Секция «Молекулярная биология»

Oncomarker modulated RNA cleavage Using threshold Binary DNAzyme

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Cancer is the second leading cause of death worldwide, according to the World Health Organization. The disadvantages of existing treatment methods, such as invasiveness and negative side effects, as well as the rapid increase in morbidity in the world, raise the problem of new types of cancer therapy. One of the most promising approaches is oligonucleotide therapy. The aim of this study was to develop a 5-input binary deoxyribozyme threshold (5i-BDz) gate that effectively cleaves specific RNA of cancer cells only in the presence of high concentrations of biomarker microRNA and has a low efficiency at low concentrations of this marker. The advantage of this mechanism is its high selectivity since activation in the presence of high concentrations of the marker should reduce the risks in relation to healthy cells.

Deoxyribozymes (DNAzymes) are single-stranded DNA nucleotides that can catalyze several reactions as usual enzymes. All DNAzymes have a catalytic core that is packed between two "arms" - sequences of nucleotides complementary to the target RNA. RNA cleavage occurs only if the arms bind to the complementary RNA target on the specific site. This allows the catalytic core to form a tertiary structure and catalyze the RNA cleavage reaction [1]. To develop new types of gene therapy, it is necessary to solve several problems related to poor accessibility, off-targets, and the efficiency of RNA targets. An example of a successful solution to these problems can be a binary (divided into 2 parts) DNAzyme activated by an oncomarker microRNA. Such design can cleave important targets, such as the mRNA of the housekeeping gene and should lead cancer cells to death [3,4].

We have developed a binary DNAzyme having 5 input threshold gates (5i-BDz). In our design, the arms binding the cancer marker and the arms binding the target RNA have 4 loops preventing the activation of the agent with low concentration of markers (see Fig.1). Such loops require an input of five times the number of molecules of overexpressed cancer markers to activate catalytic activity regarding the mRNA of the housekeeping gene. According to our results (see Fig.2), the threshold system hypothesis is well respected. Design is activated only in the presence of a five-time concentration of the cancer marker (20nM) compared to simple designs ("yes gate"-0.5 nM), and designs activated by a smaller number of marker molecules.

This opens up a range of opportunities for the field of specific cancer-treating molecular robotics and gene therapy.

References

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2) Nedorezova D.D. et al. Deoxyribozyme-Based DNA Machines for Cancer Therapy // ChemBioChem. 2020. Vol. 21. № 5. P. 607–611.
3) Oliveira A.G.G. de et al. RNA-Cleaving DNA Thresholder Controlled by Concentrations of miRNA Cancer Marker // ChemBioChem. 2021. Vol. n/a. № n/a.

Illustrations

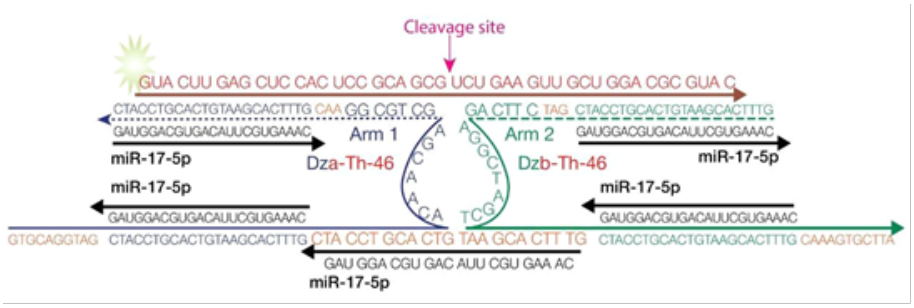

Рис. 1. Design of 5-input thresholding Binary deoxyribozyme (BDZ-5i) targeting artificial RNA-46 which is a fragment in the ORF of the house keeping gene DAD-1 (Defender against cell Death).

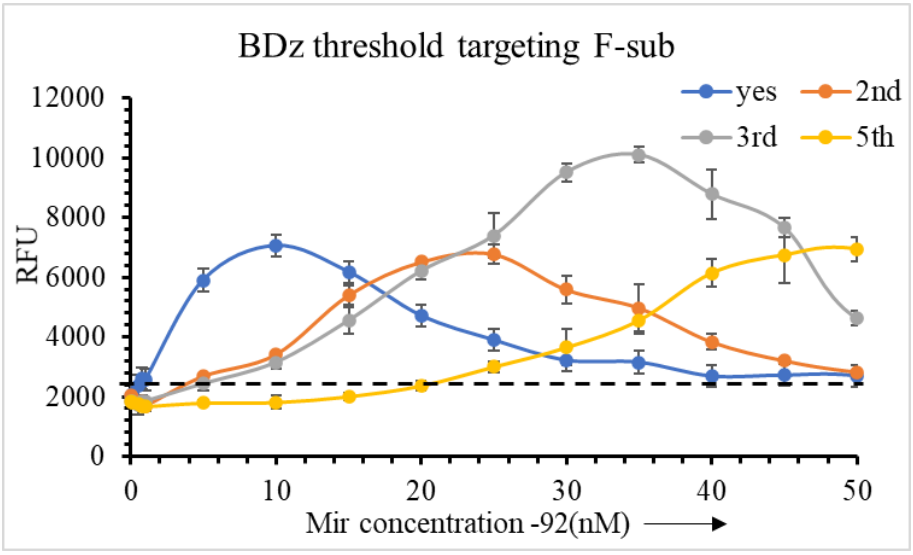

Рис. 2. Evaluation of the F-sub cleavage activity of the four different gates, the reaction was incubated at 37^0C for 1 h, followed by fluorescence spectrophotometry recording and analyzing. The data are average values of three independent measurements.