

RESEARCH ARTICLE

Human meibomian gland organoids to study epithelial homeostasis and dysfunction

Chuyue Yu^{1,2,†}, Xichen Wan^{2,†}, Jinsong Wei^{1,†}, Zhaoting Xu¹, Xingru Wu¹, Xiaoye Wang¹, Yabo Mi¹, Yiming Zhang¹, Dan Wu³, Xujiao Zhou², Qihua Le², Jianjiang Xu², Chen Zhao², Xinghuai Sun², Xingtao Zhou², Jiaxu Hong^{2,4,5,6,*}, Bing Zhao^{1,7,*} 

¹School of Basic Medical Sciences, Institute of Biomedical Innovation, The First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang 330031, China

²Department of Ophthalmology, Eye & ENT Hospital, State Key Laboratory of Brain Function and Disorders, Fudan University, Shanghai 200031, China

³Department of Facial Plastic and Reconstructive Surgery, Eye & ENT Hospital of Fudan University, Shanghai 200031, China

⁴NHC Key Laboratory of Myopia and Related Eye Diseases, Shanghai 200031, China

⁵Shanghai Key Laboratory of Rare Disease Gene Editing and Cell Therapy, Shanghai Engineering Research Center of Synthetic Immunology, Shanghai 200032, China

⁶Department of Ophthalmology, Children's Hospital of Fudan University, National Pediatric Medical Center of China, Shanghai 201102, China

⁷Z Lab, bioGenous BIOTECH, Shanghai 200438, China

†= equal contribution.

*Correspondence: jiaxu.hong@fdeent.org (J. Hong), bingzhao@ncu.edu.cn (B. Zhao)

Abstract

Meibomian glands (MGs) are holocrine glands that secrete lipids to maintain the homeostasis of the ocular surface, and their dysfunction leads to dry eye disease. Herein, we established long-term 3D organoid culture for murine and human MGs, which retained the cell lineages and lipid-producing ability. The organoids mimicked the drug treatment responses and generated functional MGs after orthotopic transplantation. Inspired by organoid cultures, we found FGF10 eye drops could rescue all-trans retinoic acid-induced MG dysfunction in mice. Besides, nicotinamide uniquely hampered the human MG organoid expansion by inhibiting FGF10 signaling. Single-cell atlas and lipidome not only aligned the delineated cell types and featured lipids between human MGs and organoids, but also highlighted MAPK signaling inhibition enhanced acinar cell differentiation and functional maturation of MG organoids. In summary, this study established an organoid platform to explore epithelial homeostasis and dysfunction of MGs, facilitating drug development and regenerative medicine for dry eye disease.

Keywords meibomian gland, organoids, tissue stem cells, FGF10, nicotinamide, MAPK signaling, single-cell atlas, orthotopic transplantation

Introduction

Meibomian glands (MGs), the holocrine sebaceous glands (SGs) located in the eyelids, are essential for the homeostasis of the tear film and ocular surface. MGs are characterized by a central duct surrounded by acini that are responsible for lipid production. Under homeostasis, the basal cells in acini could self-renew and differentiate to acinar cells, which are also called meibocytes. The

meibocytes begin to synthesize lipid while migrating toward the center of the acini. Finally, the meibocytes rupture to release the lipid content. Delivered by the central duct to the eyelid margin, the secreted lipid forms the outermost layer of the precorneal tear film to prevent tear evaporation and environmental insults (Mathers, 2004).

Meibomian gland dysfunction (MGD) is the leading cause of evaporative dry eye disease (Hwang et al., 2017),

which is featured by poor vision quality, eye irritation, ocular surface inflammation, and discomfort, affecting 38%–68% population worldwide. However, the only treatment for MGD is palliative care (Sun et al., 2020). Various risky factors were reported, including aging, drug treatment, and congenital defect (Bu et al., 2019, 2021; Caffery and Josephson, 1988; Hom et al., 1990; Liu et al., 2016; Nien et al., 2011; Sullivan et al., 2000, 2002), while the underlying pathology of MGD would be the stem cell exhaustion. To this end, the culture of MG stem cells, which could self-renew and differentiate into functional meibocytes *in vitro*, would be promising for MGD therapeutics. However, existing 2D immortalized MG epithelial cells are difficult to simulate *in vivo* responses (Liu et al., 2010; Xie et al., 2018). Thus, it is urgent to create an *ex vivo* model for exploring the homeostasis and dysfunction of MGs.

