Dry eye disease (DED) is a multifactorial disease characterized by ocular surface desiccation and related ocular irritation. The prevalence of DED is estimated to be 7% to 34% of the US population, and the number of affected persons is increasing with the aging of our population. The hallmark of DED is tear film instability, caused by a reduction of aqueous tear production and an increase in tear evaporation, either alone or in combination. The most common cause of evaporative DED is tear film instability, caused by a reduction of aqueous tear production and an increase in tear evaporation, either alone or in combination.

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Fibroblast Growth Factor Receptor 2 (FGFR2) Is Required for Meibomian Gland Homeostasis in the Adult Mouse

Lixing W. Reneker,¹ Lanlan Wang,¹ Rebecca T. Irlmeier,¹ and Andrew J. W. Huang²

¹Mason Eye Institute, Department of Ophthalmology, University of Missouri School of Medicine, Columbia, Missouri, United States
²Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, Missouri, United States

PURPOSE. Little is known about the signaling mechanisms controlling meibomian gland (MG) homeostasis and the pathogenic processes leading to MG atrophy and dysfunction in dry eye disease (DED). We investigated the role of fibroblast growth factor receptor 2 (FGFR2) in the MG homeostasis of adult mice.

METHODS. A triple transgenic mouse strain (Krt14-rtTA; tetO-Cre; Fgfr2floxflox), referred to as Fgfr2CKO mice, was generated in which the Fgfr2 gene is ablated by Cre recombinase in keratin 14 (Krt14)-expressing epithelial cells on doxycycline (Dox) induction. FGFR2 expression in normal human and mouse MGs was evaluated by immunohistochemistry. Pathologic MG changes in transgenic mice with conditional deletion of FGFR2 were examined by lipid staining, histology, and immunostaining.

RESULTS. FGFR2 was highly expressed in normal human MGs and adult mouse MGs. Two-month-old Fgfr2CKO mice fed Dox-containing chow for 2 weeks developed severe MG atrophy. MG acinar atrophy in the Fgfr2CKO mice was associated with reduced lipid (meibum) production and the development of clinical findings similar to those in humans with evaporative DED related to MG dysfunction (MGD). Immunohistochemical analyses showed that FGFR2 deletion severely affected proliferation and differentiation of MG acinar cells but affected MG ductal cells to a lesser extent.

CONCLUSIONS. FGFR2 deletion results in significant MG acinar atrophy and clinical manifestations of MGD in Fgfr2CKO mice, suggesting that MG homeostasis is FGFR2 dependent. The Fgfr2CKO mice with inducible MG atrophy can serve as a valuable animal model for investigating the pathogenesis of MGD and developing novel therapeutic strategies for MGD-related DED.

Keywords: meibomian gland dysfunction, FGFR2, dry eye disease, meibomian gland atrophy, knockout animals
genes contain alternative splicing sites at the C-terminal IgIII domain, resulting in two isoforms known as Fgfrb and Fgfrc. An in vivo mouse genetic study showed that conditional gene deletion of one of the isoforms of FGFR2, namely Fgfr2b, in epidermal cells, causes striking abnormalities in hairs and SGs without affecting animal survival.14 These findings suggest that FGFR2b signaling activity is required for the normal development and homeostasis of SGs.

Very little is known about the signaling pathways that regulate MG homeostasis.13,14,17 Specifically, the role of FGFR2 and its related ligands in MG development and homeostasis has not been explored. To determine whether FGFR2 signaling is crucial for MG homeostasis in mice, we used an inducible conditional gene knockout strategy to delete Fgfr2 in keratin 14 (Krt14)-expressing epithelial cells and generated a mouse model of MG atrophy inducible with doxycline (Dox) (Krt14-rtTA; tetO-Cre; Fgfr2flox/flox, referred as Fgfr2CKO) (Fig. 1). Herein, we describe the histopathologic features of MG atrophy in this triple-transgenic mouse strain and demonstrate for the first time that the induced loss of FGFR2 (both b and c isoforms) in 2-month old mice leads to severe MG atrophy, substantiating that FGFR2 plays a pivotal role in MG homeostasis and tissue maintenance. To the best of our knowledge, this mouse model constitutes the first animal model of inducible MG atrophy, phenotypically resembling MG atrophy in human MGD-related DED. Our findings also suggest that the Fgfr2CKO mouse model of Dox-induced MG atrophy can serve as a valuable tool for delineating the pathogenic mechanisms of MGD and for evaluating potential therapeutic interventions for MGD-related DED.

