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# Functional study of snoRNAs activity using transcriptomic analysis of CRISPR/Cas9-modified human cells

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*Motivation and Aim*: Small nucleolar RNAs (snoRNAs) represent a special class of small regulatory RNAs, participating in post-transcriptional modification of ribosomal RNA in eukaryotes. Box C/D snoRNAs present one of the subclasses and with the help of conserved elements in their structure provide targeted 2'-O-methylation of rRNA nucleotides [1]. SnoRNAs possess multiple non-canonical functions as well, such as modulation of pre-mRNA alternative splicing and an ability to be processed into short-derived RNAs resembling miRNAs [2].
*Methods and Algorithms*: We obtained plasmid constructs expressing CRISPR/Cas9 components guiding double-strand breaks into GAS5 (Growth Arrest Specific 5) gene regions encoding target snoRNAs. 293FT-derived single cell clones were characterized for mutations and target gene expression. The 2'-O-methylation level of corresponding rRNA nucleotides was estimated with a specially designed RT-PCR analysis. RNA-Seq method allowed carrying out a differential gene expression profiling.
*Results*: RNA-Seq analysis revealed both upregulated and downregulated genes for cell lines with either U75 or U77 snoRNAs being modified using CRISPR/Cas9. These genes were then categorized into groups using Enrichr online tool [3]. One of the most highly presented groups were found to be controlled with transcription factors SUZ12 and EZH2, which are involved in the processes of the chromatin remodeling. Common upregulated genes groups for both cell lines were distinguished, the group of high interest being associated with the innate immune response processes. Interestingly, this group was more diverse for the cell line with mutant U75 snoRNA.
*Conclusion*: The results obtained suggest that immune response might be activated due to the inability of mutant snoRNAs to form stable canonical ribonucleoprotein complexes with nucleolar proteins. Induced snoRNA structure alterations could result in binding with novel protein partners and acquisition of new functions.
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