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REVIEW ON ANTISENSE TECHNOLOGY AND THEIR APPLICATIONS

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Abstract: Most of the Diseases are often connected to the less or more production of certain "Proteins". If the production of these proteins is discord many diseases can be treated or cured. Antisense technology is a method that can deny the protein production. It used to design new therapeutics for diseases in which the production of a specific protein plays a circle role. RNA plays a central role in the expression of all genes. Any sequence within RNA can be recognized by complementary base pairing, synthetic oligonucleotides. Antisense technology is a tool that is used for the Inhibition of gene expression. The principle behind it is that an antisense nucleic acid sequence base pairs with its complementary sense RNA strand and prevents it from being translated into a protein. Antisense oligonucleotides are short, chemically modified strands of 15-20 nucleotides, which hybridize to a specific complementary area of mRNA of a disease related target gene, thus blocking its expression in protein synthesis. This article gives brief introduction regarding the antisense technology.

***Index Terms*– Antisense technology, Mechanism, Gene expression, Applications**

1. Introduction

Antisense compounds are Synthetic in nature and have negative charge, these molecules have different chemical properties, and by manipulation in their structure it makes more powerful Oligonucleotides and less toxic without showing any effect in non-target site. DNA makes RNA and RNA makes Protein, Gene expression suggests that synthetic compounds, which bind to RNA, should modulate protein production because protein expression affects disease. The concept of using synthetic oligonucleotides is to control the expression of genes that impact disease has been pursued for long years. The tool that is used for the inhibition of gene expression is called as Antisense Technology. Antisense nucleic acid sequence base pair with its complementary sense RNA strands and thus prevents its translation in to protein.[1]

Example of sense and Antisense RNA is:

1) 5' A C G U 3' mRNA

2) 3' U G C A 5' Antisense RNA

Antisense Oligonucleotides are single stranded chemically modified oligonucleotides have 14-20 nucleotides in length. The negative charge impedes diffusion across lipid bilayers. These are small pieces of DNA or RNA, which bind to specific molecules of RNA. They block the ability of RNA to make protein and block the production of proteins needed for cell growth.[2]

2. Basic Concept

Short Fragments of nucleic acid used in the Human genome are called as Oligonucleotides. These are used as tools to study gene function. Development of antisense oligonucleotides needs FDA approval. In antisense technology the use of sequence in which specific mRNA can inhibit its expression and then induce a blockade in the transfer of genetic information from DNA to Protein.[3] Antisense Oligonucleotides are commonly used in Laboratory and clinics. Antisense oligonucleotides discovered to induce RNA processing and modulate protein expression over two decades ago. Antisense Oligonucleotides are short, synthetic, single stranded oligonucleotides that can change RNA and reduce its effects, it can modify protein expression. when Antisense Oligonucleotides target pathogens, they have more chances of success than therapies. In clinic they need more optimization of delivery, target and safety. Antisense therapy is a form of treatment for genetic disorders.[4]

Unmodified DNA molecules degraded before thy reach their target site. Antisense drug molecule modified during the drug delivery phase and its development, mostly the target of Antisense are located inside cells and the movement of nucleic acid across cell membrane is difficult also that's why the modification in DNA has done. Antisense Oligonucleotides are small piece of DNA or RNA, which bind to specific molecules of RNA. It blocks the ability of RNA to make a Protein. Antisense Oligonucleotides used to block the production of proteins needed for cell growth.[5]

