Antisense Oligonucleotides Mediated Therapy for Neurodegenerative Disease

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Abstract

Many neurodegenerative diseases like Alzheimer's disease, Huntington's disease, Duchenne's muscular dystrophy and spinal muscular atrophy are linked to aggregated, toxic proteins. Antisense oligonucleotide-based strategies (ASOs) are the most direct method of targeting gene expression. Synthetic oligonucleotides bind to the target mRNA by Watson-Crick hybridization and can either promote the degradation of RNA or inhibit it. In 2016, two ASO therapies for spinal muscular atrophy and Duchenne muscular dystrophy were approved by the FDA.

Introduction

Many neurodegenerative diseases: Alzheimer's disease, Huntington's disease, Duchenne's muscular dystrophy, and spinal muscular atrophy, are linked to the aggregation of toxic proteins in the nervous system. Although significant strides have been made in studying the mechanisms of neurodegenerative diseases, the consequent advancements in therapies for treating them have been slower. Antisense oligonucleotide-based strategies (ASOs) are the most direct method of targeting gene expression. ASO strategies utilize synthetic oligonucleotides which bind to the target mRNA by Watson-Crick hybridization and can either promote or inhibit the degradation of this RNA, leading to a knock down of gene expression.

In 2016, two ASO therapies for spinal muscular atrophy and Duchenne muscular dystrophy were approved by the FDA. This marked a shift in the direction of treatment strategies towards antisense oligonucleotides (ASOs). ASOs can target gene expression through a variety of mechanisms including altering the splicing of pre-mRNA, blocking mRNA translation or preventing the assembly of ribosomal complexes. The main complication in ASO therapies is that oligonucleotides cannot cross the blood brain barrier and thus, require invasive forms of delivery. This article will discuss mechanisms of ASO-therapy, challenges in its clinical applications, FDA-approved ASO therapies, and future development.

Mechanisms of ASO-Therapy

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While maternal stress can negatively impact the developing fetus in a plethora of ways, stress experienced in childhood, whether due to severe traumatic events or socioeconomic standing, can also be detrimental to development. The amygdala, in particular, is highly susceptible to sensitivity due to early life stressors that these children experience. Furthermore, children who experience early life stressors show significant deficits in the affective domain and in brain regions with extended postnatal development such as the hippocampus, amygdala, and prefrontal cortex (Pechtel et al. 2010). Early life stressors seem to interfere with the neurogenesis, synaptic overproduction, and pruning of synapses/receptors, thus impairing neural plasticity and growth in the critical brain areas listed above (Pechtel et al. 2010). The corpus callosum, which connects various aspects of cognitive, motor, and sensory functioning at different stages across development, decreases in size due to early due to early life stressors.

In addition to recruiting cellular enzymes, ASOs can also directly cleave target RNA if they are designed with their own enzymatic activity. This is usually done by associating DNAzymes and ribozymes with ASOs. ASOs can also modify RNAs to alter their stability and promote or inhibit degradation. ASOs also participate in direct translation inhibition by sterically blocking ribosomes. This steric block is formed when ASOs bind to mRNA and prevent the association of the 40s and 60s ribosomal subunits during translation. Furthermore, ASOs can modulate the splicing of RNA into mature mRNA transcripts. ASOs destabilize splice sites by binding to intron-exon junctions thereby preventing the binding of splice factors. If the disorder is known to be caused by a splicing defect, it is suggested that ASOs with this mechanism of action are used to return to normal function. Usage of ASOs in this case can either promote a return to the original reading frame or can simply exclude the mutated DNA segment of the gene.

Current ASO Therapies

As of 2019, the FDA has approved only 3 ASO-mediated therapies for neurodegenerative diseases:

eteplirsen for Duchenne muscular dystrophy, nusinersen for spinal muscular atrophy and inotersen for familial amyloid neuropathy.

Duchenne muscular dystrophy is caused by a mutation in the gene DMD (human Duchenne Muscular Dystrophy) that codes for the protein dystrophin. Usually, DMD mutations result in a premature truncation of dystrophin. The ASO used by eteplirsen acts on the pre-mRNA of DMD and excludes exon 51. This causes a re-establishment of the reading frame and results in partial restoration of DMD function.

Spinal muscular atrophy (SMA) is the result of a deficiency of the 'Survival of Motor Neuron' (SMN) protein caused by a loss of function mutation in both copies of the SMN1 gene located on chromosome 5. In the treatment of spinal muscular atrophy, the gene SMN2 is targeted to offset the SMN protein deficiency. SMN2 is a homologous gene to SMN1 except it does not contain exon 7. The severity of SMA increases as the copy number of the SMN2 gene decreases. The ASO nusinersen prevents the splicing silencer that removes exon 7 from the SMN2 gene. As a result, the SMN2 gene produces the SMN protein.

