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Selective DNA-machine for SNP detection in KRAS

Ereshko D.S. (Alferov University)**, Ace V.D.** (ITMO University)**, Patra C.** (ITMO University)
Supervisor – Eldeeb A.A. (ITMO University)
Scientific supervisor – Phd, professor Kolpashchikov D.M. (University of Central Florida)

Introduction. These days one of the promising methods in modern medicine, particularly in cancer treatment, is gene therapy. However, it has some limitations including efficiency, selectivity, and leading to side effects. To overcome these issues and improve specificity, our goal is to develop a 4-armed DNA machine capable of distinguishing between wild-type and mutated KRAS, a common oncogene. Our construct is a DNA machine based on Deoxyribozyme (DNAzyme) technology, utilizing a binary DNAzyme that can detect the mutation by cleaving a fluorescence reporter substrate (F-sub) when it binds to the mutant KRAS mRNA. The machine has been designed to have an efficient melting temperature, ensuring specificity for the mutation. By utilizing this DNA machine, we aim to enable targeted gene therapy that selectively induces cell death only in the presence of the KRAS single nucleotide polymorphism (SNP). This offers a potential advancement in cancer treatment, as it provides a more precise and effective approach in treatment.

Main Part. Gene therapy agents aimed to address gene defects and modify cell properties by manipulating gene expression through knockdown in somatic cells. However, despite its potential, agents have multiple adverse effects primarily because they lack specificity [1]. To reduce unwanted outcomes, the objective is to activate these agents exclusively in the presence of particular oncomarkers. In this project our aim is to develop and optimize a 4-arm DNA machine capable of distinguishing between wild-type and mutated KRAS with SNP, which can be detected in several cancers, among pancreatic, colorectal and non-small cell lung cancer.

KRAS plays a vital role in relaying external instructions to the cell nucleus. It effectively regulates critical cellular functions like growth, maturation, and programmed cell death via its encoded protein. Unfortunately, mutations in KRAS disturb these processes, resulting in unregulated cell growth and metastasis.

A DNAzyme is a catalytic DNA molecule that has enzymatic activity. They are composed of a specific sequence of nucleotides. The nucleotide sequence is designed to form a three-dimensional structure that functions as the catalytic core, responsible for the DNAzyme's enzymatic activity. These reactions often involve the cleavage or modification of nucleic acid substrates. The enhanced binary DNAzyme comprises an additional two pairs of arms that serve specific purposes. One set of arms is designed to bind to the mRNA of the mutant KRAS analyte, whereas the other set binds to a fluorescent substrate (F-sub). This configuration enables the machine to effectively identify and respond to the presence of SNP in the KRAS gene. When the engineered machine detects the mutation in the KRAS gene, it triggers the activation of the catalytic core. This, in turn, leads to the cleavage of the fluorescent substrate (F-sub), resulting in the generation of detectable fluorescence. The intensity of this fluorescence can be accurately measured using a spectrophotometer, allowing for precise determination of the presence and extent of the SNP.

In this design it was decided to place the mutation right next to the catalytic core, which according to our hypothesis makes the core more unstable and the reaction more selective. In addition, we designed several free arms with additional mismatches in different parts of the arm to monitor changes in selectivity and activity of the reaction.

The Machines were tested with synthetic SNP and wild type analyte of KRAS at a concentration of 1 nM. It was incubated for 3 hours at 45 °C in a 200 mM Mg buffer. Also, F-sub only and machines without analyte were added as negative controls to show fluorescence background. We visualized results using a spectrophotometer.

The results of the experiment clearly demonstrate the effectiveness of the enhanced binary machine in detecting and responding to the presence of a SNP in the artificial KRAS gene. The machine exhibited a higher level of cleavage when paired with the sequence containing the SNP, indicating its ability to specifically recognize and respond to the mutant analyte. These findings validate the potential of the DNA machine as a reliable tool for cancer detection and treatment.

Conclusion. Development of an advanced DNA machine on the early stage has the potential to revolutionize cancer detection faster and chipper than other existing methods. Furthermore, by targeting specific mutant KRAS gene, this machine can activate gene therapy agents only in the presence of it, minimizing impact on healthy cells.[3] Additionally, the ability to selectively cleave vital components in cancer cells further enhances the treatment's specificity. These findings validate the potential of the 4 arms DNA machine as a reliable tool for cancer detection and treatment. Continued advancements in genetics and molecular biology offer hope for improved patient outcomes and contribute to the fight against cancer.

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