FIGURE 1. Schematic of Dox-induced conditional deletion of Fgfr2 gene. Double transgenic mice (K14rtTAtetOCre) were created to express Cre recombinase, on Dox induction, in epithelial cells that express Krt14 promoters. The double transgenic mice were then bred to Fgfr2flox/flox mice to generate triple transgenic mice (K14rtTAtetOCre-Fgfr2flox/flox). Ablation of Fgfr2 in K14-expressing cells is induced by feeding the triple transgenic mice Dox chow for various lengths of time.

MATERIALS AND METHODS

Acquisition of Donor Eyelid Tissues

Tarsal plates removed from fresh cadaver’s upper and lower eyelids of a healthy Caucasian female donor (36 years of age) were obtained from Mid-America Transplant (St. Louis, MO, USA). The use of human tissue in research conformed to the provisions of the Declaration of Helsinki and was exempted by the Washington University Human Subjects Protection Office. Prior to removal of eye tissues, medical and ocular histories of the donor had been deidentified and reviewed to ensure no evident ocular or systemic diseases. The removal of donor eyelid tissues and corneas was performed under a standard protocol for procurement of ocular tissues.

Mouse Strains and Genotyping

All transgenic mice were bred at the Animal Science Research Center of the University of Missouri (Columbia, MO, USA). Animal experiments were conducted in accordance with the institutional guidelines on the Care, Welfare and Treatment of Laboratory Animals, and the protocols were approved by the University of Missouri Institutional Animal Care and Use Committee. The experiments conformed to the standards in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Transgene alleles were screened by PCR using tail DNA, following the conditions and primer pairs recommended for JAX mice (Krt14-rtTA stock#008099 and tetO-Cre stock#006224) and previously reported for Fgfr2flox mice.18,19

Generation of Conditional Knockout Mice on Dox Administration

Compound transgenic mice of K14rtTAtetOCreFgfr2flox/flox were generated via natural mating following the scheme illustrated in Figure 1. In this deletion system, Cre expression in Krt14- (or K14)-expressing cells can be initially induced in mice of any age by systemic administration of Dox, and consequently the floxed Fgfr2 is ablated by Cre recombinase (Fig. 1). In our study, conditional knockout of Fgfr2 was induced in 2-month-old adult mice by ad libitum Dox chow feeding, for 4 days to 2 weeks, at the dosage of 1 g Dox/kg chow (Dox diet #AD3008; Custom Animal Diets, Bangor, PA, USA). Littermates fed with regular chow without Dox served as control animals.

Oil-Red-O Staining for Lipids

Fresh mouse eyelids were collected from transgenic and control mice, immediately fixed with 4% paraformaldehyde
and in newly differentiated meibocytes (white arrow) and in newly differentiated meibocytes (white arrow) and in newly differentiated meibocytes (white arrow). 5-Bromo-2’-Deoxyuridine (BrdU) incorporation was performed following the conditions previously described by Fromm et al. \(^{20}\)

**Immunofluorescence and Immunohistochemistry**

Paraffin sections were deparaffinized in xylene, rehydrated in a decreased ethanol series, and subjected to antigen retrieval in 10 mM sodium citrate buffer by boiling for 10 minutes. \(^{21,22}\) Sections were treated with 3% hydrogen peroxide in PBS for 20 minutes to block endogenous peroxidase activity. Cyrosections (7 μm) were dipped in PBS to remove OCT, fixed with 4% PFA for 10 minutes, and then rinsed with PBS three times. Tissue sections were blocked with 3% horse serum in PBST (PBS plus 0.1% Tween-20 for paraffin sections) or PBS plus 0.5% Triton X-100 (for cryosections) for 1 hour at room temperature and then incubated overnight at 4°C, with primary antibodies diluted in the same buffer. Slides were washed with PBS, incubated at room temperature for 1 hour with either fluorophore-conjugated or biotinylated secondary antibodies, and then washed with PBS. For immunofluorescence, cell nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI), and slides were mounted with Mowiol (Sanofi-Aventis, Bridgewater, NJ, USA) for viewing under a Leica microscope equipped with a charge-coupled device (CCD) camera for photography. For immunohistochemistry, sections were incubated with Vectastain Elite ABC Reagent (PK-6100; Vector Laboratories, Burlingame, CA, USA), and color was developed using 3,3’-diaminobenzidine as a substrate (D4293; Sigma-Aldrich Corp., St Louis, MO, USA). Sections were counterstained with hematoxylin for cell nuclei.

