Network and pathway analyses in zebrafish immature brain after seizures

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Introduction: To investigate the molecular mechanisms underlying seizures, we analyzed the response of different seizure-induced protocols in the zebrafish brain by transcriptome analysis. For a comprehensive understanding of the biological mechanisms affected by the transcripts that were counter-regulated by the treatments, interactive networks and pathways analyses were performed using a systems biology approach.

Materials and Methods: Wild-type zebrafish larvae at 7dpf were separated into three groups: control (CG, n=3), acute seizure (AS, n=2) and status epilepticus-like (SE, n=3). Larvae from groups AS and SE were exposed to PTZ 15mM for 20 minutes and 3 hours, respectively. Messenger RNA libraries were achieved after using Illumina's Sample Prep Kit and validated libraries were sequenced in the Illumina HiSeq 2500 System. Gene interactions and correlation networks were identified with the Ingenuity Pathway Analysis (IPA) software content version March/2017 (Ingenuity Systems, Mountain View, CA). The fold change threshold was >3 or ≤-3 and the signaling pathways were considered statistically significant with a $p<0.05$. All IPA available databases at the time of the analysis were used in this study.

Results: The most activated signaling pathway for AS vs. CG and SE vs. CG was the AMPK Signaling, but in opposite directions, since in the SE it was activated (positive z-score), and in the AS inhibited (negative z-score). However, comparisons between AS vs. SE showed that RhoGDI Signaling (p=1.06E-07) and Signaling by Rho Family of GTPases (p=1.20E-07) are the top-pathways inhibited (negative z-score) and activated (positive z-score), respectively, in the SE group.

Discussion: Transcriptome studies followed by signaling pathway analysis showed that AS and SE have distinct molecular responses. The AMPK signaling pathway is responsible for coordinating several intracellular processes, like cell growth and metabolism reprograming, and is activated when intracellular ATP reach lower levels [1]. This signaling pathway was also reported in epilepsy as acting on epileptogenesis and synaptic plasticity regulation [2]. The RhoGDI family of genes regulate signaling through RhoGTPases by inhibiting the disassociation of Rho members from GDP, thus keeping these factors in an inactivated state [3]. We found that RhoGDI pathway is downregulated and the Rho Family of GTPases is up-regulated in the SE compared to AS group. It is known that the Rho family of GTPases have an important role in the morphogenesis of the dendritic spines of neurons [4] and contribute to the synaptic plasticity [5] that is observed in epilepsy.

Conclusion: This study emphasizes the relevance of the zebrafish as a model for investigating seizures.

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