3. Mechanism of Action

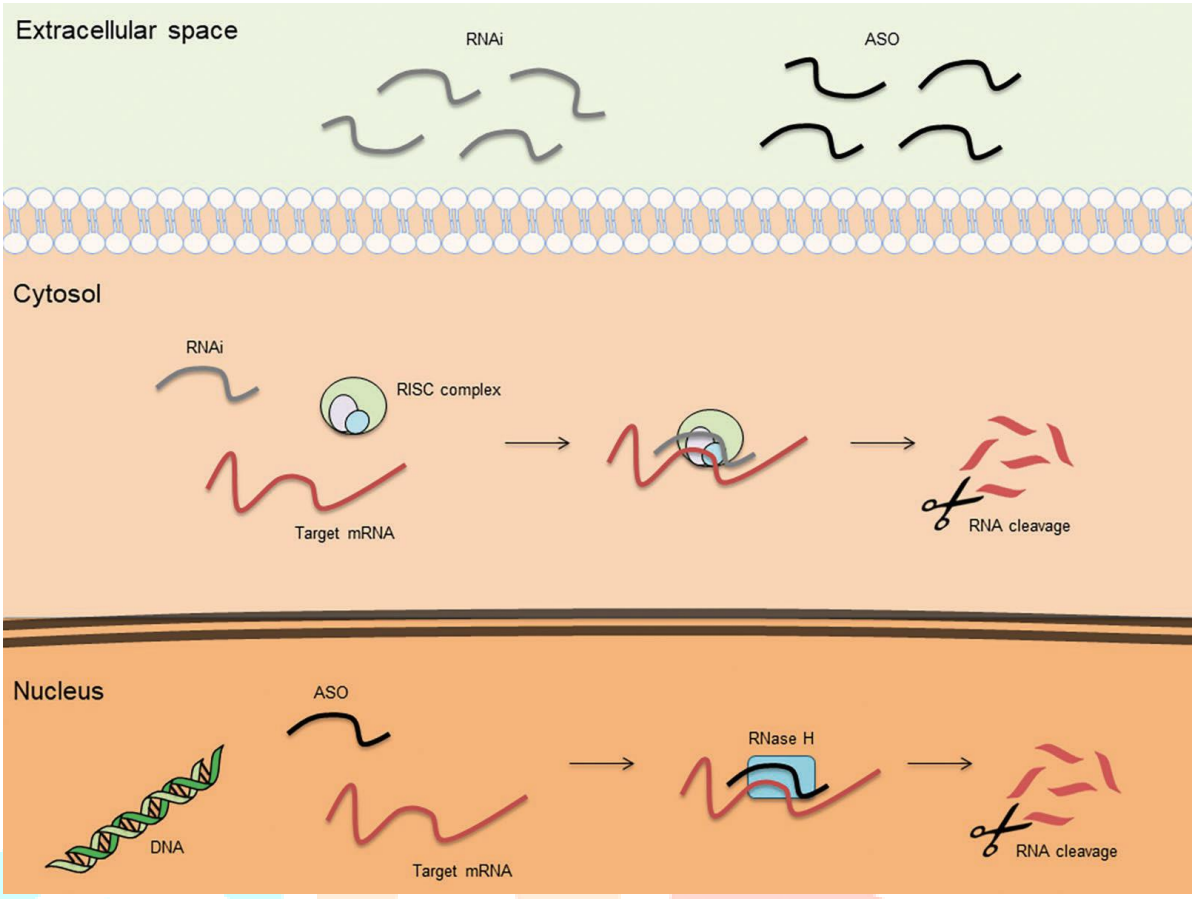
Therapeutic ASOs are synthetic single-stranded DNA analogs, usually 15–30 bp in length. Their sequence (3' to 5') is antisense and complementary to the sense sequence of the target nucleotide sequence. Unmodified oligonucleotides after quick degradation by circulating nucleases are excreted by the kidney; unmodified oligonucleotides are generally too unstable for therapeutic use. Therefore, chemical modification strategies have been developed to overcome this and other obstacles in ASO therapy program. This was achieved by replacing one of the non bridging oxygen atoms in the phosphate group of nucleotide with either sulfur groups (phosphorothioates), methyl groups (methyl phosphonates) or amines (phosphoroamidates), Phosphorothioate substitution (PS) was the earliest and the most commonly used modification that renders the internucleotide linkage resistant to nuclease degradation. In addition to their endogenous nuclease resistance, PS modification has two other distinct advantage.[6] It support endogenous RNAse H activity to degrade the target mRNA or mutant toxic mRNA and thereby diminishing its toxic protein product and then PS linkages also improve the pharmacokinetic characteristics by their sequence-independent but length-dependent binding with plasma proteins. In this context, protein binding can be favorably exploited to alter the half-life and availability of ASO to the target site.

Although substantial advantages are conferred by PS backbone modification of nucleotides, such modifications were shown unfortunately to elicit strong platelet activation, aggregation and thrombi formation in animal models and invitro experiments in human platelets. To overcome the various nonsequence-specific side effects of first generation ASOs and to improve further nuclease resistance and target-binding affinity, second generation ASOs were developed. The most commonly used modification in these ASOs is 2' ribose modifications that include 2'-O-methoxy (OMe), 2'-O-methoxy-ethyl (MOE), and locked nucleic acid (LNA).[7]

Third-generation ASOs have been developed to enhance their delivery to the target sites. In this technology, the oligo load is covalently bound to a carrier or ligand, such as lipid particles, liposomes, nanoparticles, and more recently, the sugar N-acetyl galactosamine to enhance safer delivery to the target site. Hybridization of ASO to the intracellular target mRNA can result in specific inhibition of gene expression by two main mechanisms. The most common mechanism is by induction of endogenous RNAse H activity (ASO-RNase H) that cleaves the mRNA-ASO hetero-duplex.

Example: This leads to degradation of the target toxic mRNA while leaving the ASO intact. Such antisense effect is catalytic and a single ASO can participate in the destruction of many mRNA molecules.

