for either wild-type or variant ZIKV structural genes, can be used for immunogenicity and protection. When two doses of these modified mRNA LNPs, which code for preM-E genes, are provided, they produce virus-like particles. As a result, high levels of antibody-neutralizing titers take place, which ultimately provide protection against ZIKV.

Conclusion

This review paper presents an analysis of various antisense oligonucleotides (ASO) techniques and their pharmacological applications. ASO offers a wide range of processes, including RNAi, RNase H-mediated cleavage, splicing modulation, non-coding RNA inhibition, gene activation, and programmed gene editing, to regulate gene expression. ASO drugs have tremendous therapeutic potential and have gained recognition in recent years. However, the efficient delivery of oligonucleotides, especially to extrahepatic tissues, remains a significant challenge. This review provides an overview of oligonucleotide-based drug platforms, focusing mainly on chemical modification, bioconjugation, and nanocarrier-based approaches to address the delivery challenge. These approaches offer more selective action, conceptual simplicity, low toxicity, fewer side effects, and permanent cure activities. Hence, this review advocates the use of antisense oligonucleotides as a viable treatment option for various diseases.

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