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DEVELOPMENT OF DNA-MACHINE FOR DOUBLE STRANDED DNA CLEAVAGE

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Introduction.

CRISPR/Cas9 has become very popular among scientists because of its simplicity and accessibility among scientists. Its greatest advantage is that it works on the principle through Waston-Crick base-pairing between sgRNA and target DNA. Unlike ZFN and TALEN, CRISPR does not require protein engineering, easy to construct and simple to use. Despite all the advantages of the CRISPR/Cas9 system, it cannot yet be used for gene editing because of its off-target effect[1]. This is why we are proposing a new nucleic acid-based gene therapy method for the double-stranded DNA cleavage. We hypothesize that its protein-free structure, design and Waston-Crick base pairing principle will allow it to achieve high specific double-stranded DNA cleavage and provide the lower cost of the suggested method.

Main point. DNAzymes are showing promising development and success in the last few years starting from a developed probe to the binary RNA Cleaving DNAzymes and finally the DNA-hydrolyzing DNAzyme. The DNAzyme (13pb2) is a DNA oligonucleotide that Acquires catalytic activity, for ssDNA at a specific Cleavage site, This Catalytic activity is due to the existence of the catalytic core that can be formed after binding of the flanking regions to that sequence around the cleavage site . The aforementioned DNAzyme (13PB2) hydrolyzes the phosphodiester bond between the Guanine and Cytosine upon the formation of the core in presence of Zn+2 ions [2].

In this study, We propose A multicomponent DNAzyme-based machine that consists of two single-stranded DNAzyme each of them complementary to each target on the sense strand and on the antisense strand of the DNA target molecule. The DNM is equipped also with single-stranded LNA openers to unwind the Double-stranded DNA molecule and hybridize to it until the cleavage is implemented.

All of these arms come with the advantage of high selectivity since it will never unwind the dsDNA and be activated in case of mismatches or not fully complementary sequences.

However, the DNAzyme (13PB2) still has limitations with the cleavage rate as its low compared to RNA cleaving DNAzymes, also the dependency on Zn+2 Ions which can’t be found in high quantities in Vivo, unlike the RNA Cleaving DNAzymes That depends on the Mg+2 which can be found in cells in concentration 2mM.

Conclusions. The system was tested on single-stranded DNA artificial target, Testing of the system was successfully performed on single-stranded DNA Targets and cleavage was obtained after 4 hours incubation in 37 °C, The cleavage reaction was performed in a cleavage buffer (70mM HEPES, 1mM ZnCl2, 20mM MnCl2, 40mM MgCl2, 150mM NaCl). The results were viewed in PAGE urea gel electrophoresis 7M.

References:

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