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Small non-coding RNAs of extracellular vesicles of parasitic worms as regulators of human target genes

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Motivation and Aim: Small non-coding RNAs, including microRNAs, regulate gene expression through interaction with the 3'-UTR of target mRNAs, affecting their stability and/or translation. Parasitic organisms also secrete microRNAs in extracellular vesicles (EVs), which are able to penetrate human cells and modulate cellular processes [1–3]. The identification of such therapeutic parasitic microRNAs is especially important because many parasitic infections are negatively correlated with the morbidity and symptoms of autoimmune inflammatory diseases such as Asthma or Crohn's disease.

The trematode *Opisthorchis felineus* parasitizes the bile ducts of fish-eating mammals, including humans. Human infection leads to biliary epithelial neoplasia, on the other hand, to an alleviation of Asthma symptoms. The mechanisms by which parasites alleviate acute inflammation, regulate the immune response, and stimulate the neoplasia development have not been elucidated, in particular, the role of parasitic extracellular vesicles and non-coding RNAs has not been studied.

Aim: Identification of small non-coding RNAs of EVs of *Opisthorchis felineus* trematodes that exhibit specific bioregulatory activities in relation to human cells.

Methods and Algorithms: UMI-containing DNA libraries from small RNA of *O. felineus* EVs were constructed using the MGIEasy Small RNA Library Prep Kit V2.0 and sequenced 1X50 bp on BGISEQ-500 (BGI, China). DNA-libraries to study mRNAseq of human H69 cholangiocytes were constructed using the NEBNext Ultra II Directional RNA Library Prep Kit, the libraries were sequenced 2X150 base pairs on DNBseq (BGI, China). The quality of the data after sequencing was controlled using the FastQC program. Mapping of small RNA libraries to the reference genome of *O. felineus* was carried out using bowtie2. The miRDeep2 algorithm was used to identify known microRNAs. The list of known trematode microRNAs and their precursors was compiled according to the paper [4]. The conservation of major EVs microRNAs was studied using miRBase database. To search for target genes, a combination of TargetScan and miRDB was used.

To analyze the mRNAseq data from human cholangiocytes (H69) after exposure to extracellular vesicles of *Opisthorchis felineus*, sequence mapping was carried out on the human genome GRCh38 using STAR algorithm (v.2.7.10b). To analyze differential gene expression, the Wald test with a threshold of 0.05 from the DESeq2 (v.1.42.0; R package) was used. A correction for multiple comparisons (Benjamini-Hochberg) was applied to the p-values obtained, and genes were identified as differentially expressed (DEGs) when Padj <0.05 and a change in gene expression of more than 1.5 times. Principal component analysis to assess the degree of library clustering was carried out using PCAtools (v.2.14.0, R-package). Pathway enrichment analysis was performed

using Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Molecular Signatures Database (MsigDB) databases and msigdb (v.1.10.0), as well as clusterProfiler (v .4.10.0) R-packages.

Results: After sequencing of DNA libraries of small RNAs from EVs, an average of 3 million reads per library was obtained. 97 mature microRNAs were found in EVs. The most represented microRNAs were Ofe-Mir-277-P2_3p (18.5 %), Ofe-Mir-71-P1_5p (17.7 %), Ofe-Bantam_3p (11.8 %), Ofe-Mir-10 -P1_5p (11.3 %), Ofe-Mir-219_5p (6.0 %), Ofe-Mir-7-P1_5p (3.5 %). Differences were identified in the relative abundance of miRNAs in EVs compared to that of adult whole worms. Thus, the most represented microRNA in adult whole worms was Ofe-Mir-10-P2a (48 %), while in EVs the content of this microRNA was 4.41 %. In contrast, the most abundant miRNA in exosomes, Ofe-Mir-277-P2_3p, was presented less than 0.02 % in adult worms. Thus, we observed an enrichment of exosomes with certain microRNAs, which may indicate their functionality during interaction with host cells.

Among the major miRNAs of *O. felineus* EVs, there were both conserved miRNAs and those specific to parasitic worms. For example, seed-region of Ofe-Bantam-3p was identical only in those from Platyhelminthes and Nematoda species. In contrast, Ofe-Mir-10-P1 and Ofe-Mir-10-P2a are highly conserved and are also present in humans (Mir-125) with an identical seed-region. It can be assumed that *O. felineus* microRNAs can act on the same target genes as their human homologues.

Among the predicted target genes in the human genome for Ofe-Mir-277-P2, enrichment was observed in the genes of epithelial-mesenchymal transition pathway (p-value = 0.00063), protein secretion (p-value = 0.016), myogenesis (p-value = 0.046), PI3K-Akt signaling pathway (p-value = 0.041) and regulation of the actin cytoskeleton (p-value = 0.041). For Ofe-Mir-10-P1, enrichment among human target genes was observed in the pathway associated with proteoglycans in carcinogenesis (p-value = 0.00374).

As a result of sequencing the transcriptome of human H69 cholangiocytes (6 libraries), an average of 71 million reads per library were obtained. Analysis of transcriptomes revealed 773 differentially expressed genes (DEGs) after treatment of cells with trematode EVs (expression changes more than 1.5 times, Padj < 0.05), with 365 down-regulated genes and 408 upregulated genes. The Fisher test showed the enrichment of cholangiocyte DEGs with predicted target genes of parasitic microRNAs.

Conclusion: Thus, we have demonstrated the data indicating the regulatory role of parasitic microRNAs in the regulation of human gene expression. The study of parasitic secretory microRNAs provides the opportunity to identify new anti-inflammatory and biologically-active compounds created during co-evolution of the parasite and the host to modulate the immune response or stimulate the regeneration in tissues.

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