Primary antibodies were purchased from the following sources: anti-FGFR2 (ab10648; Abcam, Cambridge, MA, USA); anti-keratin 10 and anti-keratin 14 (PRB-159P and PRB-155P, respectively; Covance, Emeryville, CA, USA); anti-keratin 16 (NBP2-45538; NovusBio, Littleton, CO, USA); anti-p63 (GTX102425, GeneTex, Irvine, CA, USA); anti-PPAR \(_\delta\) (NBP2-45538; NovusBio, Littleton, CO, USA); anti-p63 (GTX102425, GeneTex, Irvine, CA, USA); and anti-BrdU (M-0744; Dako, Carpinteria, CA, USA). Fluorophore-conjugated secondary antibodies were obtained from ThermoFisher Scientific (Rockford, IL, USA) and biotinylated secondary antibodies were from Vector Laboratories.

**Statistical Analysis**

For quantitative analysis of BrdU index in MG ductal epithelial cells, two eyelids of different mice were excised from either control or Fgfr2\(^{-/-}\) fed with Dox for 6 days. A minimal of four cross sections along a MG central duct and a minimal of six ducts on each section were included in analyses. Data were expressed as mean ± SEM, and \(P\) values were calculated using paired Student’s \(t\)-tests, with \(P < 0.05\) being significant.

**RESULTS**

**FGFR2 Expression in Human and Mouse MGs**

To investigate the importance of FGFR2 in MG homeostasis, we first examined FGFR2 expression patterns in the tarsal plate of human and mouse eyelids. We noted high FGFR2 levels were expressed in both acinar and ductal epithelial cells of human and mouse eyelids (Fig. 2). The finding of mouse MGs having a similar FGFR2 expression pattern to that of human MGs suggests that FGFR2 signaling is required for MG homeostasis in both species, similar to what has been reported in mouse SGs. \(^{14}\)

**MG Atrophy as a Result of Fgfr2 Conditional Deletion on Dox Induction**

Conditional deletion mediated by keratin 5 (Krt5) promoter has shown that Fgfr2\(^{-/-}\) is required for SG homeostasis in the skin of adult mice. \(^{14}\) However, to our knowledge, the role of FGFR2 in MG homeostasis has never been investigated in that...
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mouse model. Keratin 14 (Krt14) is a keratin pair of Krt5 and is known to be expressed in various ocular surface epithelial tissues, including MGs. To investigate whether FGFR2 plays a role in MG homeostasis, we generated a triple transgenic mouse model (Krt14TA-tetOCre-Fgfr2Z/?) , as depicted in Figure 1, in which conditional deletion of Fgfr2 only occurs via activation of Krt14 promoter on Dox feeding. At 2 months of age, the mice were fed either regular chow (control mice) or Dox chow (Fgfr2 conditional knockout or Fgfr2Z/ mice). The triple transgenic mice fed with regular chow display no evident MG pathologies but develop MG atrophy on feeding with Dox-containing chow. After 7 to 10 days of Dox chow feeding, Fgfr2Z/ mice were reluctant to keep their eyes open wide and showed symptoms of ocular irritation. With extended Dox feeding (14 days), more advanced symptoms were noted, with macerated periorbital hairs and eyelids, suggesting excessive eye rubbing due to ocular irritation (Fig. 3). Control mice did not exhibit such symptoms. Lipid (meibum) production by MG acini in the upper eyelids was assessed by eyelid whole mounts stained with ORO. In control mice, meibum was seen in the central duct and showed signs of ocular irritation, including loss of corneal luster and macerated eyelids, after extended (2 to 3 weeks) Dox chow feeding (D). Such signs of ocular irritation were likely due to increased tear evaporation and tear film instability.

