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POTENTIAL ROLE OF ANTISENSE OLIGONUCLEOTIDES IN REGULATION OF GENE EXPRESSION TO TARGET CANCER: A STEP FORWARD

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ABSTRACT

Antisense Oligonucleotides have been widely used to specifically and selectively downregulate gene expression at the messenger RNA level. Treatment of cancer is a secretive problem in the current era in spite of significant advancement in drug delivery systems and identification of new therapeutic molecules. The ration of lab to market translation of therapeutic research is quite low in anticancer therapy due to hidden and unnamable disease causing and disease progression mechanisms. Oncogene activation disturbs normal cell processes such as cell growth and apoptosis, which ultimately leads to cancer. Different ways, such as RNAi, antisense Oligonucleotides, Translation suppressing oligonucleotide and external guide sequences which silences oncogene are key techniques under investigation currently. The emerging way of formulating nanoparticles containing antisense Oligonucleotides and the targeting approaches is illustrated with the brief information on key materials used for the same purpose. Gene targets identified and investigated by various companies and research groups are enumerated and reviewed for their potential to consider antisense gene therapy. In this review, our efforts have been devoted to summarize and discuss on the potential role of Antisense Oligonucleotides the different targeting approaches used for targeted drug delivery to Cancer.

KEYWORDS: Antisense Oligonucleotides, gene expression, anticancer therapy.

INTRODUCTION

ANTISENSE OLIGONUCLEOTIDE

An antisense oligonucleotide is single stranded molecules which are synthetically prepared. They contain strands of deoxyribonucleic acid (DNA) sequences comprising 18–21 nucleotides which are complementary to their target mRNA complementary to the mRNA sequence of the target gene.[1] An antisense oligonucleotide refers to a synthetic strand of deoxyribonucleotide (DNA) analogue that hybridizes with the corresponding mRNA. It is reported that antisense oligonucleotides have been used to modify the expression of specific genes.[2] The mRNA in RNA-DNA duplex is a substrate for cellular Ribonuclease H (RNase H) an enzyme that destroys the RNA. RNase H cleaves the RNA-DNA duplex region of the mRNA thus induce a blockade in the transfer of genetic information from DNA to protein. An antisense oligonucleotide is a single-stranded deoxyribonucleotide that is complementary to the target mRNA.[3] Oligonucleotide-based antisense techniques represent the most successful approach to achieving suppression or elimination of a genetic message. Oligonucleotides are a topic of interest for scientists, chemists, biochemists, pharmaceutical scientists and physicians. They may be native compounds, yet there are also synthetic molecules used as therapeutic agents or in polymerase chain reaction. These compounds may be selectively bonded to the specific RNA region based on nucleobase complementarily, and consequently the translation process may be inhibited. Therapeutic oligonucleotides have as potential treatments for cancer, AIDS, Alzheimer's disease and cardiovascular disorders. An antisense oligonucleotide is many potential applications as they target mRNA which is ubiquitous and more accessible to manipulation compared to DNA. The mRNA of any gene can thus be theoretically inhibited due to the availability of gene sequence information from the human genome. An antisense refers to modification of DNA or RNA with the target that its components form a complimentary copy of normal, or “sense,” messenger RNA (mRNA). The hybridization of the nucleic acid sequences for specific mRNA targets will inhibit the normal cellular process through many different mechanisms.[4]

MECHANISM OF ACTION

Antisense oligonucleotide is consist of 15–20 nucleotides, which are complementary to their target mRNA. These are two major mechanisms contribute to their antisense activity. The first is that most Antisense oligonucleotide are designed to activate RNase H, which cleaves the RNA moiety of a DNA-RNA hetero duplex and therefore leads to degradation of the target mRNA.[5] Antisense oligonucleotide is taken up by cellular endocytosis, hybridize with

the target mRNA resultant in the arrangement of Antisense oligonucleotide-mRNA hetero duplex leading in most of times to either activation of RNAse H or sterichindrance of ribosomal subunit binding. Both these mechanisms result in selective degradation of bound mRNA and ultimately target protein knockout. RNase H reliant oligonucleotides be able to induce the degradation of mRNA when targeted to any region of the mRNA. still, the steric-blocker oligonucleotides physically turn away the progression of splicing only when targeted to the AUG initiation codon region.[6] Other mechanisms by which Antisense oligonucleotide can act is by ingoing the nucleus directly and altering maturation of mRNA, splicing activation, AUG formation inhibition, arrest of translation and double strand RNAse activation.[7]

ANTISENSE TECHNOLOGY

Antisense refers to short DNA or RNA sequences, termed oligonucleotides, which are designed to be complementary to a specific gene sequence. The goal is to alter specific gene expression resulting from the binding of the antisense oligonucleotide to a unique gene sequence.[8] In principle, antisense technology is supposed to prevent protein production from a targeted gene. The exact mechanism by which this occurs remains uncertain. Proposed mechanisms include triplex formation, blocking RNA splicing, preventing transport of the mRNA antisense complex into the cytoplasm, increasing RNA degradation, or blocking the initiation of translation.[9]

