A GENE EXPRESSION ATLAS BY SINGLE-CELL AND SPATIAL TRANSCRIPTOMICS AS VALUABLE RESOURCE IN LIVER FLUKE RESEARCH

Oliver Puckelwaldt, Svenja Gramberg, Tobias Schmitt, Simone Haeberlein

Institute of Parasitology, Department of Veterinary Medicine, Justus Liebig University Giessen, Giessen, Germany

Fasciolosis is a zoonotic disease caused by liver flukes of the genus *Fasciola* spp. Up to date, there is a significant lack of knowledge on the parasite's cell types and cell-specific gene expression repertoire. Novel developments in transcriptomic techniques like single-cell (sc) transcriptomics and spatial transcriptomics (st) allow for large scale analysis of the transcriptomics landscape also of non-model organisms. We planned to establish a comprehensive gene expression atlas for *F. hepatica.*

Both techniques utilize barcoded oligonucleotides that allow the retracing of sequencing data to their respective source. For scRNA-seq, this results in transcriptome data on a single cell level. The st method involves capturing and barcoding of transcripts *in situ* using oligonucleotide-coated glass slides. This way, data on gene expression can be spatially resolved and mapped into the tissue context.

Clustering the scRNA-seq data based on transcriptional information identified 15 cell clusters for adult liver flukes, which represent biologically relevant cell types. We could validate these cell types including gastrodermal cells expressing cathepsins, neoblasts expressing nanos2, and neuronal cells. Eight different tissues could be resolved with st, such as intestine, tegument and reproductive organs. By differential expression analysis, marker genes for each sc and st cluster were identified and validated by RNA *in situ* hybridisation. Clusters were further functionally assessed by functional gene ontology (GO) enrichment analysis. This revealed characteristic biological processes and molecular functions associated for each cluster. Furthermore, this new atlas enabled us to identify several drug target genes (such as ß-tubulins, protein kinases and calcium channels), drug resistance genes, and transcription factors with cell type- or tissue type-specific expression.

Taken together, this work provides the first transcriptomes for the liver fluke *F. hepatica* on a single-cell and spatial resolution. Our expression atlas serves as novel tool for the unravelling of cell biological secrets in this parasite.