Histologic examination of MG atrophy after 1 week of Dox feeding revealed a moderate reduction in the number of MG acini in Fgfr2Z/ mice compared with the number of MG acini in control mice (Fig. 4). The conjunctival and corneal epithelia looked similar in control and Fgfr2Z/ mice. Immunostainings of mucin 5AC (Muc5AC) as a goblet cell marker and of Krt13 as a conjunctival epithelial marker (Supplementary Fig. S1) further substantiated the aforementioned findings. After 2 weeks of Dox feeding, Fgfr2Z/ mice had developed severe MG atrophy. However, PAS staining revealed no discernible difference in goblet cell density between control and Fgfr2Z/ mice. In contrast to the conjunctival epithelium, the corneal epithelial layer after 2 weeks of Dox feeding appeared to be thinner in Fgfr2Z/ mice than in control mice (Figs. 4C, 4D). Cross sections of the upper eyelids of Fgfr2Z/ and control mice were stained with ORO to correlate reduced meibum production with the morphologic and histologic changes of MG atrophy (Figs. 4E, 4F). Lipid staining confirmed that MG atrophy in Fgfr2Z/ mice was associated with a severe decrease in acinar size and a reduction of meibum volume in the central duct (labeled d) in Fgfr2Z/ mice fed Dox chow for 10 days. Sections were counterstained with hematoxylin. M, muscle; HE, hair follicle.

To evaluate the efficiency of Dox induced gene deletion, we examined the levels of Cre and FGFR2 in the eyelids of control and Fgfr2Z/ mice (Supplementary Fig. S2). After 4 days of Dox feeding, Cre was expressed in most MG acinar cell nuclei but with variable amounts (Supplementary Fig. S2B). The expression of Cre in ductal cells was delayed, but high levels were detected after 6 days of Dox feeding (Supplementary Fig. S2C). In response to Cre expression, FGFR2 level was reduced significantly in the MG acini of Fgfr2Z/ mice fed with Dox chow compared with that in control mice (Supplementary Fig. S2D).
or terminally differentiated meibocytes. Compared with differentiated meibocytes and then down-regulated in mature that regulates lipid synthesis, is up-regulated in newly human MGD. Consistent with reduced meibum synthesis and secretion and Dox induction (Supplementary Figs. S2F, S2F).

Ductal atrophy (Figs. 6A, 6B). 

Acinar atrophy (Figs. 6A, 6B). 

Krt14 expression in different MG structures, such as the acinus, ductule, and the central duct (Fig. 5). As the diagram in Figure 5 illustrates, cross sections of the upper eyelid in the distal MG area and of the lower eyelid close to MG orifice were selected to investigate any changes in acini around the ductules and acini around central duct. MG acinar atrophy was readily visible in Fgfr2 CKO mice fed with Dox for 1 week compared with the control mice. The ductal structures in mutant mice were less affected than acinar tissue. The acellular area (occupied by meibum) within the ductule or central duct was reduced or nearly absent in Fgfr2 CKO mice because of decreased meibum production.

Immunofluorescence of FGF2R2 was diminished in both MG acinar and ductal cells but was still detectable in the conjunctival epithelium of Fgfr2 CKO mice after 10 days of Dox induction (Supplementary Figs. S2F, S2F').

To further evaluate the phenotype of MG atrophy in Fgfr2 CKO mice, we examined the immunofluorescence of Krt14 expression in different MG structures, such as the acinus, ductule, and the central duct (Fig. 5). As the diagram in Figure 5 illustrates, cross sections of the upper eyelid in the distal MG area and of the lower eyelid close to MG orifice were selected to investigate any changes in acini around the ductules and acini around central duct. MG acinar atrophy was readily visible in Fgfr2 CKO mice fed with Dox for 1 week compared with the control mice. The ductal structures in mutant mice were less affected than acinar tissue. The acellular area (occupied by meibum) within the ductule or central duct was reduced or nearly absent in Fgfr2 CKO mice, a phenotype consistent with reduced meibum synthesis and secretion and ductal obstruction with exfoliated epithelial plugs known in human MGD.

