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Development of DNA Nanodevices for Cancer Marker-dependent activation of therapeutic function

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Introduction. In the realm of modern medicine, gene therapy stands as a promising approach, particularly in cancer treatment. However, it faces challenges such as efficiency, selectivity, and potential side effects. Traditional oligonucleotide gene therapy (OGT) agents, such as antisense, siRNA, and CRISPR/Cas9, have shown limited clinical success in anti-cancer treatments. These agents often lack the specificity needed to differentiate between cancerous and normal cells, leading to potential side effects and reduced efficacy [1]. The development of more precise and selective therapeutic strategies is crucial for advancing cancer treatment. To address these issues, we developed a DNA machine capable of distinguishing between wild-type and mutated KRAS, a common oncogene. Only in the presence of mutated KRAS the antisense oligonucleotide is formed to fulfill the function of cancer cell suppression. Our goal is to enable targeted gene therapy that selectively induces cell death only in the presence of the KRAS single nucleotide polymorphism (SNP), offering a more precise and effective approach to cancer treatment.

Main Part. KRAS plays a crucial role in transmitting external signals to the cell nucleus, regulating essential cellular functions such as growth, maturation, and programmed cell death. Mutations in KRAS disrupt these processes, leading to uncontrolled cell growth and metastasis [2]. Our DNA nanodevice is engineered to recognize a pathogenic SNP on cancer marker RNA, specifically the KRAS gene, which is frequently mutated in various cancers.
Our DNA nanodevice consists of two modules, a sensing and a therapeutic. The sensing module contains two deoxyribozymes, Dz1 and Dz2, which are responsible for recognizing and cleaving a mutated RNA sequence. Dz1 and Dz2 are two DNA strands, each with a catalytically active core and two RNA-binding arms that specifically bind to the mutated RNA sequence. The binding is facilitated by complementary base pairing between the arms and the target RNA. The mutation is positioned near the catalytic core of the deoxyribozymes to enhance selectivity. Upon binding to the mutated RNA, Dz1 and Dz2 cleave the RNA at specific sites, releasing a short fragment. The released fragment acts as an activator for the therapeutic module by connecting ASOa and ASOb to generate active binary antisense oligonucleotide (biASO). Upon encountering the target mRNA, the activated biASO hybridizes to the mRNA, leading to its RNase H-assisted degradation.
The device was tested for the ability of its sensing module to selectively recognize and process mutated KRAS RNA versus wild-type KRAS RNA, releasing more cleaved activator by 4.6 times, in the presence of mutated KRAS (for 30 min reaction under physiological conditions). It was also proven that the standalone actuation module functions only in the presence of the short cleavage product that bears the pathogenic SNP compared to longer or wild-type makers that do not activate the device, giving a signal of 25.4% target cleavage over the background noise. Moreover, by addition of the sensing module to biASO, we achieved oncomarker-dependent activation of biASO even in the presence of long length mutated KRAS but not in the presence of wild-type KRAS. This proved that the addition of a sensing module improved the sensing and processing of the oncomarker sequence, resulting in a “smart” DNA nanodevice capable of decision making.

Conclusion. This approach leverages the precision of DNA nanotechnology to ensure that the therapeutic action is only activated in the presence of the cancer-specific mutation, thereby minimizing damage to healthy cells. Our DNA nanodevice could potentially be used to selectively target and degrade mRNA in cancer cells with a specific KRAS mutation, utilizing a sensing module with deoxyribozymes to recognize the mutation and a therapeutic module with biASO to induce

mRNA degradation. Results demonstrated that the device could distinguish between mutated and wild-type KRAS, achieving up to 4.6 times more cleavage products with the mutated form and showing enhanced selective activity.

List of references:

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