An Accelerated Mouse Model of Inflammatory Dry Eye Disease

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Introduction/Background

- Meibomian glands (MGs) in eyelids (Fig. 1A) are enlarged sebaceous glands connected to hair follicles. The function of MGs is to secrete lipids which form the outer layer of the tear film of the eye (Fig. 1B). This layer maintains tear film stability by preventing tears from evaporating.

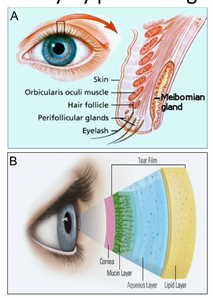

Figure 1. Schematic drawing of Meibomian gland (MG) (A) and tear film (B). The mucin/gel layer (green) is made by goblet cells in conjunctival epithelium, the aqueous layer (blue) is formed by the fluid from lacrimal glands, and the lipid layer (yellow) contains meibum secreted from Meibomian glands in eyelids (A).

- Meibomian gland dysfunction (MGD) is a chronic abnormality of the Meibomian gland, commonly characterized by a change in the quantity or quality of the lipid secretion.
- MGD is among the most frequently diagnosed eye diseases and is a major cause of Dye Eye Disease (DED), but very little is known about the pathogenic processes leading to MGD and DED.

Hypothesis

- We have known from previous study in Dr. Reneker's lab that activation of fibroblast growth factor receptor 2 (FGFR2) in MGs is essential for maintaining MG homeostasis.
- FGFR2 belongs to a family of receptor tyrosine kinase (RTK). Activation of RTK often leads to activation of downstream kinase cascade involving Raf-MEK-ERK1/2.
- The purpose of this study is to investigate the role of ERK ½ in the MGs of adult mice.

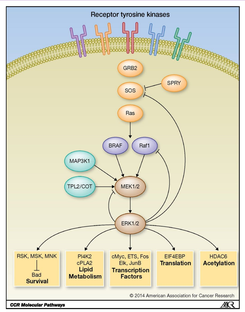

Figure 2. Signal transduction pathway activated by RTKs. ERK1/2 is a key downstream effector that leads to the activation of many downstream target genes.

Methods

- A compound triple transgenic mouse strain (Krt14-rtTA; tetO-Cre; ERK2$^{flox/flox}$) was first generated and then bred to a ERK1-null (ERK1$^{-/-}$) mice to generate a quadruple transgenic line (ERK1$^{-/-}$; Krt14-rtTA.; tetO-Cre; ERK2$^{flox/flox}$). In ERK1$^{-/-}$ background, upon doxycycline (Dox) induction, ERK2 is ablated by Cre recombinasein keratin 14 (Krt14) expressing epithelial cells. This process generates conditional ERK1/2 double knockout mice (referred to as ERK1/2^{DKO}).

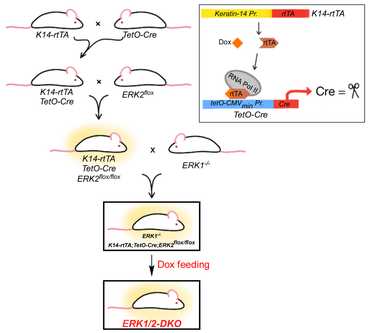

Figure 3. Diagram illustrating how compound transgenic mice are generated and ERK1/2^{DKO} mice are induced by Doxycycline (Dox) treatment.

- The pathological changes in the MGs of the ERK1$^{-/-}$ control (no Dox) and ERK1/2^{DKO} (Dox) mice were examined and compared by histology and immunostaining.

Results

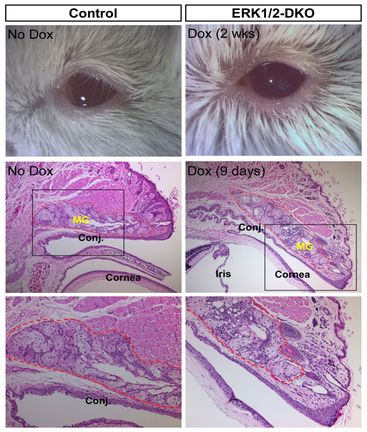

Figure 4. Eyelid appearance and histology of control and ERK1/2^{DKO} mice. Eyelids in ERK1/2^{DKO} mice looked red and swollen, showing the symptoms of inflammation. Histology (H&E staining) revealed abnormal and degenerative phenotype in the MGs of DKO mice.

