MO030

IDENTIFICATION OF REGULATED MRNAS AND MIRNAS IN GLOMERULI ISOLATED FROM AN FSGS-LIKE ZEBRAFISH MODEL

Anna Iervolino[1,2], Tim Lange[2], Sabrina Siccardi[1,3], Florian Siegerist[2], Francesca Pia Caruso[1,4], Michele Ceccarelli[1,4], Karlhans Endlich[2], Giovambattista Capasso[1,3], Nicole Endlich[2]

[1]Biogem S.c.a.r.l., Department of Translational Nephrology, Ariano Irpino, Italy, [2]University Medicine Greifswald, Department of Anatomy and Cell Biology, Greifswald, Germany, [3]University of Campania "Luigi Vanvitelli", Department of Translational Medical Sciences, Napoli, Italy and [4]University of Naples "Federico II", Department of Electrical Engineering and Information Technology (DIETI), Napoli, Italy

BACKGROUND AND AIMS: The zebrafish (Danio rerio) is a powerful animal model to study glomerular morphology and the function of the permselectivity of the glomerular filtration barrier.
Since zebrafish larvae develop quickly and can be bred to become transparent, in vivo observation of these animals is possible. At 48 hours post fertilization, zebrafish larvae develop a single glomerulus which is attached to a pair of tubules. Like in mammals, the glomerular filtration barrier consists of a fenestrated endothelium, the glomerular basement membrane and interdigitating podocyte foot processes bridged by a slit diaphragm. By using genetically modified zebrafish strains with fluorescently labeled podocytes, it is possible to study alterations of the glomerulus during the development of renal disease like focal segmental glomerulosclerosis (FSGS) directly in vivo. FSGS is characterized by podocyte loss, the effacement of their foot processes as well as scarring of the glomerulus. To study FSGS in zebrafish larvae, we induced podocyte detachment by the use of a zebrafish strain expressing the enzyme nitroreductase converting metronidazole into a toxic substance specifically in podocytes. The aim of our study was to collect glomeruli for the identification of mRNAs as well as miRNAs by RNA_Seq that are up- and down-regulated in the glomeruli of this FSGS-like disease model.
METHOD: The transgenic zebrafish strain Cherry (Tg(nphs2:GAL4); Tg(UAS:Eco.nfsB-mCherry); mitfaw2/w2; mpv17a9/a9) which expresses the prokaryotic enzyme nitroreductase (NTR) fused to mCherry, a red fluorescent protein, under the control of the podocyte-specific podocin (nphs2) promoter in a transparent zebrafish strain, was utilized. After addition of metronidazole (MTZ) into the tank water, MTZ is converted into a cytotoxin by NTR leading to dose-dependent apoptosis exclusively in podocytes. Cherry larvae were treated at 4 days post fertilization (dpf) for 48 h with 80 μM MTZ. MTZ-treated and control larvae were homogenized at 6 dpf. The cell suspension was diluted, and red-fluorescent glomeruli were collected using a micropipette and a microscope. Total RNA was isolated, and integrity was checked by a Bioanalyzer. Libraries were generated with a MACE kit and True Quant small RNA seq kit by GenXPro. Constructs were amplified by PCR and sequenced on an Illumina Hiseq 2000. Normalization and statistical analysis for differential gene expression were done using DESeq2.
RESULTS: Zebrafish larvae showed severe whole-body edema, proteinuria, loss of podocytes and an increased mortality rate after MTZ-treatment. The glomerular histology resembled mammalian FSGS. We found that only the RNA of manually collected glomeruli had an excellent quality. Using RNA_Seq, we identified a total of 16941 genes. DESeq2 analysis showed 494 up-regulated and 473 down-regulated genes. Gene ontology (GO) enrichment analysis of up-regulated genes revealed a total of 167 that are significantly enriched in GO terms (e.g. metabolic processes, immune response and ion transport). Down-regulated genes were enriched in 14 GO terms and most of them are linked to normal glomerular function and the slit diaphragm. DESeq2 analysis identified 200 miRNAs of 777 small RNAs. Some of these miRNA are already described to be regulated in different glomerular diseases like FSGS, lupus nephritis, IgA nephropathy and diabetic nephropathy.
CONCLUSION: We analyzed isolated glomeruli from transgenic zebrafish larvae that developed a FSGS-like disease. By sequencing, we have found mRNAs and miRNAs that were significantly regulated after the onset of disease. Detailed knowledge of these mRNAs and miRNA-based gene regulation will help to uncover the pathomechanism as well as to develop therapeutics for the treatment of FSGS.