**The role of mir-221 and hsp90ab1 gene in seizures in the zebrafish immature brain**

M. C. S. Nunes1, W. Souza2,V. H. S. Zago1, R. A. Oliveira1, A. S. Vieira\*, A. H. B. Matos3,
C. S. Rocha2, B. Carvalho4, I. Lopes-Cendes2, C. V. Maurer-Morelli1

1Zebrafish Laboratory, 2Biostatistics and Computational Biology Laboratory, 3Molecular Genetics Laboratory, Department of Medical Genetics, School of Medical Sciences – UNICAMP, 4 Department of Statistics, Institute of Mathematics, Statistics and Scientific Computing- UNICAMP and Brazilian Institute of Neuroscience and Neurotechnology (BRAINN).

**Introduction:** MicroRNAs (miRNAs) have been recognized as key molecules underlying seizures as well as associated with epileptogenesis [1]. In order to unravel molecular mechanisms, zebrafish has been successfully used by researchers as an animal model for genetic studies for a while [2]. This study aimed to integrate miRNA and mRNA transcript profiles by applying massive sequencing approach in order to identify molecular mechanisms underlying seizures in the zebrafish seizure model.

**Materials and Methods:** Zebrafish larvae at 7 days post fertilization were divided into three experimental groups: CTL - animals exposed to bath medium for 3 hours (*n*=3); AS - acute seizure, animals exposed to pentylenetetrazol (PTZ) for 20 minutes (*n*=2) and *SE* - status epilepticus–like, animals exposed to PTZ for 3 hours (*n*=3). Each sample (*n*) was composed by pooling 20 larva heads. Total RNA was extracted, and validated mRNA and miRNA libraries (*Illumina TruSeq Stranded mRNA LT* and *TruSeq Small RNA Sample*) were achieved followed by high throughput screening (*Illumina HiSeq 2500*). Bioinformatics analyses were first performed to filter the mRNA and miRNA differentially expressed on the samples (p<0.01), and secondly to perform an integrated analysis to cross the data between a determined miRNA and their targets. In our analyses, we utilized the public databases miRBase (mirbase.org) and TargetScanFish (targetscan.org).

**Results:** We previously reported the miRNAs differentially expressed [3] for each comparison (CTL *vs* SE; CTL *vs* AS and AS *vs* SE). For each miRNA, we selected one target gene that exhibited an inverse correlation regarding expression. Here we highlight the microRNA *mir-221* that is differentially expressed in the SE *vs* AS comparison and its target, the *hsp90ab1*gene.

**Discussion:** A recent article reported *mir-221* as an important miRNA in pro-epileptogenic processes in the human brain [1]. Interesting, this microRNA is orthologous in humans and zebrafish. We chose the orthologue gene, *hsp90ab1,* as a target for *mir-221* since both are inversely expressed. In the literature, this gene is related to the mTOR signaling pathway, important to the gene expression regulation of the GABAergic and glutamatergic receptors, neuroinflammation, antiepileptic drugs resistance, and morphine addiction.

**Conclusion:** In this work, we performed an integrated analysis of miRNA and mRNA massive profiles in zebrafish brain after two protocols of PTZ-induced seizures. We highlighted the *mir-221* and its target gene *hsp90ab1* due to its possible importance in epilepsy. The following steps will be performing real-time PCR for independent validation, and to perform in-situ hybridization in order to localize these molecules in the zebrafish brain. By using the zebrafish model, we hope to increase the understanding of the molecular mechanisms underlying seizures and provide targets that are potentially therapeutic for seizures.

**References:** [1] Cattani, et al. Epilepsia (2016).

[2] Dodd, et al. Human Molecular Genetics (2000).

[3] Zago et al. JECN 2015,22(3):94