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Indexing

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P-01.2-05
The role of post-transcriptional editing of herpes simplex virus 1 miR-H2 during latency

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Herpes simplex virus 1 (HSV-1), an important human pathogen, has been shown to encode 29 microRNAs (miRNAs), the function of which is yet to be revealed. The functions of miRNAs are defined by their sequences, as only one nucleotide difference can dramatically change the specificity of miRNA for its target. Using advanced bioinformatics tools, we recently found that miR-H2, an HSV-1 miRNA targeting important virus gene ICP0, is extensively edited by the function of the adenosine deaminase acting on RNA (ADAR) in latently infected human ganglia. The ADAR proteins deaminate adenosines to inosine (recognized as guanosine), they have a critical role in homeostasis, and it is possible that expression of ADAR proteins in neuronal tissue specifically regulates the establishment and maintenance of latency. This editing function could indicate that the virus is using cellular processes to broaden the scope of possible miRNA targets, including viral and host targets, or to affect their stability. Furthermore, to comprehensively investigate this phenomenon and biological relevance, we used biological approaches to test the relevance of a few most important bioinformatically predicted targets of edited HSV-1 miR-H2. In addition, to extensively analyze this phenomenon during infection with other viruses, we analyzed many publicly available small-RNA deep-sequencing data sets including samples infected with Epstein–Barr virus, Kaposi's Sarcoma-associated herpesvirus, cytomegalovirus, human herpesvirus 6 and human papillomavirus and our preliminary results show that many viruses employ this phenomenon to specifically change the crucial part of the miRNA sequence important for binding to its target or have effect on host miRNAome during the course of the infection. Understanding this novel aspect of non-coding RNA biology will not only shed light on an incredibly complex life cycle of HSV-1 but also might reveal important cellular pathways.

P-01.2-06
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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Use of the CRISPR/Cas9 system for molecular biological and gene engineering purposes is the issue of the 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. The 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.

P-01.2-07
Antibodies from human milk hydrolyze microRNAs

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Milk is a unique biological fluid; it contains all components necessary for the development and protection of newborn. Milk is not just a mixture of such substances, as proteins, lipids, carbohydrates and nucleic acids. Of particular interest are milk immunoglobulins, possessing various catalytic activities, such as protease, phosphatase, DNase, RNase and others. In this work, the ability of milk IgG and sIgA to hydrolyze various RNA substrates was investigated. MicroRNAs that regulate the expression of many genes are found in many biological fluids, including milk. It was shown that miRNAs regulate the expression of genes associated with the development of the newborn's immune system. Milk immunoglobulins possess RNase activity in the hydrolysis of various miRNA substrates, both highly expressed in milk and unrepresented in milk, as well as homooligoribonucleotides and cellular ribosomal RNA. In addition, microRNA isolated from human skimmed milk and various fractions of milk: cell sediment, lipid fraction, and milk plasma were studied. Analysis of the isolated RNAs was performed using an Agilent 2100 Bioanalyzer on RNA 6000 Pico and Small RNA chips. Using reverse transcription with stem-loop primers and subsequent quantitative real-time PCR, the expression of 25 miRNAs in different fractions of milk was evaluated. This work was supported by a grant from the Russian Science Foundation 18-74-10055.