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DEVELOPMENT OF A HIGHLY SELECTIVE BINARY ANTISENSE OLIGONUCLEOTIDE-BASED CONSTRUCT TO CLEAVE THE TARGET MRNA OF THE GFP GENE

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Inroduction. Conventional cancer therapies have many side effects, are invasive and often leads to recurrence of the disease. Therefore, the necessity of developing new approaches such as oligonucleotide gene therapy are needed. Oligonucleotide gene therapy is the use of functional nucleic acids, like antisense oligonucleotides (ASOs), to treat cancer. ASOs are short, single-stranded DNA molecules that can bind to the target mRNA according to the principle of complementarity. The enzyme RNase H can interact with the resulting DNA|RNA heteroduplex and degrade the targeted mRNA, leading to gene knockdown and cell death. Targeting cancer marking lead to inhibition of the progression but doesn't kill the cell, so an efficient target is to knockdown a housekeeping gene which is vital for cell survival [3]. Based on this mechanism, our colleagues previously designed a binary antisense oligonucleotide (biASO) capable of cleaving the mRNA of the green fluorescent protein (GFP) gene only in the presence of a cancer marker biomolecule [2].

Main part. Our main goal is to create a design based on biASO, which 1) activates therapy only at high concentrations of markers, 2) is not activated in the presence of similar sequences containing single nucleotide substitutions (mismatches).

MicroRNAs (miRNAs) are small non-coding sequences 18–25 nucleotides long that are involved in the regulation of gene expression. The miRNA-17 family is involved in a wide range of malignancies, and their overexpression is a marker of many types of cancer. However, miRNAs are short chains, and similar sequences can be metabolites of normal cells, which in turn increases the requirement for the selectivity of drugs based on antisense oligonucleotides [1].

We implement new input (oncomarker miRNA-17) binding sites to the design to activate mRNA degradation in the presence of high concentrations of input miRNA-17 molecules. This will give our design a threshold logic in relation to the concentration of oncogenic markers, which will lead to increase the sensitivity of the approach to cancer cells.

To demonstrate selectivity, we used the miRNA-20 marker molecule, which had an identical sequence to miRNA-17 but contained two single nucleotide substitutions at the beginning and in the middle of the binding site with biASO chains. In the presence of miRNA-17 sequences had the ability to cleave GFP mRNA, which was not observed in the presence of miRNA-20, allowing us to draw conclusions about the selectivity of the system.

Conclusion. In this work, we have design constructs based on biASO, which initiate the mRNA degradation by RNAse H only at high concentrations of markers and is not activated in the presence of similar sequences, due to which our construct meets the goals. In the future, we plan to optimize this design to achieve selectivity in the presence of a single nucleotide substitution and to test the efficiency of the constructs in cells.

References:

1. Bobbili, M. R., Mader, R. M., Grillari, J., & Dellago, H. (2017). OncomiR-17-5p: alarm signal in cancer? Oncotarget, 8(41), 71206.

2. Drozd, V. S. Binary Antisense Oligonucleotide Agent for Cancer Marker-Dependent Degradation of Targeted RNA / V. S. Drozd, A. A. Eldeeb, D. M. Kolpashchikov, D. D. Nedorezova // Nucleic Acid Therapeutics, 2022. – P. 412-420.

3. Nedorezova DD, Dubovichenko MV, Belyaeva EP, Grigorieva ED, Peresadina AV, Kolpashchikov DM. Specificity of oligonucleotide gene therapy (OGT) agents. Theranostics 2022; 12(16):7132-7157. doi:10.7150/thno.77830.