УДК 577.29

DEVELOPMENT OF A DNA-BASED NANOCONSTRUCTION TO DETECT LOW CONCENTRATIONS OF CANCER-ASSOCIATED MOLECULES

Kulikova A.V. (ITMO University), **Ace V.D.** (ITMO University), **Patra C.** (ITMO University),
Scientific supervisor – PhD, professor Kolpashchikov D. M.
(University of Central Florida)

Introduction. Gene therapy is one of the most promising areas of medicine today, particularly in oncology. Gene therapy agents can avoid many of the disadvantages of existing therapies, such as invasiveness and side effects. However, gene therapy agents also face problems of low efficiency and selectivity [1]. The solution to this problem could be the creation of a DNA nano construction capable of recognizing marker molecules specific to different types of cancer (oncomarkers) and performing a therapeutic function by silencing the target housekeeping gene [2]. The present study involved the analysis of literature sources and the selection of oncomarkers with high expression in selected cancer cell lines as well as the development and optimization of the DNA nanomachine for the detection of low concentrations of selected markers *in solution*.

Main part. The solution to existing problems of gene therapy agents may be a DNA nanorobot - an oligonucleotide structure comprising four key modules that perform the main functions of a therapeutic agent: automatic delivery, selection of cancer cells, logical analysis (comparable to a computer operation) and therapeutic activity - knockdown of the target housekeeping gene. To distinguish cancer cells, it is necessary to select molecules that are overexpressed by cancer cells compared to healthy cells, or genes whose mutations are associated with cancer (oncogenes). In the course of the literature and database searches, the following cancer markers were selected as the main candidates: *ERBB2*, *KRAS* and microRNA 21 [3, 4]. KRAS is a proto-oncogene, somatic mutations of the KRAS gene are commonly found in many types of cancer, including leukemia, colorectal cancer, lung cancer and others. ERBB2 is normally the human epidermal growth factor receptor 2 and proto-oncogene; amplification or increased expression of the gene for this protein plays an important role in the pathogenesis and progression of some aggressive types of breast, ovarian and other cancers. High expression of microRNA 21 has been confirmed in many types of cancer, including lung, breast, blood, and others.

The design of two DNA nanomachines based on DNAzymes has been developed. DNAzymes are synthetic short DNA sequences with catalytic activity in the presence of Mg ions. A conventional DNAzyme consists of a catalytic core that cleaves RNA directly and substrate binding arms that are complementary to the target RNA sequence. However, if the catalytic core is split into 2 parts and another pair of arms complementary to the marker sequence is added, a selective catalytic reaction can be achieved. We have developed two DNA nanomachines with two pairs of arms capable of cleaving a) fluorescent substrate (F-sub) and b) RNA with fluorescent label (FAM) the DAD1 gene only in the presence of ERBB2 gene.

The constructed DNA machine targeting F-sub was tested at a concentration of 10 nM and an ERBB2 concentration in the range of 0,1 to 20 nM. The solutions were incubated for 3 hours at 37 degrees in a buffer containing 200 mM magnesium. The results of the detection efficiency were tested using a spectrophotometer and showed that the DNA machine could detect oncomarker molecules with concentrations 500 pM and higher. The machine targeting DAD1 was tested at a concentration of 100 nM and an ERBB2 concentration ranging from 1 to 100 nM under conditions of 2 mM magnesium and incubation for 24 hours at 37 degrees. The detection efficiency was tested by denaturing PAGE electrophoresis. The results showed that DNA nanomachines successfully detected

10 nM ERBB2 and a maximum efficiency of DAD1 RNA cleavage was achieved with ERBB2 DNA concentration equals 40 nM.

Conclusion. The results showed that the constructed DNA machines are able to detect low concentrations of the synthetic ERBB2 sequence. However, the concentrations of oncomarkers in living cells can be much lower. Future work will include optimizing the design of a DNA machine to detect lower concentrations of the oncomarker ERBB2 and testing the effectiveness of a machine to detect isolated total RNA.

List of references:

[1] D. D. Nedorezova, M. V. Dubovichenko, E. P. Belyaeva, E. D. Grigorieva, A. V. Peresadina, and D. M. Kolpashchikov, 'Specificity of oligonucleotide gene therapy (OGT) agents', *Theranostics*, vol. 12, no. 16, pp. 7132–7157, 2022, doi: 10.7150/thno.77830.

[2] D. D. Nedorezova, A. F. Fakhardo, D. V. Nemirich, E. A. Bryushkova, and D. M. Kolpashchikov, 'Towards DNA Nanomachines for Cancer Treatment: Achieving Selective and Efficient Cleavage of Folded RNA', *Angew. Chem. Int. Ed.*, vol. 58, no. 14, pp. 4654–4658, 2019, doi: 10.1002/anie.201900829.

[3] E. Birkeland *et al.*, 'KRAS gene amplification and overexpression but not mutation associates with aggressive and metastatic endometrial cancer', *Br. J. Cancer*, vol. 107, no. 12, pp. 1997–2004, Dec. 2012, doi: 10.1038/bjc.2012.477.

[4] D. Yu and M. C. Hung, 'Overexpression of ErbB2 in cancer and ErbB2-targeting strategies', *Oncogene*, vol. 19, no. 53, pp. 6115–6121, Dec. 2000, doi: 10.1038/sj.onc.1203972.