УДК 577.29

ENHANCING RNA DEGRADATION WITH FUNCTIONAL NUCLEIC ACIDS: A MULTIVALENT APPROACH

Ннаньеруго В.О. (Университет ИТМО**), Дубовиченко М. В. (**Университет ИТМО**)**

Научный руководитель – Эльдиб А.А. (Университет ИТМО)

Introduction. Functional nucleic acids (FNAs) encompass nucleic acids that extend beyond their traditional genetic roles, exhibiting specialized recognition and catalytic functions. These natural or synthetic molecules achieve their activity through conformational changes, hybridization, and distinct chemical interactions [1]. These molecules, including aptamers, nucleic acid enzymes, and antisense oligonucleotides (ASOs), have been widely explored for their potential in diagnostics and therapeutic applications [1]. Among FNAs, Antisense oligonucleotide (ASO) technology holds significant promise in gene therapy; however, its efficiency in vivo is often constrained by the limited binding affinity of short ASOs (15–30 nucleotides), particularly when targeting RNA structures with stable secondary folding. To enhance RNA cleavage efficiency while maintaining specificity, we present a bivalent ASO design that facilitates binding across an extended region of folded RNA, thereby improving target recognition and cleavage.

Main part. Multivalent binding is a naturally occurring mechanism that enhances molecular interactions by increasing binding stability (avidity) without compromising specificity [2]. Despite its advantages, this approach has been underexplored in mRNA targeting by ASOs. Conventional ASOs rely on Watson-Crick base pairing to bind their RNA targets, leading to RNase-H dependent degradation. However, their therapeutic potential is hindered by selectivity and efficiency challenges [3]. While longer ASO sequences can improve RNA binding, they often compromise specificity. To address this limitation, three bivalent ASO variants were designed and evaluated: BivASO, BivASO(c), and BivASO(t). We hypothesize that the BivASO(t), with shorter sequences binding across a region of folded RNA, will achieve improved RNA cleavage efficiency and specificity compared to conventional ASOs.

To test this hypothesis, single-stranded ASOs and bivalent ASOs were designed to target RNA-58, a fragment of the strA gene responsible for streptomycin resistance [4]. RNA-58, which has a folding energy of -24.50 kcal/mol [5], was incubated with each ASO variant individually and in tandem to assess their binding interactions and RNase H-dependent cleavage efficiency.

Among the tested designs, the multivalent ASOs generally exhibited enhanced cleavage efficiency compared to their monovalent counterparts. BivASO and BivASO(t) demonstrated cleavage efficiencies of approximately 50% and 31%, respectively. The superior efficiency of BivASO is likely due to its longer binding sequence, which enhances target recognition and stability. However, when specificity was evaluated by introducing mismatched bases, BivASO(t) exhibited superior selectivity compared to the other variants. This can be attributed to its shorter binding arms, as longer nucleotide sequences tend to tolerate mismatches more readily.

Conclusions. These findings highlight the potential of BivASO(t) in enhancing RNA cleavage efficiency while maintaining high specificity. The improved performance of this design presents a promising strategy for overcoming the limitations of traditional ASOs in clinical applications, potentially advancing their use in gene therapy.

Список использованных источников:

1. Wang J. et al. Multiple functionalities of functional nucleic acids for developing high-performance lateral flow assays// Trends in Analytical Chemistry. 2022. Vol. 148.

2. Dubovichenko M.V. et al. Multivalent DNAzyme agents for cleaving folded RNA // Nucleic Acids Res. 2024. Vol. 52, № 10. P. 5866–5879.

3. Drozd V.S. et al. Binary Antisense Oligonucleotide Agent for Cancer Marker-Dependent Degradation of Targeted RNA // Nucleic Acid Ther., 2022. Vol. 32, № 5. P. 412–420.

4. Sundin G. W and Bender C.L. Dissemination of the strA-strB streptomycin-resistance genes among commensal and pathogenic bacteria from humans, animals, and plants // Mol. Ecol. 1996. Vol. 5, № 1. P. 133–143

5. Zuker M. Mfold web server for nucleic acid folding and hybridization prediction // Nucleic Acids Res. 2003. Vol. 31, № 13.