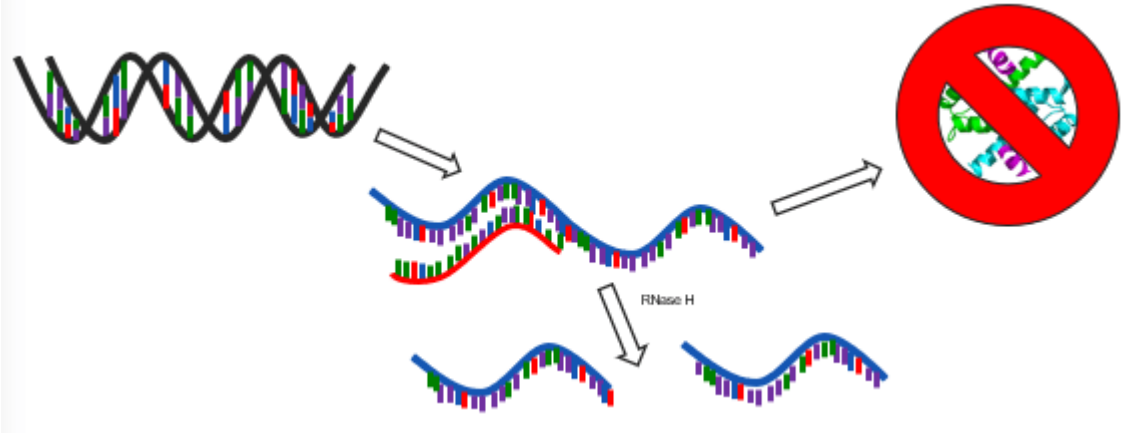
Antisense Oligonucleotides – The Significance of the DNA-like Molecules

Guest Author – Helen Whitby, PhD., Technical Specialist

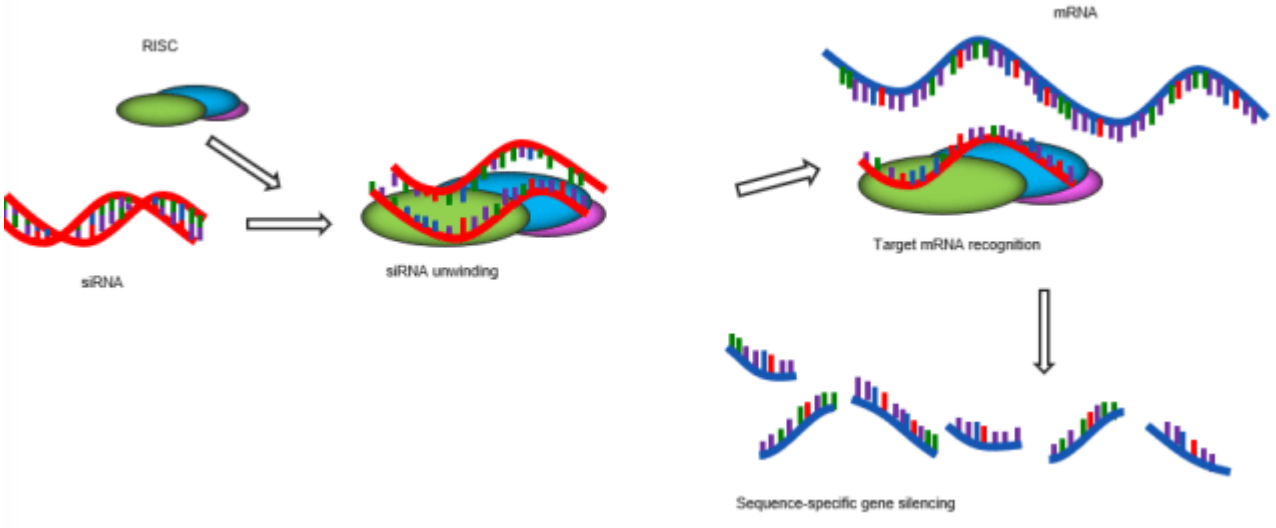
After discussing what oligonucleotides are and why they are of interest, we are now going to turn our focus at the clinical significance of oligonucleotides and why they are an interesting target for disease control. Starting with the fact that these DNA-like molecules known as antisense oligonucleotides offer our most promising target for gene therapy.