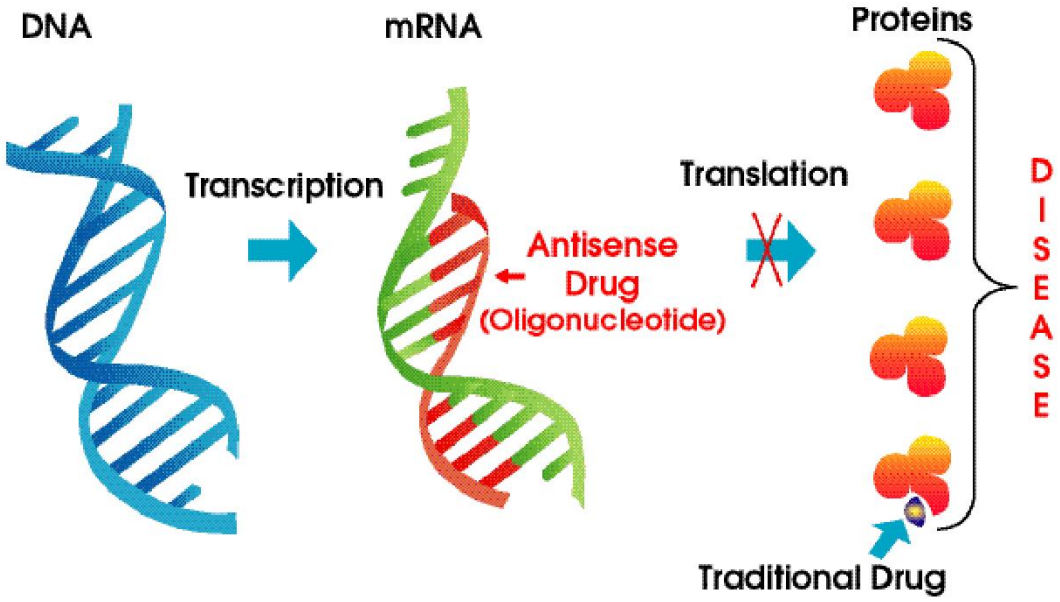

Fig 1:- Antisense Technology.

CHEMICAL SYNTHESIS OF MODIFICATIONS OF OLIGONUCLEOTIDES

Oligonucleotides are unmodified or chemically modified single- stranded DNA molecules. In general, they are relatively short (13–25 nucleotides) and hybridize to a unique sequence in the total pool of targets present in cells. Although it is not a complicated matter to synthesize phosphodiester oligonucleotides, their use is limited as they are rapidly degraded by the intracellular endonucleases and exonuclease.[10] On the basis of these modifications, Antisense Oligonucleotides are classified into three generations: Antisense Oligonucleotides with alteration of the phosphate group, those with sugar modification and those with altered backbone. In all the cases the base is not modified, since it is responsible for binding with complementary nucleotides of the target mRNA.

First-generation antisense oligonucleotides

These are formed when one of the non-bridging oxygen atoms in the phosphate group is replaced by a sulphur atom to form phosphorothioates, or a methyl group to form methyl phosphonates respectively. When compared to phosphodiester oligonucleotides, they display ease of synthesis, long plasma half-life and are nuclease-resistant. They have the ability of recruiting RNase H[11] and display appropriate pharmacokinetics. Phosphorothioate oligonucleotides are the most widely used Antisense Oligonucleotides. The FDA-approved Antisense Oligonucleotides drug However, Antisense oligonucleotide display a tendency of unspecific binding *in vivo* due to interaction with the cell surface and intracellular protein resulting in immune stimulation and complement activation.[12,13,14]

Second-generation antisense oligonucleotides

They are formed by introducing alkyl modification at the 2'-position of the ribose sugar. This modification enhances nuclease resistance and improves the binding affinity towards target mRNA. Widely used second-generation Antisense oligonucleotides are 2'-*O*-methyl and 2'-*O*-methoxyethyl oligonucleotides.[15] Since the 2'-position is modified, these agents are unable to activate RNase H enzyme and thus they exert activity by stearic blocking. Chimeric Antisense oligonucleotide is synthesized to enable RNase H activation. This newly formed Antisense oligonucleotide consists of a central block of phosphorothioate deoxynucleotide surrounded by nuclease-resistant arms on both sides thus preventing degradation by nucleases.[16,17] Compared to the first-generation, the second-generation Antisense Oligonucleotides are reported to have better tissue uptake, longer *in vivo* half-life and lower toxic effects.

