Novel approach to the delivery into the cells and light-activation of the guide RNA for the genome editing CRISPR/Cas9 system

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A. Yakovlev[I,II], E. Akhmetova[I,II], N. Danilin[I,II], O. Semikolenova[I,II], I. Vokhtantsev[I], D. Kim[I], D. Zharkov[I], A. Venyaminova[I], D. Novopashina[I,II]

[I]Institute of Chemical Biology and Fundamental Medicine Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia, [II]Sirius University of Science and Technology, Sochi, Russia

Using of the CRISPR/Cas9 system for molecular biological and gene engineering purposes is the issue of a day. Effective delivery of the components of system in cells is obligatory for their successful application. The main goal of our study is the development of novel approach for the delivery of guide RNA (crRNA) in the cells. Proposed approach is based on the usage of additional photocleavable oligodeoxyribonucleotide complementary to guide RNA and bearing ligands facilitating the penetration of the whole construction through the cell membrane. The presence of photocleavable linkers in oligodeoxyribonucleotide conjugate permits to destroy them by UV-irradiation after cells' penetration, to liberate guide RNA and to initiate the CRISPR/Cas9 system functionalizing in the cells. This approach is proposed for the first time in our study. Oligodeoxyribonucleotides containing three photocleavable linkers and their 3'-functionalized analogs were synthesized by solid-phase phosphoramidate method using phosphoramidite on the base of 1-(2-nitrophenyl)-1,2-ethanediol. Non-modified stable oligodeoxyribonucleotides and their 3'-functionalized analogs were also prepared as controls. The conjugates of 3'-amino-, 3'-alkyne and 3'-phosphate containing oligonucleotides with cholesterol, pyrene, peptide and GalNac were prepared using different methods of conjugation. The kinetics of modified oligonucleotide photocleavage and thermal stability of the duplexes of the additional oligodeoxyribonucleotides and their conjugates with guide RNA were investigated. The possibility of activation of designed CRISPR/Cas9 system by UV-irradiation was demonstrated using model DNA plasmid. Proposed approach to the delivery into the cells and light-activation of the genome editing CRISPR/Cas9 system can be applied in future for photocontrollable gene editing in cells. The reported study was funded by RFBR, project number 19-34-51026.