Секция «Генетика»

Antisense Oligonucleotides-Mediated Gene Silencing for Cancer Therapy

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Nucleic acid-based technologies are a promising area in gene therapy [1]. One of the most successful of these is the antisense oligonucleotides (ASOs) technology. The widespread adoption of this technology is due to FDA approval for treating various diseases [2], ongoing clinical trials [3], and the recognition of its effectiveness within the scientific community. ASOs are short DNA-like molecules that bind to target messenger RNA (mRNA). This causes the activation of an RNase H enzyme, which cleaves ASO/mRNA duplex, preventing the production of disease-related proteins [4]. This gene manipulation technique has potential for use in the treatment of malignant neoplasms. It is worth noting that scientists tried to use ASOs for cancer treatment, but these drugs weren't approved for clinical usage. One of the reasons for this can be wrong choice of target, so to address this challenge we hypothesized that lowering the expression of the most vital gene mRNA can lead to apoptosis in cancer cells.

Systematic investigation in literature and DepMap portal database allowed to select 13 target genes: DHX8, DHX9, CSE1L, DARS1, DHPS, COPB1, AP2M1, DYNC1I2, EEF1A1, EEF2, EIF2S1, EIF2S3 and PSMB2. Bioinformatic analysis identified mRNA open regions, allowing the creation of multiple design options for each gene, enhancing the likelihood of ASO-target interaction. As a result, 38 different oligonucleotide designs have been generated for further testing. Primary screening involved MTT cytotoxicity testing 24-48 hours after transfection of oligonucleotides via liposomal delivery on the SKOV-3 human ovarian adenocarcinoma cell line. Oligonucleotides exhibiting the highest cytotoxicity were selected for secondary screening. Cell vitality was evaluated using Calcein AM fluorescent dye to measure intracellular esterase activity. The two most toxic ASOs targeting DARS1 and DYNC1I2 genes were identified, inducing cellular stress level around 65% at 250 nM after 24 hours (Fig. 1). RT-qPCR analysis revealed that the knockdown of these genes led to a reduction in expression by approximately two times after 24 hours. Cell cycle arrest occurred in the G2/M phase, and presumably the cells were unable to restore the loss of these genes, so after 48 hours we observed an increase of Sub-G phase (Fig. 2).

Thus, in this research, we have determined two noteworthy agents, which show a great ability to induce cellular stress, which leads to a significant decrease in viability.

References

1) Kim YJ, Sivetz N, Layne J, Voss DM, Yang L, Zhang Q, Krainer AR. Exon-skipping antisense oligonucleotides for cystic fibrosis therapy. Proc Natl Acad Sci U S A. 2022 Jan 18.

2) Stein CA, Castanotto D. FDA-Approved Oligonucleotide Therapies in 2017. Mol Ther. 2017 May 3;25(5):1069-1075.

3) Database of privately and publicly funded clinical studies: https://www.clinicaltrials.gov/

4) Bajan, S.; Hutvagner, G. RNA-Based Therapeutics: From Antisense Oligonucleotides to miRNAs. Cells 2020, 9, 137.

Illustrations

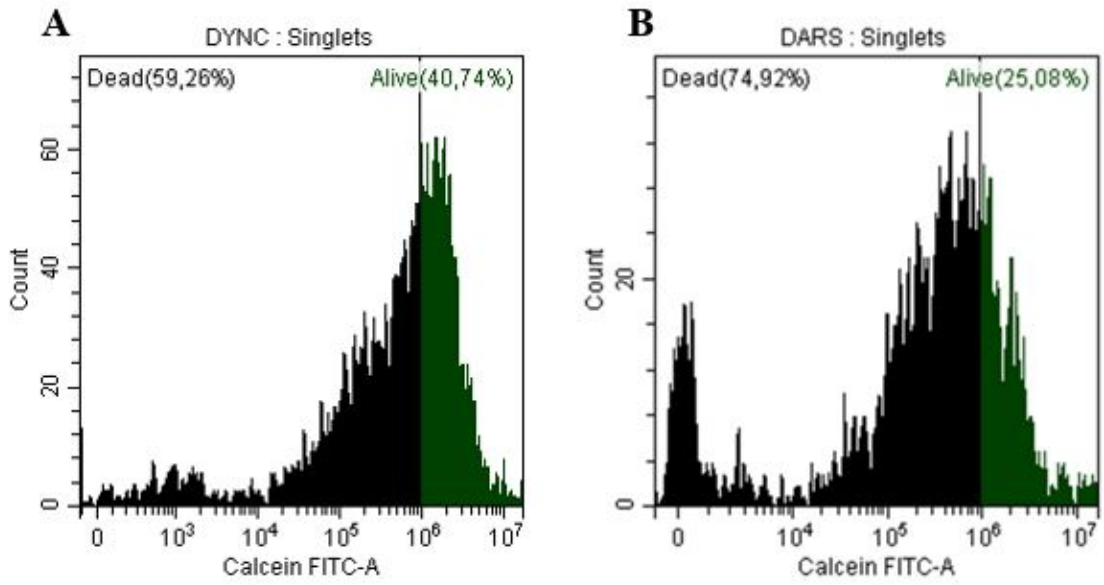

Рис. : Figure 1 - Esterase activity results after 24 hours. A - DYNC1I2, B - DARS1

