Final Report on "Cellular and Computational Studies of Tissue Reorganization in Retinitis Pigmentosa"

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Motivation

The title of this application is **Cellular and Computational Studies of Tissue Reorganization in Retinitis Pigmentosa.**" This application combines computational modeling with cellular and molecular studies to understand the retinal mechanisms involved in cone-mosaic reorganization in *Retinitis Pigmentosa (RP).* We are doing Systems Biology of the self-assembly of a tissue during disease.

Background

In the visual system, rods and cones are the primary light sensing cells. Cones are responsible for daytime and color vision whereas rods respond in low light levels. Most mutations responsible for the *R*P selectively affect rods. However, cones undergo degeneration secondary to the loss of rods, often leading to total blindness. It is estimated that 1/ 4,000 people are affected by RP. Hence, understanding the possibility of cone survival has become a major research challenge in RP. In Dr. Lee's studies, she found reorganization of the cone mosaic in the S334ter-line-3 rat (a transgenic model developed to express a rhodopsin mutation similar to that found in human RP). The rods' death triggers a reorganization of the cone mosaic by migration into an orderly array of rings. In their new mosaic, cones survive until old age. The overall hypothesis of this application is that the driving forces for cone migration are due to a complex interaction between rods, cones, Müller cells, and the retinal pigment epithelium (RPE). We hypothesize that trophic factors secreted by these cells mediate this interaction. Based on this hypothesis, we have been able to build a so-far successful computational model for the manifold interaction in the outer retina. This model accounts for and predicts the outcome of a host of cellular and molecular experiments, some of which we have performed preliminarily. Without this model, we could not hand-wave an explanation for many peculiar aspects of the data and thus, they did not make sense. This model, grounded on the field of Systems Biology, is now helping understand the data and make predictions for new experiments. Therefore, with this model, we may be able to understand the cellular mechanisms controlling the mosaic of surviving cones in RP retinas. Consequently, the model may lead to treatment of RP by suggesting strategies for cone repopulation.

New outcome and impact

Aim 1. To Test Whether the Probability of Rod Cell Death Decreases with the Density of Neighboring Rods.

From the healthy-rod data, the model generates a map of the concentration of the trophic factor and thus, the probability of finding a dying rod in arbitrary points of the retina (data not shown). A further test of the model hypothesis will be to inject *N*-methyl-*N*-nitrosourea (MNU). MNU causes local apoptotic death of photoreceptors in a dose-specific manner. The model predicts a reduction of trophic factor in the MNU-affected zone, which thus, should be concentric with a ring of rod death.

Our data showed that intravitreal injection of MNU in normal animals led to local hot spots of photoreceptor deaths. The size of the holes in the outer nuclear layer (ONL) appeared to be related to the concentration of MNU after the injection (Fig. 1). With photoreceptor deaths, MC processes invade the holes. In our experiment, we measured the density of MC processes in the center of the MNU-affected areas. Counts referred to processes encountered within a area of 500 μm^2 from the center of MNU-induced holes. The density of MC processes was higher in the lower density of neighbor rods (Fig. 1).

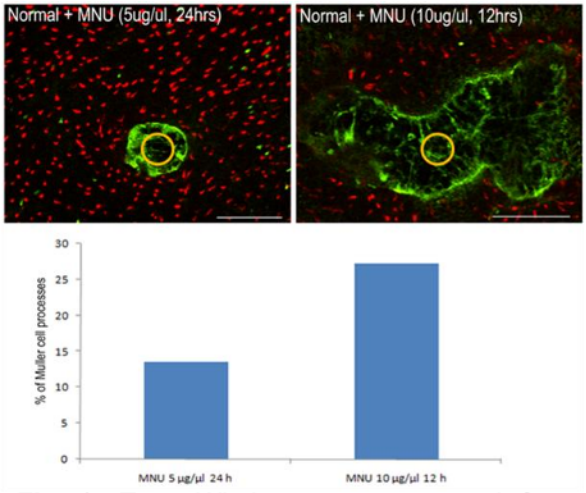
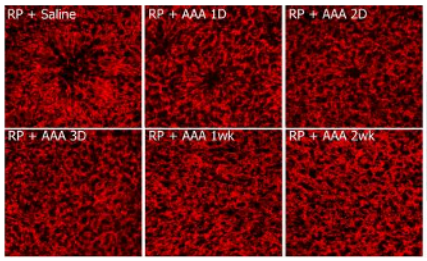


Fig 1. Top. *Wholemounts processed for rhodopsin (red) and GS (green) staining in MNU-treated retina. Processes of MCs (green) are remodeled and placed inside of MNU-induced holes. Scale bars, 50 µm.* ***Bottom.*** *Histogram showing the densities of MC processes in the holes at the top. Counts refer to processes encountered within a area of 500 µm^2 from the center of the MNU-*

Aim 2. To Probe in the Early Stages of RP, If the Spatial Distribution of Cones Depends on the Spatial Distribution of Rod Death. (We are still working on Aim2).

