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Title of Presentation: Role of Receptor Interacting Protein Kinases in the Retinal Degeneration of S334ter and P23H Retinal Degeneration Mutant Rat models in Retinitis Pigmentosa

Purpose: Retinitis Pigmentosa (RP) is a clinically and genetically heterogeneous group of neurodegenerative diseases affecting photoreceptors and causing blindness. Understanding the cell-death mechanisms in this group of genetic disorders is a key to pathology of RP. Recent studies have suggested that RP is caused by retinal degeneration in rod cells as well as the subsequent death in nearby healthy cone cells. Despite this finding, the characteristics and pathway of cones cell-death have remained unclear. Previous studies showed that the caspase-dependent pathway apoptosis is the main cell-death pathway of RP yet, it seems caspase-independent pathways also are involved in the cell-death process. This study is aimed at investigating the presence of necroptosis, the programmed necrosis, in cones cell-death in two models of photoreceptor degeneration transgenic rat models, P23H and S334ter, with rhodopsin mutation in protein folding and sorting respectively.

Methods: To investigate the cell-death mechanism in cone cells, the activity of necroptosis was tracked by receptor-interacting protein kinase 1 (RIP1) marker. A wide range of transgenic rats in different ages including P10, P17, P28, P40, P46, P55 and P70 was collected. Fresh retinas were removed from the eye cups to perform qPCR and western blot. In addition, the fixed eye cups with removed lens were collected to execute immunohistochemistry and TUNEL staining. Statistical analyses were performed using two-tailed student t-test, $p\text{-}value < 0.05$ to compare the mean of two groups and one-factor ANOVA followed by Tukey-Kramer post-hoc adjustment to compare the mean of multiple groups of data, $p\text{-}value < 0.05$ as discussed in the Results section.

Results: The results of a one-factor ANOVA test showed that there is a statistically significant difference in RIP1 and PNA, the specific marker of cone cell, colocalization rates between different postnatal groups [$F(8,101)=37.85$, $P<0.05$] in the P23H rat model. A post-hoc Tukey test showed that RIP1 is most active at P17 and P28. Similarly, the results of a one-way ANOVA test showed the statistically significant impact of different postnatal in colocalization rate in S334ter [$F(8,119)=26.01$, $P<0.05$]. Tukey adjusted post-hoc test showed statistically higher colocalization rate in P28 compared to P10, P22 and P40. Similarly, colocalization rate on average is higher in P17 and P28 in S334ter model. In addition, the results of western blot on RIP3 for the P23H model showed the presence of RIP3 in all days and the highest RIP3 expression was observed on P17 in P23H rat model. Similarly the results of western blot on RIP3 for S334ter model showed RIP3 activities on all postnatal days with the highest activity on P28. It was also found that there is no colocalization of RIP1 and rhodopsin, the specific marker of rod cell, in any of the postnatal days. This result might suggest that there is no active necroptosis cell-death pathway on rod cells. Further investigation is in progress to rollout the activity of RIP3 cell-death in rod cells.

Conclusion and Future work: This study showed that RIP1 cell-death pathway is active in different postnatal in both P23H and S334ter rat models. The results of western blot and qPCR also showed presence of RIP3 in both P23H and S334ter rat model in all postnatal. The colocalization of RIP3 and PNA and also RIP3 and rhodopsin are in progress.