**PPARγ, Krt10, and Krt16 Expression by Immunohistochemistry**

In normal MGS, meibocytes within an MG acinus undergo a pattern of differentiation, starting from undifferentiated proliferating basal cells in the periphery and progressing to suprabasal nucleated meibocytes that synthesize lipids. During this differentiation process, peroxisome proliferator–activated receptor gamma (PPARγ), a lipid-activated hormone receptor that regulates lipid synthesis, is up-regulated in newly differentiated meibocytes and then down-regulated in mature or terminally differentiated meibocytes.

Compared with control mice, Fgfr2 CKO mice fed Dox chow for 1 week exhibited a significant decrease in the number of PPARγ-positive meibocytes, which correlates with the finding of acinar atrophy (Figs. 6A, 6B).

Normally, as meibocytes proceed through their usual holocrine differentiation, the acinar cells increase in size as a consequence of accumulating lipid droplets, and then rupture and release their lipid cargo into the ductule space. Before the lipid (meibum) in the central duct is delivered to the ocular surface to form the outer layer of tear film, most of the cellular remnants are disintegrated. In the control mice, this process was evident by the lack of PPARγ expression in mature meibocytes with high lipid content inside the duct (Fig. 6A). In Fgfr2 CKO mice, the meibocytes in the central duct contained more densely packed and nucleated cell debris, suggesting that FGR2 deletion inhibits differentiation and maturation of meibocytes and disrupts the normal holocrine mechanism (Fig. 6B).

To confirm the observation that ductal structures are less or minimally affected compared with acini in Fgfr2 CKO mice, we examined by immunohistochemistry the expression of Krt16, a marker for ductal epithelia (Figs. 6C, 6D). In control mice, Krt16 was expressed at a much higher level in MG ductules and central duct than in acini. In Fgfr2 CKO mice, despite severe MG acinar atrophy, the intensity of Krt16 immunostaining in the ductal tissues was at a similar level as in control mice (compare Fig. 6D with 6C). This result suggests that, in contrast to MG acinar cells, ductal epithelial cells are not significantly affected by FGR2 deletion.

Jester et al. previously demonstrated that expression of Krt16 (which pairs with Krt10) extends from the fully keratinized epidermis (skin) into the ductal epithelium at the MG orifice in young adult mice and that Krt1 expression extends posteriorly toward the conjunctiva. Krt10 expression in the MG central duct was also shown by Call et al. recently. We found that Krt10 was expressed in ductal epithelial cells in both control and Fgfr2 CKO mice (Figs. 6E, 6F). However, the central ducts of Fgfr2 CKO mice showed a reduction of meibum volume and an increase of cellular debris (Fig. 6F). These results suggest that MG acinar cell differentiation and maturation are FGR2 dependent. In contrast, ductal epithelial cells are not significantly affected by the loss of FGR2.

