Analyses of Mouse RPE Morphology Give Discriminatory Categories

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***Short Abstract* — Age-related macular degeneration (AMD) is the main cause of vision loss in the elderly and is a looming epidemic in our aging society. Presently there is no way to determine how a patient's eye will progress, and no effective treatment for AMD. Retinal pigment epithelium is a crucial site of AMD pathology and undergoes morphological changes as the eye ages and AMD progresses. We collect RPE morphological data from mouse eyes and conduct statistical studies to establish the relationship between the RPE morphology and the age and disease progression of the eye. This work provides a foundation for a potential diagnostic and prognostic tool for AMD.**

***Keywords* — retinal pigment epithelium, age-related macular degeneration, functional principal component analysis, multi-dimensional scaling**

I. Introduction

SITUATED between the neursensory retina and the choroid vessel bed, retinal pigment epithelia (RPE) is crucial for maintaining the wellbeing of photoreceptor cells, and is the principal site of pathogenesis of AMD[1]. To test if RPE morphology changes during and after retinal degeneration as a bystander effect, we compare the morphology of RPE cells from C57BL/6J (wild type), as well as congenic RD10 and RPE65 mouse eyes of various age groups. Using functional principal component analysis (FPCA) and multidimensional scaling (MDS) techniques, we classify RPE sheets into major categories of genotypes and age groups.

II. METHODS

Cell borders of RPE/choroid flatmounts were stained with anti-ZO-1. Photoshop CS2 was used to photomerge the images. Morphometric analysis of cell shape, area, number of neighbors, etc was performed with CellProfiler[2]. Density curves of cell size and aspect ratio were estimated using the penalized likelihood method. FPCA generated principal component scores, used to construct the classification rule. Four classification methods, linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), k-nearest neighbor (KNN), and support vector machines (SVM), were applied to the results of PCA and MDS analysis. Leave-one-out cross validation was used to assess predictive accuracy.

III. RESULTS

Using cell shape (as described by aspect ratio) and cell size (area), FPCA segregates all RPE cell morphology into 4 distinct classes: young C57BL/6J, old C57BL/6J, young RD10, and old RD10. Postnatal 70days best segregates the age groups for both genotypes into young (<70 days) and old (> 70 days). From 88 eye images, FPCA results in a 88 × 4 matrix of the first four PC scores, used to construct classification rules. The predictive accuracies were 96.6% (85 are correctly classified among 88 mice), 95.5%, and 95.5% for LDA, QDA, and SVM, respectively[3].

Using all 123 mouse eyes, further dividing the ages into four age groups, and separating the RPE cells according to their spatial locations, we performed PCA and MDS analyses to show that 1) of all the >20 morphometric measurements, none is particularly significant as 'signature' of genotype or age; 2) spatial information is important in discriminating rd10; 3) different regions reveal different aspects of morphology changes; and 4) postnatal 180 days is when large cell deformation occurs in mouse eyes. Classification accuracies depend on the metric and spatial location of the RPE cells[3].

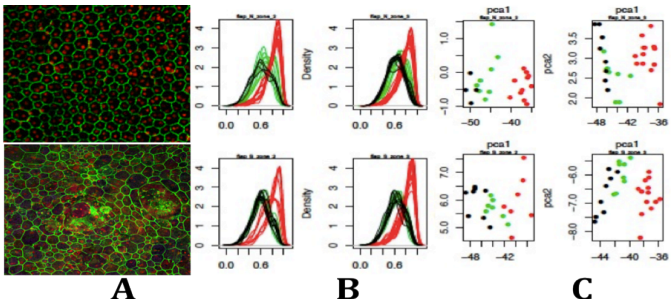

Figure 1: A: Small sections of RPE sheet images (Top: wild type or normal RPE, Bottom: AMD). B: density of cell shape (eccentricity) from four different regions of the same eyes (black: C57BL/6J, red: RD10, green: RPE65). C: sample PCA score plots for age 180 days with the same color code for genotypes.

IV. Conclusion and Discussion

Extensive and systematic statistical analyses of the RPE morphology give discriminatory categories in mouse eyes. In young wild type mice the RPE morphology resembles a regular hexagonal array of cells of uniform size. Old wild-type eyes develop a subpopulation of large cells. A clear disruption of the regular cell size and shape appears in RD10 mutants. Aspect ratio and cell area together give rise to discriminatory principal components that classify age and genotype. Here we demonstrated the use of RPE sheet morphometrics as a clear indicator of retinal disease stage despite age as a confounding factor. These same analyses may be applied to patients noninvasively with suitable imaging instruments.

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