***Modified siRNAs, ribozymes and CRISPR/Cas system as strategies for influenza A virus inhibition. Application and comparison of RNA-targeting methods.***

Influenza A virus is a human pathogen posing a serious threat to public health, causing seasonal epidemics and sporadic pandemics. According to World Health Organization infectious respiratory disease induced by influenza A virus causes annually 3-5 million severe cases and 250-300 thousand deaths worldwide. Pathogen transmits easily and every year affects about 5-10% of human population. It is especially life threatening for newborns, young children, elderly and people suffering from other serious medical conditions. In these cases infection may lead to severe complications and even death. Virus is also a major threat to food and livestock industry. Currently, there are two ways of combating pathogen- prevention with vaccination and antiviral drugs, both of them have remarkable limitations. Antigen changes and emergence of new strains reduces vaccine effectiveness, while development of a new vaccine is time-consuming. Antiviral therapy is based mainly on viral proteins inhibitors; however their application is reduced by drug-resistant viral mutations. During last few years, new approaches on antiviral treatment by RNA inhibition are under development, but none of them is close to gain FDA approval.

Basic research on potential influenza virus inhibitors is necessary. The main objective of proposed research is to develop, optimize and compare RNA-targeting strategies in order to inhibit influenza virus proliferation. In particular we would like to study:

1) siRNA-mediated influenza virus inhibition and effect of siRNA chemical modification on this process,
2) ribozymes as tool for inhibition of influenza virus and relationship between hammerhead ribozyme and target sequences as well as efficiency of viral RNA cleavage,
3) assessment of siRNA and hammerhead ribozyme (shRNA-hammerhead ribozyme chimeric construct) combined approach in terms of anti-influenza activity,
4) utility of CRISPR/Cas system for targeting RNA and inhibition of the influenza virus.

In proposed research pandemic strain A/California/04/2009 (H1N1), representative of (H1N1) subtypes circulating among humans, will be used. For design of inhibitory molecules we implement careful selection of target regions in segment 5 influenza virus (+)RNA, based on viral RNA secondary structure predicted in bioinformatics' analyses and experimentally defined by our research team. Selected regions are highly structure-conserved among type A influenza strains and especially important from the perspective of their proliferation. Preliminary results of our research indicate that they are optimal regions for interaction with particular inhibitory molecules. Limiting targets to one RNA segment and selection of three conservative and functionally important motifs gives the opportunity to achieve significant inhibitory effect and valuable comparison. If all strategies directed against the same structural motifs of viral RNA result in virus inhibition, it will be a strong proof of their crucial role in influenza virus proliferation.

Proposed basic research has the potential to serve as a base for further analyses allowing design of tools with improved features for therapeutic use by other researchers. Proposed methods haven't been used for influenza virus inhibition yet. Obtained results will allow to increase potential of RNA-targeting strategies and broaden knowledge of factors determining their effectiveness. Moreover, using several different strategies against the same regions of viral RNA permits comparison of RNA inhibition efficacy of these methods. Conducted research will provide information which of exploited strategies is more effective (results in efficient RNA degradation), specific and brings long-lasting effects. Also which of the methods is more prone to introduction of chemical modification and property improvements, and which is rather more dependent on viral RNA target region selection. Targeting structurally conserved and functional motifs of viral RNA is a new strategy, especially important for fast-transmitted and pandemic strains of influenza virus. Results of the experiments will have significant impact on understanding pathogen biology from the perspective of RNA and role of selected segment 5 structural motifs. Acquired knowledge will be a major contribution to influenza virus proliferation inhibition research.