**Decrease of MG Basal Cells in Fgfr2 CKO Mice**

When apoptosis was examined by TUNEL assay, a few positive nuclei (indicating apoptosis) were only found in the duct, but not in the acini, in both control and Fgfr2 CKO mice, suggesting that MG atrophy in mutant mice is not caused by cell death (data not shown). To further explore the pathogenic mechanisms of MG atrophy in Fgfr2 CKO mice, we examined the
ductal basal cells but rarely seen in acinar basal cells. We
Dox chow for 1 week, PCNA-positive nuclei were found in
these cells are in the proliferative state. In
basal epithelial cells of MG acini and ducts, suggesting that
eyelids. In control mice, PCNA-positive nuclei were found in
Krt14 immunofluorescence (green) to demarcate the MGs in
Immunofluorescence of PCNA (red) was colocalized with
expression pattern of another cell proliferation marker,
(compare Fig. 7A with 7B). We then investigated the
expression of basal and proliferating cell markers. (A, B)
Transcription factor p63 in MGs detected by immunohistochemistry on
crysections. The basal cells in MG acini (dark brown nuclei indicated
by arrows in A) and ducts (data not shown) express p63 (A). In
FGFR2CKO mice fed with Dox for 4 days, the number of basal cells
around the MG acini was markedly reduced (B). (C, D) Immunofluorosençe of Krt14 (green) and proliferative cell nuclear antigen
(PCNA) (red). Krt14 expression marks the areas of MGs in eyelids. In
control mice (C), intense PCNA immunofluorescence was found in
acinar (a) and ductal (d) basal epithelial cell nuclei (red as indicated by
tebite arrow in C), suggesting that these cells are in the proliferative
state. Within a MG acinus, PCNA immune intensity decreases when
basal cells are differentiated into meibocytes (arrowhead in C). In
FGfr2CKO mice induced by Dox for 1 week, PCNA-positive nuclei were
rarely seen in acinar basal cells, but detected in ductal basal layer
(white broken-line circle in D). (E, F) BrdU-labeled cells were found in
MG acinar and ductal basal cell layer in control mice (arrows in E). In
FGfr2CKO mice fed with Dox for 6 days, BrdU incorporation was seen in
MG ductal basal cells (arrows in F), but rarely detected in the acini. HF
hair follicles.

number of basal cells in MG acini using p63 as a marker.
Transcription factor p63 is a member of the p53 family and a
key regulator of epithelial cell fate.24–26 We found that basal
cell density in MG acini of Fgfr2CKO mice was largely reduced
after 4 days of Dox induction compared with the control mice
(compare Fig. 7A with 7B). We then investigated the
expression pattern of another cell proliferation marker,
proliferating cell nuclear antigen (PCNA) (Figs. 7C, 7D).
Immunofluorescence of PCNA (red) was colocalized with
Krt14 immunofluorescence (green) to demarcate the MGs in
eyelids. In control mice, PCNA-positive nuclei were found in
basal epithelial cells of MG acini and ducts, suggesting that
these cells are in the proliferative state. In Fggr2CKO mice fed
Dox chow for 1 week, PCNA-positive nuclei were found in
ductal basal cells but rarely seen in acinar basal cells. We
further confirmed that cell proliferation in MG ductal epithelium of Fgfr2CKO mice by colocalizing PCNA immuno-
fluorescence with Krt17. Krt17, similar to Krt 16 (as shown in
Figs. 6C, 6D), is also preferentially expressed in MG ductal cell
(Supplementary Fig. S3). Taken together, these results suggest
that FGFR2 deletion primarily inhibits proliferation of the MG
acinar basal cells.

BrdU incorporation assay was performed to examine the
effect of FGFR2 on cell cycle progression in MG acinar basal
cells (Figs. 7E, 7F). In control mice, BrdU-positive nuclei,
diagnostic of cells at S-phase of cell cycle, were found in the
basal cell layers of MG acini and in central ducts. Compared
with the control mice, a drastic reduction in BrdU-labeled cells
was noted in MG acini of Fgfr2CKO mice, suggesting that FGFR2
deletion blocks cell cycle progression in MG acinar basal cells.
In contrast, BrdU incorporation in the ductal basal cells of
FGfr2CKO mice did not seem to be affected. The BrdU labeling
index in ductal epithelium was 7.31 ± 0.65% in control and
5.90 ± 0.55% in Fgfr2CKO (P = 0.08), indicating the difference
was not statistically significant. The findings of BrdU labeling
are consistent with those of PCNA immunofluorescence. Taken
together, our cell proliferation studies imply that FGFR2
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signaling is crucial for acinar basal cell proliferation to maintain MG homeostasis in adult mice.

To explore the downstream signaling pathways of FGFR2, we further examined the levels of ERK1/2 (or ERK) proteins and their active form, the phosphorylated ERK (or pERK), in the MGs of control and Fgfr2CKO mice fed with Dox for 4 days (Supplementary Fig. S4). We found that ERK proteins were present in both MG acinar and ductal cells in control mice (Supplementary Figs. S4A, S4C), but pERK (the active form) was mostly localized in the nuclei of MG acini. Despite MG atrophy in Fgfr2CKO mice, pERK could still be found in the nuclei of MG acinar cells of both control and Fgfr2CKO mice (Supplementary Figs. S4B', S4D'), suggesting that FGFR2 may regulate MG basal cell proliferation via ERK-independent pathways.