The ASO molecules currently undergoing trials in familial amyotrophic lateral sclerosis (FALS) belong to this category. The second group of ASO mechanism of actions include translational inhibition by steric hindrance, exon skipping, exon inclusion, destabilization of pre-mRNA in the nucleus, or targeting the destruction of microsomal RNAs that control expression of other genes. Strategy utilizing steric hindrance or modulating splice site (nusinersen in spinal muscular atrophy [SMA]) does not utilize RNAse H activity.[8]



Basic mechanisms of action for therapeutic antisense oligonucleotides (ASOs) and RNA interference (RNAi).

4. Technology

Therapeutic objective of antisense technology is to block the production of disease which is caused by proteins. This can be done by creating a synthetic "antisense" or complementary nucleotide sequence of DNA or RNA that interacts and binds to the "sense" or original mRNA sequence. This "mRNA"- antisense complex" can no longer be translated and the disease causing protein will stop its production. The antisense polygalactouronase cDNA sequence is cut with restriction enzymes and fused in the inverted orientation to a plasmid, containing an upstream promoter and a downstream terminator sequence. The cauliflower mosaic virus (CaMV) promoter is chosen because it produced the right amount of antisense RNA to provide an adequate delayed softening time. The degree of production of antisense RNA in the plant cells is dependent on a number of factors and the type of promoter sequence chosen is one of them.

E-Coil serves as the plasmid host. After that the hybrid gene is transferred to the Agro bacterium Tumefaciens by triparental mating with E-Coil. This type of mating is a recombinant DNA method which allows genetic information to exchange from one parent to the other. The gene of interest is now present in Agro bacterium Tumefaciens plasmid (Ti plasmid) and is situated beside the T-DNA.[9]

A complimentary DNA sequence is cloned and inserted in front of the sequence of interest. This means that the last base of the inserted DNA is complimentary to the first base of the original DNA. The second last base is complimentary to the second base and so forth. When transcription takes place in this modified strand of DNA, the mRNA becomes double stranded. Depending on the size of the inserted DNA, the mRNA could be double stranded along all its length or could be partially double stranded.[10]

5. Applications

5.1. Antisense technology has already been successful in suppressing the gene for the protein that makes tomatoes – Poil Flavor saur tomatoes were transgenic tomatoes constructed to have artificial DNA that coded for aRNA that was complementary to the RNA that coded for the protein that caused spoiling. The aRNA suppressed the expression of this spoilage gene by 10% which was enough to save the tomatoes from rotting while being shipped to grocery stores. The tomatoes are no longer on the market due to complications in the harvesting process.[11]

5.2. Cellular rennin angiotensin system play important role in a wide variety of cardiovascular disorders, including atheroeclerosis and vascular hypertrophy. It is difficult to demonstrate that a cellular system is operative in any given process. However, because a circulatory rennin angiotensin system exists that produces angiotensin II in tissue culture medium as well as in tissues. To approach this problem they developed oligonucleotides to inhibit the synthesis of angiotensinogen, the substrate from which cells make angiotensin II.[12]

5.3. Antisense technology is able to demonstrate the biological principle that cells can make their own angiotensin II with growth promoting effects. Now they extended this antisense work to certain cancers and demonstrated, for the first time, that neuroblastoma cells.[13]

5.4. Antisense technology is used successfully in two general areas. The first one is fundamental research where antisense oligonucleotides introduced helps to determine the role of a specific gene. Cell growth and other changes occurred due to the production of angiotensin II. Cellular renin angiotensin system played an important role in variety of cardiovascular disorders like artherosclerosis and vascular hypertrophy.[14]

5.5. Viral infections can occur when the antisense oligonucleotides are complementary to viral RNAs. Similarly, antisense oligonucleotides directed towards the oncogene product plays an important role to reduce growth of cancer cells.

5.6. The most widely used application of this technology is in gene therapy. In this case, a variety of vectors are used to introduce antisense encoding genes into larger number of cells in a patient or animal to produce long term inhibition of protein. For example, vectors having angiotensin II receptor sequences when introduced in animal models can cause long term normotension in hypersensitive animals.[15]

6. Conclusion:

The rationale use of antisense-based therapies in IBD is supported by the benefit seen in preclinical models and initial clinical studies, together with the safety profiles of the compounds. Unfortunately, however, large clinical trials have not confirmed the promising results obtained with ASOs in preclinical models. Although, it is unclear why these treatments failed in patients, it is conceivable that some factors either related to the target or route of administration may have contributed to these negative results. For example, the negative results of alicaforsen can, in part, rely on the fact that ICAM-1 is just one of the various molecules involved in leukocytes trafficking, and therefore, even in the absence of ICAM-1, other integrin could promote recruitment of activated leukocytes in the gut. Another possibility is that systemic administration of ASO could be not ideal for allowing optimal concentration of the drug within the gut tissue, where there is the main expression of the target.

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