

# COMPUTATIONAL AND EXPERIMENTAL ANALYSIS OF MIRNA GENES OF OPISTHORCHIIDAE FAMILY LIVER FLUKES

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MicroRNAs (miRNAs) are non-coding small RNA molecules which are significant part of any multicellular organism transcriptome and are involved in many important regulatory processes shaping expression of many protein coding genes [1].

The liver flukes *Clonorchis sinensis*, *Opisthorchis felineus* and *Opisthorchis viverrini* (class Trematoda, order Plagiorchiida, family Opisthorchiidae) are parasitic flatworms with complex life cycle, which consist in change of 3 hosts with human and piscivorous mammals as definitive hosts [2]. These liver flukes cause hepatobiliary disease (clonorchiasis or opisthorchiasis) in population of Central and Eastern Eurasia, including Russia, with a risk of cholangiocarcinoma development [3]. The detailed molecular mechanisms of the liver flukes ontogenesis as well as molecular mechanisms of their pathogenic effects are poorly studied. Namely the role of fluke miRNAs in these processes is not clear. So the problem of miRNAs and their genes identification in Opisthorchiidae flukes and the miRNAs molecular function determination is quite actual.

This research aims at finding and characterization of miRNA genes structure and function in the three opisthorchids flukes. Our tasks were to computationally analyze the sequence data on *C. sinensis*, *O. felineus* and *O. viverrini* miRNA-containing transcriptome portions, generated by SOLiD technology, and experimentally verify these computer predictions.

To identify miRNA sequence fragments in transcriptome data we used bioinformatics pipeline developed in our group. The pipeline included:

- (1) Pre-processing reads (quality filtering [4], adapter trimming [5]);
- (2) Filtering possible non-miRNA fragments by mapping reads to Refseq (rel. 106) mRNA and Rfam (rel. 10) non-miRNA sequences using BFAST [6];
- (3) Mapping reads that passed filters to miRBase (rel. 18) (<http://www.mirbase.org>) to search for known miRNAs;
- (4) Mapping remaining reads to *C. sinensis* genome [7];

(5) Analysis of the RNA secondary structure by UNAFold [8] for segments mapped in *C. sinensis* genome to identify candidates for novel miRNA genes specific to opisthorchids. This computational approach allowed to predict 120 candidate miRNAs. Among them there were 20 conservative miRNAs from 13 miRNA families annotated in DB miRBase. Seven conservative miRNAs (mir2a, mir-2b, mir-2c, mir-2d, mir-2e, mir-71a, mir-71b) are part of the two miRNA gene clusters, which turned to be conserve in several flukes of other Trematoda orders. Fragments of these clusters for the three opisthorchids were amplified and sequenced. The distribution of conservative and variable regions in these clusters were analyzed upon ClustalW multiple alignment (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). The 100 novel miRNAs predicted were not described earlier for any animal. The expression of 2 conservative and 2 novel miRNAs of *O. felineus* was confirmed by stem-loop RT-PCR technique [9].

The search for microRNA targets among opisthorchids fluke mRNAs was carried out with program PITA [10]. The 27 opisthorchids flukes miRNAs were predicted to have 71 targets with certain functional annotation for corresponding proteins.

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