**DISCUSSION**

By definition, MGD is a chronic, diffuse MG abnormality, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in glandular secretion.5,7,30 This condition may result in alterations of the tear film on the ocular surface, symptoms of eye irritation, clinically apparent inflammation, and ocular surface morbidities.2,31 MGD is the leading cause of DED in the United States and elsewhere in the developed world, affecting 5% to 50% of the population aged 50 or older.3,33 Age-related changes in human MGs include primary acinar atrophy and/or inspissation of MG orifices leading to decreased meibum secretion. MG atrophy and dropout can apparently occur as early as 25 years of age, and the extent of these pathogenic changes increases significantly and approximately linearly with age.3,33 At present, the underlying mechanism of age-related MG acinar atrophy is poorly understood. In this study, we developed a novel mouse model of inducible MG atrophy by conditional deletion of FGFR2. We demonstrate for the first time in mice that FGFR2 plays an essential role in MG acinar maintenance and homeostasis. Importantly, the observed MG atrophy in our mouse model as a result of loss of FGFR2 is in striking resemblance to age-related MG atrophy in humans.34 Because FGFR2 proteins are present at high levels in both mouse and human MGs (Fig. 1), our current findings thus imply that reduced or altered FGFR2-signaling activity could be one of the underlying mechanisms that lead to age-related MG acinar atrophy and glandular dropouts in humans.

It is well known that the MG acinar cells (meibocytes) secrete meibum via a holocrine mechanism.3-5 During this secretory process, the meibocytes undergo a well-defined program of cell differentiation and maturation, which can be defined morphologically as undifferentiated (basal), differentiating, mature, and hypermature meibocytes. A single MG acinus consists of a contiguous layer of proliferative basal cells in the acinar periphery that surrounds a cluster of lipid-filled meibocytes undergoing differentiation and maturation.56 Lysis of hypermature meibocytes releases meibum via a small canal (ductule) to a central excretory duct that opens at the lid margin. To maintain meibum production and secretion continuously, the meibocytes are constantly undergoing renewal that is sustained by division of the basal cells in the peripheral acinus. Our current data from the control mice (Fig. 7) also support such a notion on MG homeostasis. However, how does FGFR2 signaling control MG homeostasis at cellular level? Our findings in the Fgfr2CKO mice with Dox-induced MG atrophy suggest that cell proliferation markers, including PCNA expression and BrdU incorporation, are drastically reduced in the MG acinar basal cells (Fig. 7). Deletion of FGFR2 induced by Dox results in the loss of MG acinar basal cells, as shown by the number of p63-expressing cells (Fig. 7B) and disruption of the continuous replenishment of meibocytes and regeneration of MG clusters, thus leading to MG atrophy. This finding further suggests that FGFR2 signaling plays an essential role in maintaining acinar basal cell proliferation in MGs. Alter 2 evenly so the role that FGFR2 activity is required for the maintenance of MG acinar progenitor/stem cell population, which was recently demonstrated by Parfitt et al.57 Dox-induced FGFR2 deletion may result in a depletion of MG acinar progenitor cells in Fgfr2CKO mice. Nonetheless, whether FGFR2-signaling activity plays a similar role in human MGs and whether such activity is altered in aged human MGs await further investigation.