The development of oligonucleotide drugs is nothing new. In 1978 the first reported synthetic oligonucleotide was found to act as an efficient inhibitor of protein expression[1]; with the first antisense oligonucleotide commercialized in 1998.[2] As potential therapeutic agents oligonucleotides can be specifically designed to prevent or alter the translation of genetic code which facilitates the possibility of a highly targeted treatment; setting them aside from other biologic drugs (e.g., monoclonal antibodies) which target proteins and in turn the effects of a disease rather than the root causes.

The central dogma of biology defines in genetics and molecular biology genetic information is coded in self-replicating DNA and undergoes transfer to messenger RNAs in transcription which act as templates for protein synthesis during translation. With oligonucleotide therapeutics, Mrna mRNA is targeted. Antisense therapeutics use either DNA or RNA oligonucleotides complementary to a known mRNA strand. Through RNAse H mediated activity, the mRNA strand is degraded, thus the target protein of interest is not expressed.



Short interfering RNA is another therapeutic oligonucleotide; the robust nature of these therapeutic agents has made them a favoured silencing tool for many. Unlike messenger RNA these are double stranded oligonucleotides of around 20-25 base pairs in length comprising of both sense and antisense strands. Upon entry into the cell this siRNA is cut up by a protein complex called a dicer and is then recognized by a complex of proteins known as RNA induced silencing complexes and referred to as RISC. A strand of RNA, typically the antisense, is then loaded allowing the RISC complex to recognize this mRNA sequence which leads to sequence specific gene silencing.



Challenges for Oligonucleotides as Therapeutics:

The success of therapeutic oligonucleotides is dependent on their recognition by the target mRNA coupled with their pharmacokinetics upstream however the delivery challenges facing these interesting molecules are two-fold. Their anionic charge prevents them from permeating cell membranes leaving them susceptible to nucleases and to be effective not only does the oligonucleotide need to reach the tissue of therapeutic interest it must also reach the correct intracellular compartment for therapeutic activity. Overcoming these hurdles to delivery is typically achieved either by incorporating the oligonucleotide into some form of carrier or by chemically altering the oligonucleotide with a targeting ligand. The relative importance of the various barriers will depend on the chemical and physical properties of the oligonucleotide therapeutic being employed. For example, the biodistribution of antisense or siRNA oligonucleotides when used as individual molecules will obviously be quite different from that attained when some type of nanoparticle carrier is used.

Antisense oligonucleotides are negatively charged, and this can lead to digestion through the action of serum nucleases which post digestion leads to rapid clearance by the host. It is also known that oligonucleotides can be degraded in the lysosomes or expelled from the cells via other un-desirable pathways.[3] Conjugation of the target oligonucleotide to a ligand molecule will improve its retention in blood which in turn leads to better transition to the cell of interest. One such ligand which has shown promise through conjugation at the 5' end is Vitamin E. It was deemed a suitable candidate as it has well documented safety studies in humans and physiological movements are also well characterized. When the Toc-siRNA complex was tested for efficacy the gene silencing effect was found to be much higher than other ligand conjugates such as cholesterol conjugated siRNA for a 29mer antisense strand.[4] Aptamers are longer oligonucleotides of 40 60mers which bind to specific target molecules and because of this offer one of the most promising tools for targeted oligonucleotide delivery however as such are yet to realize this potential in a clinical setting.[5]

Oligonucleotides have been under development for several decades and to date the FDA have approved 11 drugs of which 8 are in regular circulation. The target therapeutic areas for oligonucleotides are also diversifying to include oncology, infectious disease, metabolic disorders and genetic disorders. Two further oligonucleotide drug candidates are in the pre-registration phase with over 150 more listed in clinical trials.

Have any questions regarding the above information or any other technical inquires? Reach

out to our technical specialists today through Chat Now – our free online service that can help with method optimization, chromatographic tips, product recommendations, and provide quotes for an easier buying process.

Start chatting today at www.phenomenex.com/chat.

References

1. J Pathol. 2012 Jan; 226(2): 365–379
2. Jiang K (February 19, 2013). “Biotech comes to its ‘antisenses’ after hard-won drug approval: Spoonful of Medicine”. *Nature Medicine: Spoonful of Medicine Blog*
3. Yu B, Zhao X, Lee LJ, Lee RJ. Targeted delivery systems for oligonucleotide therapeutics. AAPS J. 11, 195–203 (2009)
4. Nishina K, Unno T, Uno Y, Kubodera T, Kanouchi T, Mizusawa H, Yokota T. Efficient in vivo delivery of siRNA to the liver by conjugation of alpha-tocopherol. Mol Ther. 2008; 16:734-740.
5. Rudolph J L. Nucleic Acids Res. 2016 Aug 19; 44(14): 6518–6548