Investigation of retina and brain development in a mouse model for Familial Dysautonomia

Veronika Shchepetkina, Yumi Ueki, Marta Chaverra, Frances Lefcort
Department of Cell Biology and Neuroscience
August 4, 2016

Montana INBRE Poster Session

ABSTRACT

Familial dysautonomia (FD) is a genetic disorder affecting the development and maintenance of the nervous system, and is prevalent in those of Ashkenazi Jewish descent. FD is caused by a point mutation in the gene called IKBKAP (IKK complex-associated protein). The IKBKAP knockout results in a decreased amount of the IKK complex-associated protein (IKAP). FD patients experience symptoms such as decreased sensitivity to pain or temperature, dysfunctions of their autonomic nervous system, incoordination, hypotonia, various dysfunctions of the organs, and often die in early adulthood. In addition, FD patients experience progressive blindness due to the loss of retinal nerve fiber layer, which greatly affects their quality of life. In order to study the role of IKBAP in the retina, the retina-specific Ikbkap conditional knockout (CKO) mouse model was developed using the Crelox system. The number of retinal ganglion cells, which make up the retinal nerve fiber layer, was counted in the CKO and littermate control retinas using immunohistochemistry.

We have generated another mouse model, known as the Tuba1α-Cre, IkbkapLoxP/LoxP mouse, in which Ikbkap is deleted in the CNS in order to further investigate the implications of IKBAP on the CNS. Data from our lab has revealed evidence of behavioral alteration, a reduction in specific neuronal populations, reduction in spinal motor neuron innervation, and alteration in cortical morphology for mice with FD. Our data show that the Tuba1α mouse have enr🔆

RESULTS

Retina

● Our experiments show that the lack of Ikbkap in the retina results in decreased numbers of retinal ganglion cells. These data recapitulate the FD visual phenotype.

● Our next goal is to investigate the mechanisms causing the death of retinal ganglion cells and to test therapeutics for preventing the death of retinal ganglion cells in FD.

Brain Development

● Our data show that without Ikbkap in the CNS, the lateral ventricles are enlarged in both E14.5 and E17.5 mice. The reason for this abnormality is unknown, however we will continue investigating plausible causes of ventricle enlargement, such as abnormal cilia, disrupted neurogenesis, or increased cell death.

● Although our preliminary experiments do not show a significant difference in radial glial cell numbers, we will continue to improve our methodology in order to achieve consistent results. We will also try to localize other specific cell populations, such as intermediate neuronal progenitors, mitotically active cells, and apoptotic cells.

REFERENCES


CONCLUSION / DISCUSSION

ACKNOWLEDGEMENTS

Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R01GM63574 and R01NS206796. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. This work was also funded by a grant from the Familial Dysautonomia Foundation as well as Montana INBRE. Thank you to Alexis Thompson for her technical assistance throughout these experiments.

Fig. 1 One month retina shows that Cre is expressed in the periphery of the retina. Pax6Cre was crossed with Rosa-flox-STOP-EGFP (Cre reporter) in order to assess Cre expression. This image shows the expression pattern of Cre expressing cells in GFP. Cre is expressed more prevalently in the peripheral retina than in the central retina.

Fig. 2 Photomicrograph of Pax6-Cre mice shows reduced RGC counts 1 mm from the optic nerve head (ONH). A, RGC tissue was analyzed in four different regions, temporal, superior, nasal, and inferior. 1 mm from the ONH. B, The CKO temporal retina revealed decreased quantities of RGCs. C, Quantified results of RGC counts showed a significant decrease in neuronal populations of CKO retinas, in all regions.

Fig. 3 E17.5 αTubaf mice had enlarged lateral ventricles compared to WT mice. A, coronal sections of E17.5 WT and CKO mouse brain lateral ventricles at 3XZ. The gangliocytic eminence was used as an indicator for the axial level at which the somatosensory cortex is located. The locations of the lateral ventricles and the gangliocytic eminence are shown on the image taken from the Electronic Prenatal Mouse Brain Atlas. B, average lateral ventricle areas of WT or CKO mice measured in square pixels. 124130.6 ± 12875.7 in WT and 190770.7 ± 16531.1 in CKO p-value = 0.0335. C, coronal sections of E14.5 WT and CKO mouse brain lateral ventricles at 5X. The location of the lateral ventricles is shown on the image taken from Kaufman’s Atlas of Mouse Development. D, average lateral ventricle areas of WT or CKO mice measured in square pixels. p-value = 0.0025.

Fig. 4 Immunolabelling of Sox9 (green), a marker of radial glial cells, and Dapi (blue) in the ventricular zone of E17.5 WT or CKO mice at 20X and 63X. There is no discernible difference between the WT and CKO mice, suggesting that the absence of Ikbkap does not influence the population numbers of radial glial cells. However, the inconsistent results may be due to variable phenotypes in the mutants or an uneven stain.