In obstructive MGD, hyperkeratinization of the MG orifice is thought to lead to cystic ductal dilation and downstream disuse atrophy of the MG acini.3,38,39 However, ductal hyperkeratinization was not observed in a mouse model of age-related MGD.35 In our current mouse model, we did not see ductal dilation in Fgfr2CKO mice, and the K16 and K10 expression patterns were not affected by MG atrophy (Fig. 6). By immunofluorescence, a low level of FGFR2 was still detectable in MG ductal epithelial cells in Fgfr2CKO mice fed Dox for 6 days (data not shown). The differential deletion efficiency between MG acinar and ductal cells potentially could result from a higher FGFR2 level (Fig. 1B) and a slower cell turnover rate in ductal epithelial cells than in acinar meibocytes.57 However, the acellular areas (indicative of meibum volume) in MG ducts were visibly reduced, and the pyknotic nuclei were increased in the ductules and central ducts of Fgfr2CKO mice (Figs. 5-7). These observations suggest that meibocyte differentiation and maturation was compromised in Fgfr2CKO mice. We speculate that such an accumulation of cellular/nuclear debris in the ductal system could be a contributing factor, eventually leading to pathogenic ductal obstruction. Clinically, MGD is often associated with an inflammatory response. The causal relationship between MGD and inflammation remains somewhat debatable.5,60 In our Fgfr2CKO mouse model, inflammation was not readily evident when the mutant mice developed severe MG atrophy after 2 weeks of Dox treatment (Fig. 3D). Because Dox has been shown to have anti-inflammatory effects and is frequently used clinically for treating MGD,41,42 it is possible that Dox is suppressing inflammation in the Fgfr2CKO mice. Alternative conditional deletion systems have been investigated,35 and we are currently breeding another transgenic model using tamoxifen (instead of Dox) to turn on Cre expression. Nonetheless, we can take advantage of our current Dox inducible model to explore the pathogenic mechanisms of glandular atrophy in MGD independent of inflammation-mediated pathways.

Conventionally, DED is often considered to be a disorder of tear film, accompanied by changes in several ocular surface tissues, including the conjunctiva, goblet cells and corneal epithelium.44,45 Similar to Mgs, these ocular surface epithelial tissues also express Krt14 and are subject to FGFR2 deletion in our mouse model. However, the deletion efficiency varies among these tissues, depending on several factors such as the K14-promoter activity level, accessibility to Dox, and cell turnover rate. When FGFR2 protein level was examined by immunofluorescence in ocular surface tissues of Fgfr2CKO mice fed with Dox for 10 days, the signal intensity was reduced but still detectable in conjunctival and corneal epithelial cells (data not shown). In this study, we could not examine whether prolonged Dox exposure results in complete depletion of FGFR2 in these tissues, because the general health of Fgfr2CKO mice started to deteriorate after 2 weeks of feeding with Dox. When conjunctival epithelia of control mice and Fgfr2CKO mice fed with Dox chow for 2 weeks were carefully examined by histology, we did not observe any major changes in cell
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morbidity or goblet cell density (Fig. 4; Supplementary Fig. S1). However, the corneal epithelial layer of Fgfr2<sup>−/−</sup> mice was notably thinner than that of control mice (Fig. 4D, pointed out by arrows). Because corneal surface damage can occur in MGD patients, the corneal changes in Fgfr2<sup>−/−</sup> mice could be secondary to the severe MG atrophy induced after 2 weeks of Dox feeding. Alternatively, corneal epithelial cells can be directly affected by FGF2 deletion, as we have previously shown that FGF2 plays a critical role in control of cell proliferation during early corneal development. To further explore whether FGF2 is directly involved in maintaining normal corneal epithelium homeostasis, an inducible Cre system driven by the cornea-specific Krt12 promoter can be used in future studies to eliminate confounding factors such as the interference from eyelids and other ocular surface tissues.

In conclusion, our novel mouse model with conditional deletion of FGF2 clearly demonstrates that the FGF2-signaling pathway is critical for MG maintenance and homeostasis in adult mice. Because human MGs also express high levels of FGF2 and because MGD is the most common cause of evaporative DED, it would be exciting to further substantiate that age-related MG atrophy and glandular dropouts in aging humans are a result of deficient FGF2 signaling. Understanding the underlying mechanism of MGD can potentially lead to the development of effective management of this condition. The animal model of MG atrophy we developed not only should allow us to elucidate a previously unexplored disease process, but would also facilitate the development of novel therapeutic strategies specific for MGD, such as modulation of FGF2 signaling in MGs and ocular surface tissues.

Acknowledgments

The authors thank Bethany Irlmeier, Alyssa Labonte, and Abby Lueckenotte for technical assistance and Sharon Morey for editorial assistance.

Supported by National Institutes of Health R01 Grant EY24221 (LWR).

Disclosure: L.W. Reneker, None; L. Wang, None; R.T. Irlmeier, None; A.J.W. Huang, None.

References