Results

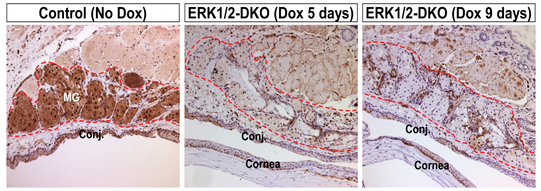

Figure 5. Depletion of ERK proteins from MGs and other ocular surface epithelial cells upon Dox induction. Immunohistochemistry against ERK proteins (brown color) indicated that ERK protein level was significantly reduced in MGs after Dox feeding for 5 days, and was eliminated in MG acini after 9 days of Dox feeding. Infiltrating of ERK^{+} cells in MGs of ERK1/2^{DKO} mice was noticed.

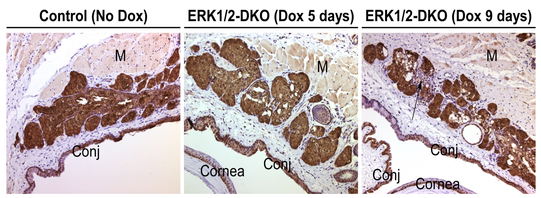

Figure 6. Keratin 14 (Krt14) expression in MGs and ocular surface epithelial tissues. Immunohistochemistry showed that MGs in ERK1/2^{DKO} mice were infiltrated by cells that do not express Krt14 (indicated by arrow), suggesting that these cells are not derived from MGs. MG atrophy and degradation were seen in the MGs of ERK1/2^{DKO} mice.

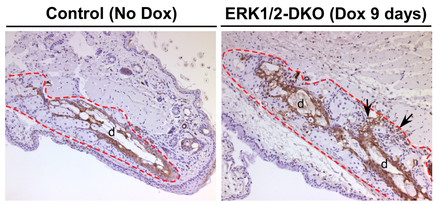

Figure 7. Immunostaining of CD45 (a marker commonly used for identifying inflammatory cells). We found that CD45 is normally expressed in MG ductal epithelial cells as shown in the control. In ERK1/2^{DKO} mice, in addition to MG ductal cells, CD45 expression is also found in cells infiltrating the MG acini (indicated by arrows).

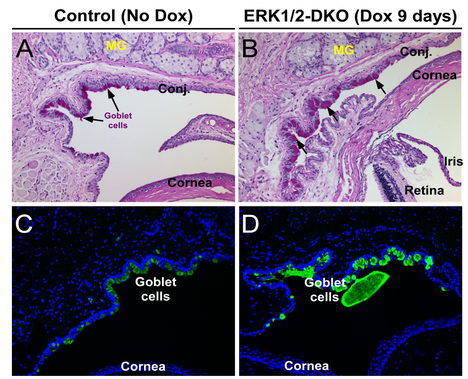

Figure 8. Goblet cells identified by PAS staining (purple red) (A,B) and mucin 5AC immunofluorescence (green) (C, D). Cell nuclei were counter-stained by either hematoxylin (A, B) or DAPI (C, D). Increase of goblet cells and mucin production in conjunctiva of ERK1/2^{DKO} mice.

Summary

- ERK proteins are highly expressed in adult mouse MGs. ERK proteins can be depleted from MGs after transgenic mice (Krt14-rtTA; tetO-Cre; ERK2$^{flox/flox}$) receive Dox for about a week. We have created an inducible ERK1/2 double knockout mouse model.
- Loss of ERK1/2 in keratin-14 (Krt14) expressing cells resulted in an inflammatory response in skin and ocular surface tissues, including the MGs.
- Infiltration of CD45+ cells in the MGs of ERK1/2^{DKO} mice resulted in degeneration of the glandular and ductal tissues.
- We are currently investigating the underlying mechanisms of inflammation and are further characterizing the infiltrating cells in the MGs of ERK1/2^{DKO} mice.

Acknowledgements

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