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DESIGN AND IN VITRO EVALUATION OF SHORT HAIRPIN RNA (SHRNA) EFFICACY

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Введение. RNA interference (RNAi) is a mechanism of gene regulation through mRNA degradation or translation repression that involves short RNA or DNA sequences. Over the past decades, the structures of molecules involved in RNA interference have been studied, cellular mechanisms have been described, and approaches to using RNAi in studying gene functions and treating diseases have been developed. For example, in 2003, it was shown that RNAi acts in mammalian cell culture [1], and in 2006, the first results of clinical trials of RNAi therapy were obtained [2].

The potential for using RNA interference in gene therapy is associated with the possibility of producing and delivering synthetic RNA molecules that are structurally similar to “natural” ones to cells. One type of such molecule is called short hairpin RNA or shRNA. shRNA constructs in plasmid DNA (pDNA) format are transcribed in the nucleus, which provides a long-lasting effect and allows regulation of gene expression, and shRNAs also exploit cellular mechanisms for specific inhibition of target genes due to the presence of a sequence complementary to single target site.

Основная часть. The efficiency of gene inhibition using RNA interference is determined, among other things, by the design of short interfering RNAs sequence. When selecting shRNA variants for inhibiting the genes of fluorescent proteins GFP, BFP and RFP, we were guided by literature data on the design of the molecule [3]. shRNA is a nucleotide sequence consisting of a sense and antisense region ~21 bp long, connected by a 3-5 bp loop, and a termination signal at the 3' end. Between the loop and the stem there is a recognition site for the Dicer protein for correct processing of the molecule. Taking into account the described characteristics, an in silico design of the shRNA nucleotide sequences was performed.

A series of plasmid DNAs with selected shRNA variants were obtained by restriction ligase cloning into an expression vector with a polymerase III promoter, with sequence confirmation by Sanger sequencing.

The efficiency of inhibition of fluorescent protein expression was evaluated by co-transfection of HEK293 cells cultured in 12-well plates with pDNAs encoding the selected proteins and pDNAs with the corresponding shRNAs. A cell sample transfected with pDNA containing a fluorescent protein sequence and pDNA containing an shRNA sequence without binding sites for the target protein was used as a negative control. The efficiency of inhibition of target gene expression was determined relative to the negative control 24 hours after co-transfection for GFP and BFP and 48 hours after co-transfection for RFP using flow cytometry. According to the results of the experiment, all selected variants inhibited expression of target proteins.

Выводы. Three shRNA variants were selected to inhibit the expression of each fluorescent protein GFP, BFP, and RFP. The selected sequences were cloned into an expression vector. The efficiency of inhibition of target proteins by the obtained constructs was confirmed by transfection of the HEK293 cell line with subsequent analysis on a flow cytometer.

Список использованных источников:

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