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Commentary

Double-stranded DNA invasion by anti-gene oligonucleotide clamps

Angana De[1] and Raman Bahal[1]

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Anti-gene oligonucleotides (AGOs) have been developed to target genomic DNA, which can regulate gene expression to curb the root cause of diseases. In this issue of *Molecular Therapy Nucleic Acids*, Umek *et al.* reported a promising AGO with a clamp design to target the double-stranded DNA (dsDNA). Umek *et al.* designed a series of mixmers made of locked nucleic acid (LNA), DNA, and phosphorothioate (PS) to target the *Huntington* (*HTT*) gene.[1] This AGO effectively knocked down *HTT* mRNA expression in patient-derived fibroblasts of Huntington's disease (HD). Therefore, this work aims to demonstrate a potential anti-gene strategy for downregulating gene expression in genetic disorders.

Targeting genomic DNA poses numerous challenges, including cellular and nuclear permeability and accessing the target site within chromatin-packed dsDNA. Sequence-specific oligonucleotides have been developed to target the DNA via Watson-Crick (WC) base pairing or Hoogsten (HN) base pairing. Strand-invading oligonucleotides utilize the WC base-pairing rule following the displacement of homologous strands. Triplex-forming oligonucleotides (TFOs) utilize the HN base-pairing rule. The combination of WC and HN base pairing leads to tail-clamp AGOs. Tail-clamp AGOs showed promising results in effective hybridization and invasion of dsDNA. The peptide nucleic acids (PNA)-based tail-clamp anti-gene nucleic acids were tested to target the dsDNA. A tail-clamp design with chemically modified gamma PNA has been successfully tested to target the genomic DNA of the *c-MYC* oncogene to inhibit transcription in multiple pre-clinical mouse models *in vivo*.[2]

Here, the author uses a clamp-based AGO mixmer to invade dsDNA efficiently. The anti-gene strategy could target the dsDNA, which inhibits the transcription of the pre-mRNA and dysregulates the mRNA and aberrant protein levels (Figure 1). Therefore, this strategy possesses several advantages: downregulation of aberrant protein from mutant DNA and single-site dsDNA targeting with less site optimization.[3] Umek *et al.* demonstrated that targeting the dsDNA of the *HTT* gene with the sequence-specific anti-gene constructs downregulates the mRNA level without off targets.

The authors methodically optimized the design of the anti-gene constructs and determined the gene expression patterns. Initially, the intercalating linker (M3) position was optimized to design the clamp nucleic acid analogs for efficient dsDNA invasion. The M3 linker comprises two naphthalene rings connected via a 1,3-diethynylphenyl moiety that links the WC arm and TFO arm. Further, they evaluated its strand invasion potential *in vitro* using a plasmid-based assay in a dose-dependent manner. Other intercalating linkers like acridine derivatives and benzoquinoquinoxaline have also been explored previously to enhance the stability of the TFO in dsDNA strand invasion.[4] Further, the PS backbone modification was evaluated, which delayed hybridization and invasion of dsDNA. However, the efficiency of the dsDNA invasion of PS-modified AGOs was similar to that of the phosphodiester. The advantage of delayed invasion of the PS-modified AGO could be its accumulation in the nucleus *in vivo* following improved binding kinetics. The authors also utilized S1 nuclease for single-strand digestion to confirm the binding of these AGOs with dsDNA. Physiological factors like pH were evaluated to determine the protonation of the cytosine and its binding kinetics during dsDNA invasion assays.

Umek *et al.* evaluated the series of anti-gene constructs for the knockdown efficacy of the *HTT* gene in HD patient-derived fibroblasts. HD is an autosomal neurodegenerative disorder caused by the expansion of CAG repeats in the *HTT* gene.[5] Therefore, targeting this specific site in the mutant DNA of the *HTT* gene could result in an efficient knockdown of its mRNA and protein. The authors demonstrated the ability of these anti-gene constructs to specifically invade a single site in dsDNA and inhibit the transcription of the *HTT* gene.

A few challenges still need to be addressed for anti-gene technology. Firstly, an *in vivo* study is required to determine the efficacy and safety profile of the anti-gene constructs. Additionally, tissue-specific biodistribution is necessary for the clinical relevance of such an anti-gene strategy. Therefore, delivery strategies like conjugating with tissue- and cell-specific ligands are required for its broader application.

In summary, the clamp AGO by Umek *et al.* can potentially target the genomic DNA of the *HTT* gene at a single site of the dsDNA. Different designs of the clamps with the linker and backbone modifications demonstrate a broader array of anti-gene nucleic acid chemistry. These findings suggest that anti-gene strategies can be implied to target various genes for treating genetic disorders as well as cancer.

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DECLARATION OF INTERESTS

The authors have no conflicts of interest to declare.

[1]Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT 06269, USA

Correspondence: Raman Bahal, Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT 06269, USA.
E-mail: raman.bahal@uconn.edu

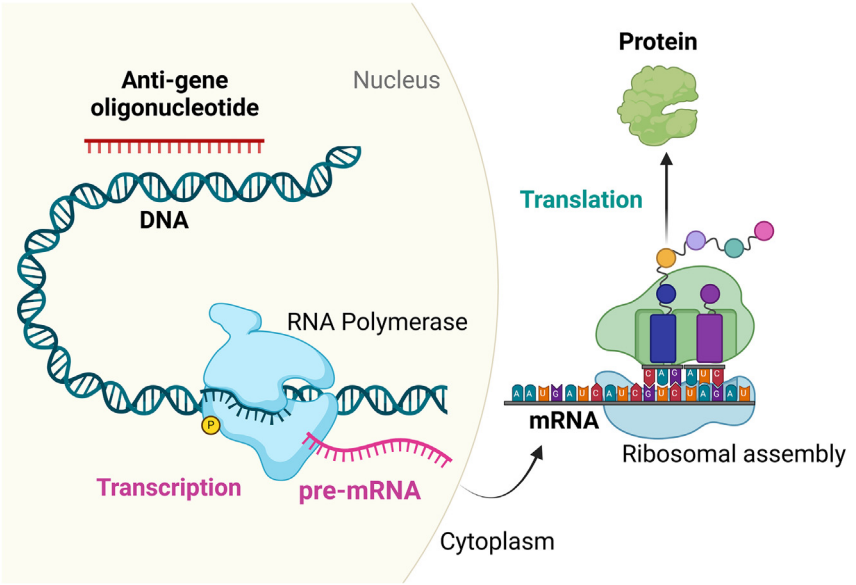

Figure 1. Binding of anti-gene oligonucleotide with the double-stranded genomic DNA in the nucleus, followed by inhibition of transcription and translation

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