AB046. The Endocytic Adaptor Protein Numb Functions in Müller Glia to Maintain Retinal Polarity and Photoreceptor Survival through the Polarity Determinant Crumbs

Maude Vinette[1,2], Marie-Claude Bélanger[1,3], Christine Jolicoeur[1], Mina Gabraie[4], Pierre Lachapelle[4,5], Michel Cayouette[1,2,3,6]

[1]Cellular Neurobiology Research Unit, Institut de recherches cliniques de Montréal (IRCM), Montreal, QC, Canada; [2]Integrated Program in Neuroscience, [3]Division of Experimental Medicine, McGill University, Montreal, QC, Canada; [4]Department of Ophthalmology, Research Institute of the McGill University Health Centre, Montreal, QC, Canada; [5]Department of Neurology and Neurosurgery, McGill University, Montreal, QC, Canada; [6]Department of Medicine, Université de Montréal, Montreal, QC, Canada

Correspondence to: Michel Cayouette, PhD. Institut de recherches cliniques de Montréal, 110 avenue des PIns Ouest, Montréal H2W 1R7, QC, Canada. Email: michel.cayouette@ircm.qc.ca.

Background: The loss of cell polarity plays a key part in retinal dystrophies such as retinitis pigmentosa (RP) and Leber congenital amaurosis (LCA), resulting in photoreceptor (PR) degeneration and vision loss. Despite not knowing the direct genotype-to-phenotype correlation, many disease-causing mutations in the polarity determinant Crumbs (Crb1), have been identified. Indeed, the loss of Crb1 in mice was shown to cause PR death, due to the loss of adhesions between PR and Müller cells at the apical surface of the retina. Unfortunately, although the role of Crb1 in neuron polarity and survival is well established, little is known about how its intracellular trafficking is regulated. With future treatments for retinal degenerative diseases in mind, the goal of this project is to understand the mechanism by which Crb1 is regulated and how it maintains retinal integrity. Previous work in our laboratory showed that Numb, an endocytic adaptor protein, is an important regulator of protein trafficking in retinal cells. We therefore hypothesized that Numb might function as regulator of Crb1 in Müller glia.

Methods: To study Numb function in Müller cells, we generated a conditional knockout (cKO) mouse line to inactivate Numb specifically in Müller cells by crossing a Glast-CreERT2 mouse line with a Numb-floxed line. At 30 days, mice were administered tamoxifen to trigger inactivation of Numb and retinas were then collected at time points varying from 2 weeks to 17 months for analysis. Firstly, we studied the retinal morphology and outer limiting membrane integrity by histology and immunohistochemistry. Using electron microscopy (EM), adhesions between Müller glia and photoreceptors were analysed and retinal function was assayed in live mice by electroretinography (ERG). To detect protein expression levels, protein extracts were prepared from cKO and control retinas for immunoblotting. To test for the presence of a biochemical interaction, Hek-293 cells were transfected with Numb and Crb1 vectors, and protein extracts were processed for co-immunoprecipitation.

Results: When Numb was deleted in Müller cells, we observed a similar retinal phenotype than what was reported in the Crb1 KO. In 3-month-old animals, we found a disruption of the outer limiting membrane and an ingression of photoreceptor cells in the inner layers of the retina. In older animals (17 months), we observed a clear thinning of the photoreceptor layer and reduced ERG responses. Immunoblotting of retinal lysates revealed that Numb cKO retinas had significantly lower expression of Crb1, suggesting that Numb function in Müller cells is critical to maintain Crb1 levels and thereby outer limiting membrane integrity. Interestingly, we found that Numb can interact with Crb1 both *in vitro* and *in vivo*, suggesting that Numb might function as an adaptor protein regulating Crb1 trafficking.

Conclusions: Based on these results, we suggest that, in the absence of Numb, Crb1 cannot be trafficked to the apical membrane of Müller cells, and is instead degraded. This ruptures the adhesion between Müller and photoreceptor cells, leading to photoreceptor degeneration. We anticipate that understanding the mechanisms by which Crb1 maintains the structural integrity of the retina will lead to new possibilities for target-based therapies against retinal dystrophies.

Keywords: Degeneration; retinal polarity; Crumbs-related dystrophies (Crb1-related dystrophies)

doi: 10.21037/aes.2019.AB046

Cite this abstract as: Vinette M, Bélanger MC, Jolicoeur C, Gabraie M, Lachapelle P, Cayouette M. The Endocytic Adaptor Protein Numb Functions in Müller Glia to Maintain Retinal Polarity and Photoreceptor Survival through the Polarity Determinant Crumbs. . Ann Eye Sci 2019;4:AB046.