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Development of DNA-based machines for selective detection of cancer-associated RNA

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Gene therapy is the method of treating genetic diseases by introducing healthy genes into the patient to replace damaged genes. This technology can potentially avoid the side effects of current therapies but still face problems of non-selective and insufficient activation. Therefore, in order to overcome such issues, developing a new highly selective agent that can suppress the expression of target genes only in cancer cells is an urgent task. For this challenge, we decided to use high expressed and mutated RNA of oncomarkers as activators. This work is devoted to the development of DNA-machines for the detection of such oncomarkers, which can later be used to activate therapy agents [1].

Oncomarkers are mainly produced by both healthy and cancer cells. Their overexpression is often an indicator of a malignant tumor. After database and literature analysis we decided to select *KRAS* and *ERBB2* proto-oncogenes as first candidates. KRAS mutated forms are commonly found in many types of cancer (leukemia etc). ERBB2 is an epidermal growth factor receptor which increased expression is crucial in the pathogenesis of some aggressive types of breasts, ovarian and other cancers.

We develop DNA-machines based on DNAzymes for detecting both of these oncomarkers. DNAzymes are single-stranded nucleotides that catalyze reactions as enzymes. Their structure includes a catalytic core that cleaves RNA directly and two substrate binding arms that are complementary to the target sequence. To increase the selectivity of DNAzymes in presence of specific marker, binary DNAzymes were developed, where catalytic core was split in two parts and additional arms that are complementary to marker were added.

We have designed two DNA-machines that have two pairs of arms for selective ERBB2 detection and capable of cleaving a) fluorescent substrate (F-sub) and b) RNA of the DAD1 gene (Fig.1). The results showed that 5nM of the F-sub targeting DNA-machine could detect 0,4 nM of ERBB2 molecules. The detection efficiency of 100 nM of the DAD1 targeting machine showed that 10 nM of ERBB2 were detected with a maximum cleavage efficiency of 30% (Fig.2). For KRAS SNP detection a construct targeting F-sub was designed. The DNA-machine needs to identify the existence of a single-nucleotide polymorphism (SNP) mutation located in 13th codon of the KRAS gene. With the help of The UNAFold Web Server machine was designed to have melting temperature equal to 64.9°C in comparison to the SNP target which prevents the machine from binding with wild-type KRAS (55.8°C) (Fig.1).

The final goal of the project is to create a highly selective anticancer DNA nanorobot. The high selectivity of agents to act against housekeeping genes will be achieved by a rationally designed sensing module, which was partially achieved in this work.

References

1) Kolpashchikov D.M. Evolution of Hybridization Probes to DNA Machines and Robots // Acc. Chem. Res. American Chemical Society. 2019. Vol. 52. No 7. P. 1949–1956.

Illustrations

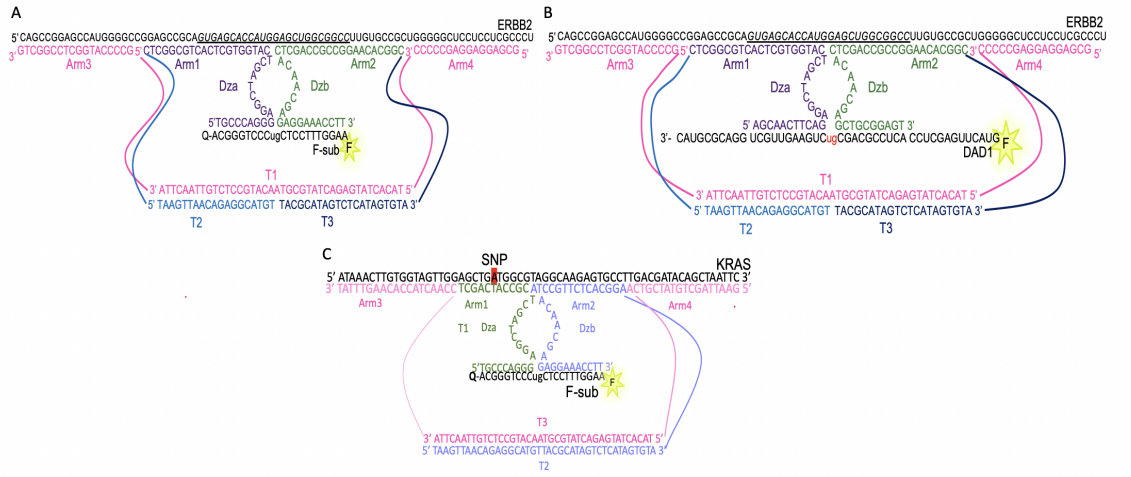

Рис. : The design of three oncomarkers detected DNA-machines targeting A) ERBB2 and fluorescent substrate (F-sub), B) ERBB2 and RNA of the DAD1 (Defender against cell Death) gene with fluorescent label (FAM), C) KRAS with SNP and F-sub.

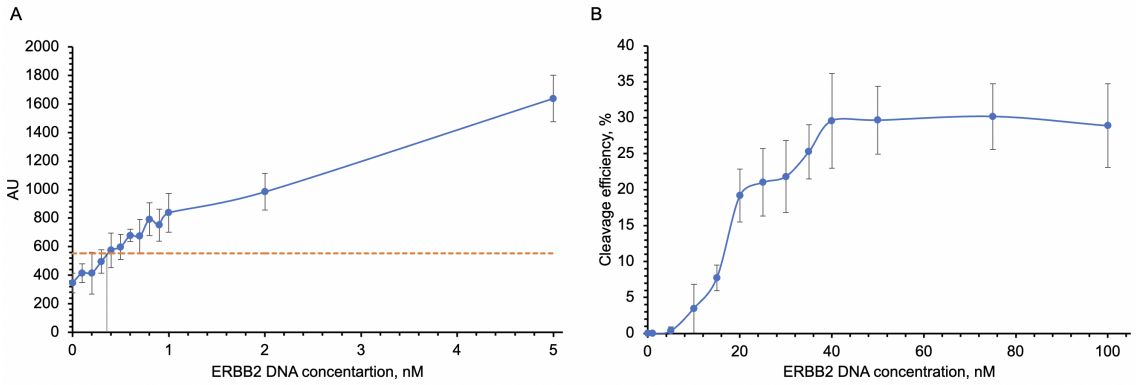

Рис. : The results of ERBB2 detection efficiency experiments for A) DNA-machine targeting F-sub and B) DNA-machine targeting DAD1. A) Machine with concentration 5 nM was incubated with artificial ERBB2 DNA (80 nt) in range of concentrations from 0,1 to 5 nM under conditions of 200 mM magnesium ions for 3 hours at 37 °C. The results of the detection efficiency were tested using a spectrophotometer. B) Machine with concentration 100 nM was incubated with artificial ERBB2 DNA (80 nt) in range of concentrations from 1 to 100 nM for 24 hours at 37 °C in solution, containing 2 mM of Mg. The detection efficiency was tested by denaturing PAGE electrophoresis.