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TOWARDS INTRACELLULAR OVEREXPRESSION AND MUTATION RECOGNITION

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

Introduction. Although there have been significant advancements in cancer therapeutic strategies, there is still a long way to go in terms of finding an effective long-term solution. Traditional anti-cancer therapies have been proven to cause hurdles to the patients and their families, and to be a burden for the healthcare systems. More sophisticated and patient-centered cancer therapeutics are currently being researched, with gene therapy being one of the leading-edge approaches. However, only a few anti-cancer gene therapy agents have made it into clinical trials, with most of these being based on therapeutic nucleic acids (TNAs) such as antisense oligonucleotides (ASOs) and small interfering RNAs (siRNAs)[1].

Main part. Despite the significant potential of TNAs, there are still many challenges that prevent them from being utilized in clinical applications, such as degradation mediated by exonucleases, non-targeted and ineffective intracellular delivery, and insufficient activation. Cell/organelle membrane barriers, lyso/endosomal entrapment, and non-selective and insufficient activation of therapeutic tools lead to off-target effects, resulting in toxicity for healthy tissues, which is a critical issue in achieving effective treatment via TNA agents. Therefore, there is a need for the development of new, highly selective TNAs that can suppress the expression of target genes exclusively in cancer cells. As a solution, we are developing our original approach to cancer treatment – the anticancer DNA nanomachine capable of vital housekeeping genes' knockdown only in the presence of cancer marker RNAs (oncomarkers)[2]. The nanomachine is equipped with four modules responsible for different functions, including sensing the molecular cancer microenvironment, computing and decision making, therapeutic activation, and intracellular delivery.

The application of classical TNAs is not feasible when targeting housekeeping genes due to the non-specific nature of therapeutic agents, which would result in the suppression of all cells in the body, including healthy ones. To address this issue of specificity to malignant cells, we focus on the rational design of the sensing module of the nanomachine. The sensing module is designed to recognize preselected, cancer-specific oncomarkers based on their expression level and the existence of mutations. Overexpression and mutations of interest do not exist in healthy cells, a fact that will allow our sensing module to distinguish between malignant and healthy cells[3]. Furthermore, the sensing module must cleave the fragment of interest of the oncomarker, such as the fragment containing the mutation, making it available for subsequent computation or therapeutic modules of the greater anticancer DNA nanomachine[4].

Until date, we have conceptualized this sensing module in an antisense oligonucleotide (ASO)-based design. Four short ASO sequences complementary to the oncomarker fragment of interest, will be bound on a common tile increasing the local concentration of all four strands. The short length of each fragment will allow for highly selective recognition of the mutated oncomarker only, while they will not be able to hybridize in case of no mutation existence in healthy cells. Additionally, carefully designed alternations of chemical modifications will allow for cleavage of the oncomarker's fragment of interest in predetermined cleavage sites, by the endonuclease RNAse H. This fragment will then move along to the subsequent modules of the anticancer DNA nanomachine due to ascending melting temperature gradient.

Conclusion. In conclusion, the sensing module is only one out of the four vital parts of

the bigger anticancer DNA nanomachine. On this sensing module we rely our nanomachine's specificity against malignant cells, which will be achieved by the high selectivity against cancer-specific mutated markers. This research aims to make advancements in the field of cancer research and lay the foundation for the development of high-tech drugs for the individualized treatment of oncological diseases.

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