Секция «Биофизика и нанобиотехнологии»

DNA construct based on DNAzymes for selective SNP detection in KRAS

Научный руководитель – Eldeeb Ahmed

Ereshko D.S.[1], *Ace V.D.*[2], *Patra C.K.*[3]

1 - Санкт-Петербургский национальный исследовательский университет информационных технологий, механики и оптики, Санкт-Петербург, Россия, *E-mail: malasha601@gmail.com*; 2 - Санкт-Петербургский национальный исследовательский университет информационных технологий, механики и оптики, Saint Petersburg, Россия, *E-mail: veronika.acc@mail.ru*; 3 - Санкт-Петербургский национальный исследовательский университет информационных технологий, механики и оптики, Saint Petersburg, Россия, *E-mail: christinapatra@hotmail.com*

Gene therapy is a developing treatment method that focuses on targeting gene defects and manipulating gene expression in somatic cells. However, current gene therapy agents have low selectivity and are associated with many side-effects. To address this issue, there is a need for gene therapy agents that can be activated specifically in the presence of a particular oncomarker, such as the KRAS proto oncogene. This project aims to develop a 4-arms DNA construct that can distinguish between the wild-type and mutated forms of KRAS by detecting a single nucleotide polymorphism (SNP) in the RNA analyte in the future.

KRAS is a crucial gene that regulates cell maturation, growth, and death. Mutated forms of KRAS have been linked to various cancer types, including pancreatic, colorectal, and non-small cell lung cancer. One specific mutation, known as SNP, occurs in the 13th codon of the KRAS gene and can contribute to the growth and spread of cancer cells in the body. The DNA construct designed for this project is based on Deoxyribozyme (DNAzyme) technology, where it acts as catalysts similar to enzymes. The structure of a DNAzyme consists of two arms that bind to the target sequence and a catalytic core that cleaves RNA in the presence of magnesium ions. Cleavage of RNA only occurs when the target RNA binds to the arms and activates the catalytic core through the formation of a tertiary structure. To enhance the selectivity of RNA identification, a binary DNAzyme was developed. This sensor consists of two separate strands that bind to a specific region in the RNA and initiate the cleavage of a fluorescence reporter substrate (F-sub). The binary DNAzyme not only provides a signal but can also amplify it by cleaving several substrates upon binding to one RNA molecule.We designed a binary DNAzyme construct with two additional pairs of arms and a mismatch that makes it more selective. One part of the construct binds to the mRNA of the mutant KRAS analyte, while the other part binds to a F-sub. In the presence of SNP detection in KRAS, the catalytic core is formed, resulting in the cleavage of F-sub and the production of fluorescence. This can be detected using a spectrophotometer.

Developed a DNA construct that differentiates mutated and Wild Type artificial KRAS. As a following step we want to achieve detection in amplicons and cell RNA and enable the detection of SNP faster and cheaper than other existing methods. This information can be utilized to activate gene therapy agents specifically in the presence of the SNP mutation, leading to the cleavage of vital targets such as the housekeeping gene and ultimately causing the death of cancer cells.

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.