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Role of Stim2a protein in the neuroprotection in Danio rerio

## Abstract

Calcium ions ($Ca^{2+}$) play a vital role in the signaling of any eukaryotic cell. An influx of $Ca^{2+}$ into the cytoplasm originates either from the endoplasmic reticulum (ER), the main store of these ions, or the external environment. Refilling of these stores is possible by store-operated calcium entry (SOCE or CCE, capacitative calcium entry); (Putney 1986). The SOCE relies on the detection of a reduced level of $Ca^{2+}$ in the ER by STIM sensory proteins and subsequent activation of Orai/TRP channels located in the plasma membrane (Hartmann et al., 2014, Shin et al., 2016), by which these ions can enter the cytoplasm and then transferred to the ER by the ATP-dependent calcium pump (SERCA). STIM proteins have been established in neuronal $Ca^{2+}$ signaling in mammalian primary neuronal cell cultures (reviewed in (Majewski et al., 2015, Wegierski et al., 2018)). However, *in vivo* data supporting neuronal $Ca^{2+}$ homeostasis and complex behavior analysis were not available because of the early lethality of S*tim2* knockout in mice (Berna-Erro et al., 2009, Garcia-Alvarez et al., 2015).

Zebrafish possess two isoforms of STIM2 – Stim2a and Stim2b encoded by *stim2a* and *stim2b*. Using the CRISPR/Cas9 technique, *stim2a* mutant zebrafish line was created, which was viable. In this present study, it was shown that *stim2a* deletion caused distinct behavioral changes in zebrafish larvae. Hyperactivity was observed in *stim2a*$^{-/-}$ zebrafish larvae. An increase in thigmotaxis (i.e., a preference for remaining close to the well) was also observed compared to the WT. Moreover, reduction of phototaxis in *stim2a*$^{-/-}$ zebrafish larvae was also found compared with WT. Furthermore, *stim2a*$^{-/-}$ zebrafish larvae reacted to the changes in light and showed higher activity in the low activity phase in the visual-motor response (VMR) test. To establish the link between the behavior with cellular events, *in vivo* changes in the $Ca^{2+}$ fluorescence (due to GCaMP5G activity) in neurons were measured. An increase in $Ca^{2+}$ oscillation frequency was observed in neurons in the optic tectum in *stim2a*$^{-/-}$ zebrafish larvae compared to the WT. Furthermore, *in vivo* $Ca^{2+}$ activity in neurons were measured after larvae were treated with glutamate, which showed a further increase in the neuronal $Ca^{2+}$ oscillation frequency. Next-

generation RNA sequencing was performed, and differential gene expression analysis was done to further establish the molecular level link. Total 392 genes were found, which showed ≥ 2-fold change in *stim2a*$^{-/-}$ zebrafish. Out of these 392 genes, 86% of genes were upregulated, and 14% were downregulated. Among the differentially expressed genes, encoding proteins of CaTK: *anxa3a, grinab, hp, hpca, mast2, pkn3, pvalb7*, and *slc25a25b* showed significant change in expression in *stim2a*$^{-/-}$ zebrafish. This indicates that a number of genes that encode proteins involved in $Ca^{2+}$ homeostasis are affected in *stim2a*$^{-/-}$ zebrafish neurons in the absence of Stim2a isoform. For this reason, single-cell RNA sequencing was performed on the pure population of cells of neuronal origin. In cells of neuronal origin in WT zebrafish, 13 different clusters were identified, representing different cell types and early neuronal subtypes based on cellular marker genes. Eleven of them were identified as a specific type of neurons, and two clusters were identified as not known. Further analysis showed 88 unique CaTK genes from all neuronal cell clusters; those could be involved in neuronal $Ca^{2+}$ signaling. However, the number of these genes were varied in each neuronal cell cluster. 15 different clusters were identified based on cellular marker genes representing different cell types in *(stim2a;stim2b)*$^{-/-}$ double mutant. Overall, in all cell clusters, a total of 102 unique CaTK genes from all neuronal cell clusters were identified. Six cell types were identified in both WT and *(stim2a;stim2b)*$^{-/-}$ double mutant. Despite the same cell type, the CaTK genes in these six clusters showed heterogeneity. Four out of eight CaTK genes, which were found to be significantly upregulated in bulk RNA sequencing data of *stim2a*$^{-/-}$ mutant, were also identified in the scRNA seq data (*grinab*, *hpca*, *mast2*, and *pvalb7*) in the cells of neuronal origin. The other four genes (*anxa3a*, *hp*, *pkn3*, and *slc25a25b*), which were found to be upregulated by bulk RNA-Seq in *stim2a*$^{-/-}$ larvae brains, were not identified in GCAMP5G positive cells by scRNA seq, indicating that these genes were expressed in other cells than cells of neuronal origin. It was found recently in our lab that the *anxa3a* gene was upregulated in the GCAMP5G negative cell population separated by FACS in the *(stim2a;stim2b)*$^{-/-}$ double mutant.