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DEVELOPMENT DEOXYRIBOZYME BASED MACHINE FOR GENOTYPING OF CANCER-ASSOCIATED KRAS MUTATION.

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Introduction. Gene therapy is one of the currently developing cancer treatment methods. Its purpose is to target gene defects or to give new properties to cells by manipulating gene expression via its knockdown in the patient's somatic cells. However, gene therapy agents have many side-effects because of their low selectivity [1]. In order to decrease the off-target effects, we want them to be activated only in the presence of a specific oncomarker. For instance, one of the proto-oncogenes is KRAS which due to a mutation leads to improper translation and eventually cancer.[2] Therefore, the aim of this project is to design and develop 4-arms DNA-machine that is able to distinguish between wild-type and mutated KRAS with only one mismatch in the DNA sequence.

Main part. KRAS is the gene that provides instructions to the cell's nucleus from outside of the cell. This protein sends signals that supervise cell maturation, growth and death. There is an unchanged (wild-type) form and some mutated forms of KRAS, which can be detected in several cancers, among pancreatic, colorectal and non-small cell lung cancer.[2] These mutated genes can cause growth and spread of cancer cells in the organism. One of them is a single-nucleotide polymorphism (SNP) mutation which produces a single nucleotide substitution located in 13th codon of the KRAS gene that we aim to identify by the DNA-machine. Our machine is based on deoxyribozyme (DNAzyme) technology. DNAzymes are single-stranded nucleotides of DNA that catalyze reactions as usual enzymes. The structure of DNAzyme includes a catalytic core which cleaves RNA directly in presence of Mg ions and two substrate binding arms that are complementary to the target sequence. RNA cleavage only occurs when the target RNA binds to the arms at a particular site and activates the reaction by forming the tertiary structure of the catalytic core. In addition, to increase the selectivity of RNA identification the binary DNAzyme was developed, a sensor which consists of two separate strands that bind to a certain region in the RNA and initiate the catalytic core to cleave a fluorescence reporter substrate (F-sub) after binding. The binary DNAzyme is not only giving a signal but also can amplify it by cleaving several substrates by binding to one RNA molecule.

We designed a binary DNAzyme machine which have two additional pair of arms. One part of it binds to the analyte mRNA of mutant KRAS and the other binds to a fluorescent substrate. In case of SNP detection in KRAS, the catalytic core is formed, and F-sub is cleaved producing fluorescence, which we can detect with a spectrophotometer. While designing the machine, its melting temperature in comparison to the SNP target using The UNAFold Web Server was calculated to be 69.2 °C. This melting temperature makes the machine more efficient and prevents it from joining with other forms of KRAS that have a much lower melting temperature.

Conclusion. This machine will allow us to detect whether a SNP mutation is present in our cell line. In this case we will be able to use this oncomarker as an activator for our gene therapy agents and cleave more vital targets such as the housekeeping gene, and should lead to the death of cancer cells, only in presents of SNP in KRAS analyte.[3]

List of references:

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