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DESIGN OF A DNA MACHINE BASED ON SHRNA FOR MARKER-DEPENDENT RNA INTERFERENCE

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Annotation.
This paper presents the concept of combined DNA technology and RNA interference (RNAi) for developing a DNA nanomachine based on shRNA capable of regulating the expression of a target gene only in the presence of a marker sequence. This system can become the foundation for the development of new RNAi agents with high efficiency and selectivity towards suppression of cancer cells.

Introduction.
Nowadays RNA interference (RNAi) has become an affordable and simple technology for studying gene functions. The technology has a therapeutic potential as well. Fire and Mello shared the 2006 Nobel Prize in Physiology or Medicine “for their discovery of RNA interference - gene silencing by double-stranded RNA." After that, three drugs based on RNAi were approved by the US Food and Drug Administration (FDA). Currently development of RNAi technology is useful for down regulating genes that are associated with disease. We see the technology has some limitations - non controlled silencing target genes. Which is why we're suggesting combining DNA nano construction and RNAi technology to give the interfering agent a new property - marker-dependent activity.

Main part.
For this purpose, we selected an interfering agent - Ago-mediated shRNA (agoshRNA) targeted to EGFP mRNA. According to our experiences in the K562 cell line which expresses the gfp gene, agoshRNA has higher potency interference than canonical (Dicer-mediated) shRNA and siRNA. Several DNA-nanomachines were designed based on agoshRNA. The general concept is the addition of nucleotides in 5’- or 3’-ends in agoshRNA reducing potency (In the study by Guihua Sun and et all in 2015 showed that adding two to four As reduced the potency). Using DNA technology and marker-dependent DNAzyme (This was developed by Kolpashchikov D.M. and his research group), which cleaves a phosphodiester bond in oligonucleotide in presence of marker sequence, it will be possible to release active forms of shRNA from DNA-machine followed by downregulation of target genes. Consequently, the technology of downregulation genes will be controlled by marker-dependent processing which initiate cleavage and release the shRNA.

Conclusions.
Thus, this system will demonstrate that combining the two technologies allows to give marker-dependent activity that will be useful for therapeutic use. After conducting an experiment on the K562 cell line which expresses the gfp gene and choosing the best design of the DNA machine, we plan to design a DNA machine to release several shRNA by one marker sequence and implement this system using oncomarker dependent activation to suppress housekeeping genes exclusively in tumor cells.

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