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Liver transcriptomics revealed species-specific responses during infections with three foodborne trematodes

Pakharukova M.Y.[1, 2]*, Lishai E.A.[1, 2], Zaparina O.G.[1], Kapushchak Y.K.[1]

[1] *Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia*

[2] *Novosibirsk State University, Novosibirsk, Russia*

** pmaria@yandex.ru*

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Motivation and Aim: Trematodes of the Opisthorchiidae family vary in their carcinogenicity to humans. *Opisthorchis viverrini* and *Clonorchis sinensis* are classified as group 1A biological carcinogens, while *O. felineus* is classified as group 3A, a non-carcinogen [1, 2]. Comparison of three closely related species with different carcinogenic potential seems to be a promising model for studying the key mechanisms in liver metabolic shifts during biological carcinogenesis and identifying the cascade of regulatory signaling events and the dynamics of pre-carcinogenic changes.

The aim of the study was to identify differences in the activation of genes and signaling pathways in the liver of *Mesocricetus auratus* hamsters infected with three trematode species.

Methods and Algorithms: The following research methods were applied (RNA isolation, cDNA library construction, Western blot analysis, histological analysis of liver sections). Liver transcriptomes of golden hamsters (HiSeq Illumina, 2X150 bp) were sequenced at 1 and 3 months postinfection. Samples for the study were prepared in an SPF Animal Facility (free from specific pathogens) and analyzed under the same conditions. This made it possible to minimize comparison errors, such as a human factor, climate differences, specific pathogen infections of laboratory animals, etc.

Data processing was carried out using the following bioinformatics approaches: analysis of differential gene expression, functional enrichment of differentially expressed genes (DEGs), analysis of cellular composition using sequencing data of single liver cells, analysis of weighted gene coexpression networks by means of FastQC, STAR and R packages (DESeq2, cluster-Profiler, bisqueRNA, PCAtools, WGCNA). DEG enrichment analysis was performed using Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Molecular Signatures Database (MsigDB) databases.

Results: Sequencing of 24 DNA libraries resulted in an average of 37 million sequences per library. *C. sinensis* infection caused changes in the expression of the largest number of DEG compared to the uninfected animals – 3148 genes; *O. viverrini* infection – 1464 genes were differentially expressed, and *O. felineus* infection – 1408 genes.

All infections are characterized by enrichment in the inflammatory response signaling pathway, fibrogenesis and cell proliferation, IL2-STAT5, TNF-α NFkB, TGF-β, Hippo, MAPK, PI3K-Akt signaling pathways. However, species-specific response to each infection were also found. Thus, *O. viverrini* infection was characterized by significant enrichment in the FoxO and hypoxia pathways, *C. sinensis* infection – P53 and relaxin signaling pathway, *O. felineus* infection – TNF-α and ErbB signaling pathways.

The contribution of individual liver cells to transcriptomic response also depended on the type of infection. *C. sinensis* caused the most pronounced changes in the response of

various types of liver cells, including pro-inflammatory macrophages and stellate cells, which was confirmed using Western blot analysis for the content of cell marker proteins alpha smooth muscle actin (aSMA) and metalloproteinase 9 (MMP9).

Semi-quantitative histological analysis of hamster liver revealed hepatobiliary lesions and severity of damage during infections, which differed significantly between groups of infected animals. Infections with *C. sinensis* and *O. felineus* are most characterized by the profibrotic changes. Whereas *O. viverrini* infection is characterized by more pronounced neoplasia of the bile duct epithelium, which is considered a precancerous state. In general, the transcriptome data are consistent with the results of semi-quantitative histological analysis, where during infection with *C. sinensis*, liver periductal fibrosis was most pronounced, in the formation of which stellate cells are mostly involved.

We also analyzed the correlation (Pearson coefficient) between clusters of co-expressed genes and structural changes in the liver, such as fibrosis and neoplastic changes in the biliary epithelium using analysis of weighted gene co-expression networks.

Conclusion: This study was the first to conduct a comparative analysis of gene expression profiles in the liver of experimental animals infected with trematodes with different carcinogenic potential *O. viverrini*, *O. felineus* and *C. sinensis*. The studied trematodes have a species-specific effect on the hepatobiliary system, influencing changes in the expression of various genes and activation or suppression of cellular signaling pathways, which leads to differences in the severity of structural lesions to the liver and can explain the carcinogenic potential of closely related foodborne trematodes.

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