Aim 3. To Determine if Müller-cell Processes Remodel into Low Density of Rods and Cones, and Contribute to the Formation and Maintenance of Cone Rings. We found that AAA eliminates the mosaic of rings of cones, with these cells and MCs redistributing themselves homogenously in RP retinas. In Aim 3, we probed the dynamics of cone diffusion to make the mosaic homogenous after disruption of their interaction with MC processes. We also probed this dynamics by examining hole sizes at different time points after the intravitreal injection of AAA (Fig. 2). We have manipulated the hole sizes and the diffusion of cones by applying MNU and AAA respectively in normal animals. Our experiments showed that the size of the rings decreases as a function of time after the application of AAA. Cones become homogenously distributed 3 days after the drug injection. This distribution of cones persists for life (Fig. 2). These results suggest that the interaction of cones with MCs is important for the spatial distribution of photoreceptors.



Condition	Ring size (μm)
RP + Saline	255.6±8
RP + AAA 1D	160±10
RP + AAA 2D	86.4±8
RP + AAA 3D	0
RP + AAA 1wk	0
RP + AAA 2wk	0

Fig 2*. Wholemounts processed for M-opsin (red) staining in AAA-treated P50 RP retinas. Rings of cones disappear 3 days after AAA injection. The table shows the size of cone rings (mean ± standard error) in the AAA-treated retinas.*

Aim 4. To Ascertain Whether Metalloproteinases Released by the RPE Contribute to Better Spatial Coverage by the Cones. We determined whether the mosaics of S- and M-cones are affected by injecting TIMP-1 in control and RP retinas. We found that when this treatment was applied to the eye of a normal animal, cones formed clusters in the retina (Fig. 3B, F). Moreover, unable to form normal distributions of cones (Fig. 3A, E), this treatment on RP eyes elicited rings of clusters of cones (Fig. 3D, H). These results indicated that TIMP-1 was disrupting the mosaic of cones in both control and RP eyes. Eyes treated with TIMP-1 will thus allow us to investigate whether metalloproteinases induce cones to cover the retina well.

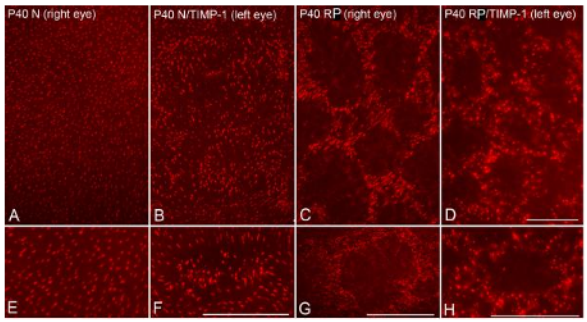


Fig 3*. Wholemounts processed for M-opsin staining in TIMP-1 treated P40 N and P40 RP (right eye–saline — A, E, C, G; left eye, TIMP-1 — B, F, D, H) retinas. Clusters of cones are observed in P40N-TIMP-1 treated retinas. Rings of Clusters of cones are observed in P40RP-TIMP-1 treated retina (n=3, each group). Scale bars, 30 μm.*

Future Direction

1. To investigate cone mosaic in RP rats using high-resolution images and to correlate the findings with clinical phenotypes and genetic mutations in patients with inherited retinal degeneration. Adaptive optics scanning laser ophthalmoscopy (AOSLO) allows for noninvasive, in vivo visualization of retinal abnormalities at a cellular level. AOSLO imaging of cones has provided unique insight into the structure and function of the human visual system and has become an important tool for both basic scientists and clinicians. AOSLO imaging of persons with mutations in the genes that encode the cone opsins have provided new insights about the cone mosaic. First, we will obtain AOSLO images of cone photoreceptors mosaic from RP rats. Then, we will use variety of statistical analyses to measure the difference in mosaic of cones.

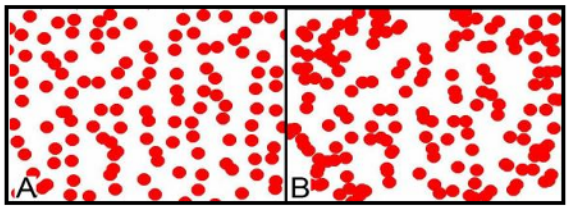


Fig 4*. In the model, cones are distributed at random normally (A), but clusters are formed with inhibition of the metalloproteinases.*

2. To understand whether re-engineered cone mosaics (using AAA, see Fig. 2) in RP can lead to visual function and if so, to find conditions to optimize it. We have preliminary data showing that rearranged cones can both survive until old age and elicit retinal-ganglion-cell (RGC) responses. Furthermore, these preliminary data show that long-term treatment with Ciliary Neurotrophic Factor (CNTF), a nerve-growth factor released by the MCs, may preserve cones and their functions. The apposition of cones and MC processes in the rings may facilitate the delivery of CNTF to the former, contributing to their survival.