Challenges in Clinical Application

While some successful therapies have been developed, one of the main issues in using ASO-mediated therapies for neurodegenerative disorders is effective delivery of the drug to the brain. Antisense oligonucleotides are too large to cross the blood brain barrier. In order to reach the brain, the therapy is delivered through the spinal cord by injection into the cerebrospinal fluid.the spinal cord is the pathway of delivery that must be used. Cerebrospinal fluid (CSF) is produced by the choroid plexus and is stored in cerebral ventricles in the brain as well as in the spinal cord. ASOs can be safely administered by injecting them directly into CSF in the spinal cord. The first phase of human clinical testing of an ASO targeting the gene SOD1 showed that the drug was successfully and safely injected into the CSF but only reduced the mutant SOD1 protein expression by about 12%.

There is also the issue of sustainability for long term usage of ASO-mediated therapies. Chemically modified ASOs can have longer half-lives. For example, the modification 2'-O'methoxyethyl can be added to ASOs. This increases binding affinity to mRNA and has a half life of 6 months or more.

ASO-mediated therapies are still relatively new and many improvements need to be made to existing therapies before they can be considered a standard treatment. Improving the specificity to targets is very important to prevent any off-target effects. During the development of ASOs, two main methods are used to study and improve specificity. The first is quantitative PCR to appraise the mRNA expression when treated with ASOs. This method can be used to study where mismatches occurred and whether or not the mismatched binding to the ASO resulted in any changes in gene expression of the target. The second method is transcriptome analysis or RNA-sequencing of mouse tissues. The tissues extracted from mice do not have the mRNA target and are studied for expression changes when treated with the ASO.

ASO-mediated therapies can also be modified to enhance their pharmacokinetic properties like binding affinity and resistance to endogenous nucleases. For example, Modifications from 2' to 4' positions constrain the sugar and result in stronger binding as well.

It is also important that long-term and side effects are studied before the therapies are implemented. There are two possible reasons for off-target effects, hybridization dependent or independent. As ASOs are streamlined to be more efficient and their effects become more widespread, the off-target effects are likely to pose a larger problem. For example, as ASO sequences get shorter, the risk of mismatched complementary binding rises and this leads to a larger risk of influencing the expression of non-target RNAs.

Future Development

As a new type of therapy, ASOs can still be refined and applied to a broad variety of diseases outside of the few known so far. For example, ASOs mediated by RNase H are the most common mechanism of ASO therapies. However, target RNA suppression can also be achieved by other mechanisms. The modulation of splicing is very promising as an alternative ASO mechanism. In this case, attuning splicing can result in an out-of-frame deletion that consequently causes nonsense decay of the transcript which overall, results in protein knockdown.

Most of the current ASO-mediated therapies work by degrading RNA; the opposite, increasing RNA expression, however, is a much more complicated endeavor. In vivo, increasing the levels of proteins is a delicate task because there are not many genes to which this strategy is applicable. Gene therapy and targeting inhibitory antisense transcripts in a process called antisense-mediated derepression are mechanisms of achieving increased protein levels, in vivo. Liang et al. (2016) used ASOs to increase the efficiency of mRNA translation. This study used ASOs that targeted open reading frames upstream of the target sequence to increase translation and thus increase protein levels.

After the approval of ASO therapies for DMD and SMA, the potential of ASOs has been significantly broadened. For example, ongoing studies are developing ASO-mediated therapies for Huntington's and Alzheimer's. Huntington's disease is caused by repeats of the sequence CAG in the gene HTT that codes for a polyglutamine section in the protein huntingtin. This same polyglutamine section is the site of mutations that lead to a number of other neurodegenerative diseases like spinocerebellar ataxias. ASOs are being developed to silence the CAG section of huntingtin, but the issue with this approach is that it may cause downregulation of nontarget sequences that contain CAG. Other approaches include using ASOs to target the mutated HD allele, specifically polymorphisms of individual nucleotides, that a large majority of HD patients have.

With regard to Alzheimer's, ASOs can be used to target the protein tau. Alzheimer's falls under the category of tauopathies, where tau is hyperphosphorylated and accumulates to form tangles in neurofibers. ASO-mediated approaches are being developed to silence tau by targeting a number of different points in the gene expression pathway.

This includes binding and blocking the start codon, splice factors or sequences. So far, the most successful approach has involved using ASOs to create an out-of-frame deletion by skipping specific exons that ultimately reduces tau protein levels.

Concluding Statements

ASO-mediated therapies have shown to be a novel approach to treating neurodegenerative diseases. A lot of development and further research needs to be done to broaden the scope of applications of this therapeutic strategy. ASOs, as a new therapy, have a lot of room for improving specificity, efficiency, and rates of activity. Further research needs to be done on enhancing ASO selectivity without increased off-target binding. As ASOs become a more prominent method of treating neurodegenerative diseases, it is important to study its applications to non-neurological disorders.

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