Third-generation antisense oligonucleotides

These are formed by the chemical modification of the furanose ring of oligonucleotides along with modification of phosphate linkages. The introduction of these changes in the structure displays enhanced nuclease stability, more affinity for target and better pharmacokinetic profiles of oligonucleotides. Peptide nucleic acid, morpholino phosphoroamidates and locked nucleic acid are the three most commonly used third-generation Antisense Oligonucleotides. PNA is formed by replacing the sugar–phosphate backbone with pseudo peptide polymer. MFs are nonionic Antisense Oligonucleotides which are formed by replacing the ribose sugar with morpholino ring and replacing the phosphodiester bond by a phosphoroamidate linkage. Locked nucleic acid is a ribonucleotide formed by connecting the 2'-oxygen of the ribose with the 4'-carbon through a methylene bridge. Third-generation Antisense Oligonucleotides have higher biological stability because of higher resistance to nucleases and peptidases, and show strong ybridization affinity with mRNA. However, third-generation Antisense Oligonucleotides do not have the ability to activate RNase H, and thus produce antisense effect by stearic hindrance to ribosomes, leading to translational arrest. Since they are uncharged, Antisense Oligonucleotides have no affinity for serum proteins, lowering the chances for unspecific interactions but, on the contrary, they exhibit rapid clearance from the body. Being electrostatically neutral in nature due to their backbone, Antisense Oligonucleotides face challenges regarding solubility and cellular uptake. Employment of delivery systems *in vitro* to increase their uptake would thus help circumvent this problem. PNA, MF and LNA have demonstrated favourable results in different *in vitro*, *ex vivo* studies. LNA can be incorporated in the DNA to form chimeric gapmers resulting in greater affinity, greater nuclease resistance and the ability to recruit RNase H.[18,19]

Table 1: Summary of comparative properties of various generations of antisense oligonucleotides.

Generation	Examples	Advantages	Limitations	Mechanism
First generation (replacing non-bridging O)	Phosphorothioates, methyl phosphonates	Nuclease resistance than phosphodiester, highaffinity for mRNA	Immune stimulation at high concentration (toxic)	RNase H activity
Second generation (alkyl modification at 2' position)	2'-O methyl oligonucleotides, 2'-O methoxy ethyl oligonucleotide, chimeric As-ODNs	High nuclease resistance, high affinity, better tissue uptake, less toxic than first generation	Incapable of activating RNase H	Stearic hindrance (chimeric As-ODN: RNase H activity)
Third generation	Locked nucleic acid, peptide nucleic acid, morpholino phosphoramidates	Higher nuclease resistance, higher binding affinity with mRNA, uncharged and so do not bind serum proteins	Rapid clearance, neutral backbones make solubility and uptake difficult (need delivery systems), incapable of activating RNase H	Stearic hindrance

APPLICATIONS OF ANTISENSE OLIGONUCLEOTIDES

The number of antisense oligonucleotides that are being studied is increasing greatly. The first antisense oligonucleotide to be marketed was Formiversin by ionis pharmaceuticals in 1998. The most critical challenge for the antisense oligonucleotides to be an effective therapeutic is for it to be delivered to the site of action and to produce expected efficacy *In vivo*.[20] There is a new trend of using topical application of antisense oligonucleotides s as the most popular mode of administration. In fact, the first clinically approved antisense oligonucleotides, Formiversin, is administered intravitreously.[21] This is used in the treatment of cytomegalovirus induced retinitis in aids patients. It is a phosphorothioate oligodeoxynucleotides and targets the major immediate early regions 1 and 2 of mRNA of the virus.[22] This is used to reduce low density lipid cholesterol and lipoproteins in patients with homozygousm familial hypercholesterolemia and is given by subcutaneous route.[23] It is chimeric antisense oligonucleotides having phosphorothioate oligodeoxynucleotides in the middle flanked by 2'-methyl modification on both ends, and targets the mRNA. Currently, there are many antisense oligonucleotides undergoing clinical trials.

CONCLUSION

The past two decades have seen an increasing use of antisense oligonucleotides for the purpose of target identification/validation and the use of the information, thus obtained, for the development of effective therapeutic interventions. Most of the structural motifs in PS-ODNs, that interfere with their antisense effects and are responsible for toxicities, have been delineated. Many desirable properties such as efficient uptake by the cells, stability against nucleases, and a strong affinity for target mRNA have been identified. Effective chemical modifications are likely to avoid the non antisense effects and further enhance the safety and efficacy of antisense oligonucleotides and thus expand their potential clinical applications.[24/5] The rather disappointing results of some of the recent clinical trials indicate a need to clearly establish the relevance of the target to the patient population being studied, an early determination of optimal biological dose and the rational use of combination strategies for the treatment of the disease.[25] Finally, the clinical end points need to be clearly defined in order to evaluate the efficacy of antisense oligonucleotides.[26]

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