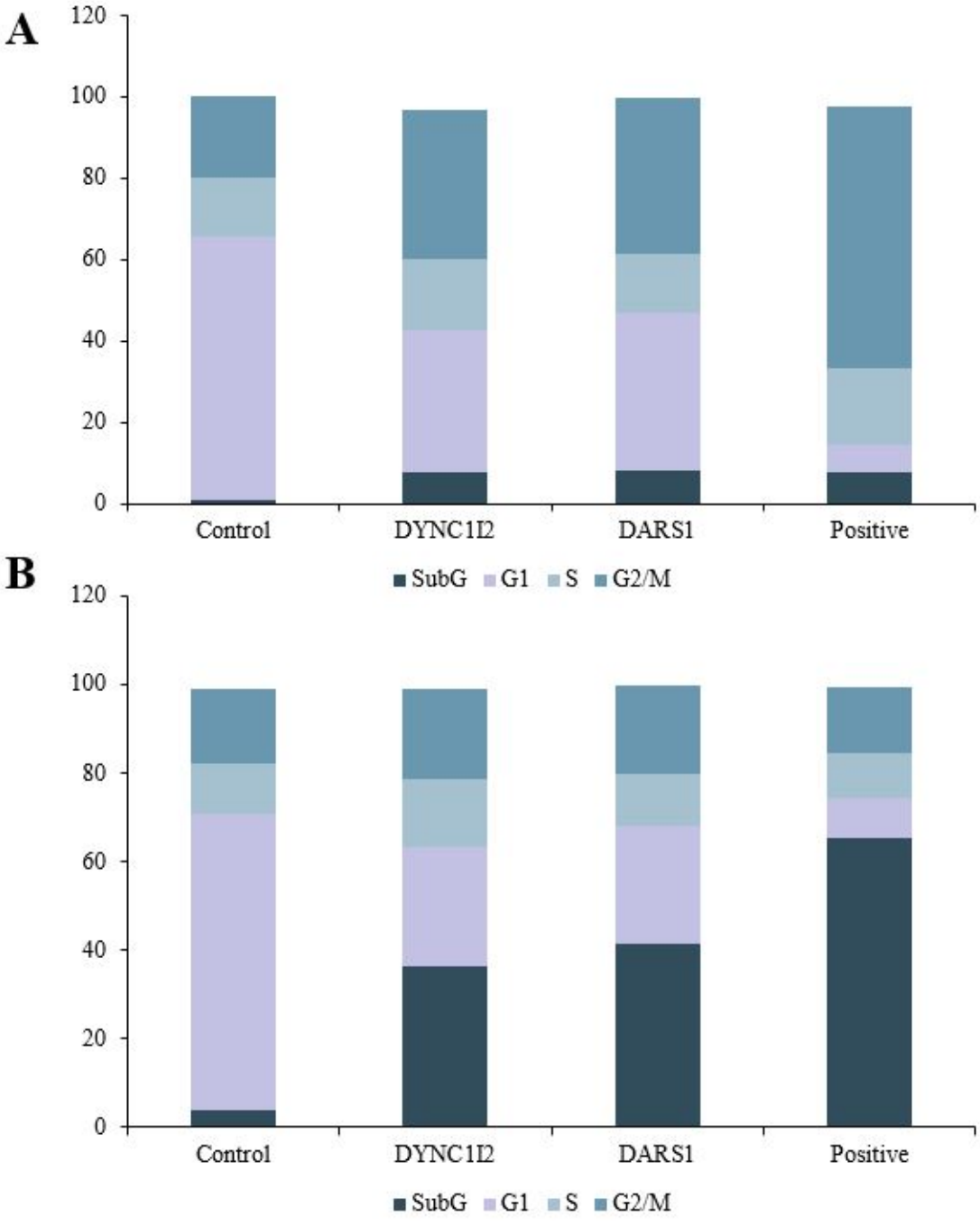

Рис. : Figure 2 - Cell cycle experiment. A - 24 hours, B - 48 hours