Organoids with three-dimensional (3D) structures derived from adult stem cells accurately mimic physiological aspects of parental tissues *ex vivo*. Thus, organoids are used to model organ development and disease progression (Clevers, 2016). However, no approach has been established to generate human MG organoids (hMGOs) yet, limiting the study of the physiology and pathology of MGs. In this study, we achieved long-term expansion of murine and human MGs as 3D organoids, which recapitulated the characteristics of tissues, and modeled the MG degenerative diseases induced by chemical injuries *ex vivo*. Notably, hMGOs could engraft and undergo functional maturation after orthotopic transplantation into mice. Furthermore, we probed the cell types and lipidome of parallel human MG tissues and organoids using scRNA-seq and lipidomic analysis, highlighting that the MAPK signaling is critical for meibocyte maturation. Taken together, this study described an organoid platform to explore the homeostasis and diseases of MGs and provided a useful platform for drug testing and regenerative medicine.

Results

Establishment of functional murine MG organoids

Before formulating the culture protocol for meibomian gland organoids (MGOs), we first examined the key signaling pathways in the MG tissues (Fig. 1A). FGF signaling is essential for mammary gland development, and loss of Fgf10 or its receptor Fgfr2 led to placode defects during embryogenesis (Kim et al., 2013; Mailleux et al., 2002). The Wnt signaling is the key driver of adult stem cells in most tissues. As the receptor for R-spondins, Lgr5 marks adult stem cells in actively self-renewing organs (Nusse and Clevers, 2017). In addition to the extracellular signals, nicotinamide phosphoribosyltransferase (Nampt) is a key enzyme that regulates the intracellular nicotinamide adenine dinucleotide (NAD) pool, which is crucial for the cellular redox stage (Garten et al., 2015). Immunostaining

showed that Fgfr2, Lgr5, and Nampt were highly expressed in MG tissues, which were marked by Krt14 (Fig. 1B). Therefore, we supplemented FGF10, R-spondin-1, and nicotinamide as the core factors in murine MG organoid (mMGO) culture medium. During 9 days' incubation, the seeded MG cell gradually formed the spheroid structure, and became solid and opaque during expansion (Figs. 1C and S1A). H&E staining showed organoids and tissues shared similar architecture: the outer layer of the organoid was lined with small and compact cells, which resembled the basal cells. While the internal cells displayed lower nucleocytoplasmic ratios and condensed cell nuclei ruptures, which resembled the differentiated acinar cells (Fig. 1D).

To further confirm the cell lineages in organoids, we stained the typical cell markers. In the MGs, the basal acinar cells (Krt5⁺) retained the ability of self-renewal, and moved toward the center of the acini to form terminal differentiated gland acinar cells/meibocytes (PPAR γ ⁺). Ideally, the organoids well preserved the cell lineages and their niche in MGs, exhibiting proliferative Ki67⁺ or Krt5⁺ basal acinar cells lining the out layers, and PPAR γ ⁺ meibocytes locating in the inner layers (Fig. 1E). Besides, the inner cell also gained cell polarity, with the apical side facing inside (Fig. 1F). Importantly, the mMGOs could be long-term expanded up to 20 passages (Figs. 1H, 1I and S1D).

The main function of MG is meibum/lipid secretion. Oil Red O staining of MGOs showed that inner layer cells were Oil Red O positive, and the inner cavity displayed much condensed staining (Figs. 1G and S1B), indicating the meibocytes secreted lipid into the cavity. To further validate the recapitulation of the holocrine secretion mode in our MGOs, we performed neutral lipid staining and transmission electron microscopy (TEM). Neutral lipid staining revealed specific accumulation of lipid droplets in the organoid center (Fig. S2A), confirming the inward migration and maturation of meibocytes. Crucially, TEM imaging definitively captured the entire holocrine process, it visualized the progression from basal cells (Layer 1) with prominent nuclei, dense heterochromatin, and scarce lipids; through differentiating meibocytes (Layer 2) featuring dramatic lipid accumulation and honeycomb-like structures; to fully mature, anucleate cells (Layers 3–4) filled with lipids ready for release into the inner cavity (Fig. S2B).

To further characterize the transcriptomic similarity between MGs and MGOs, we performed bulk RNA-seq and set the irrelevant lacrimal gland as controls. The organoids displayed a similar gene expression pattern with the MGs (Fig. S1E and S1F), and showed a significant enrichment of MG signatures: Krt6, Lrig1, and lipid synthesis-related genes Elov16, Scd1, Far1, and Hmgcs1 (Fig. S1G). Besides, we found that age and sex do not affect the MGO establishment (Fig. S1C). In summary, the long-term expandable

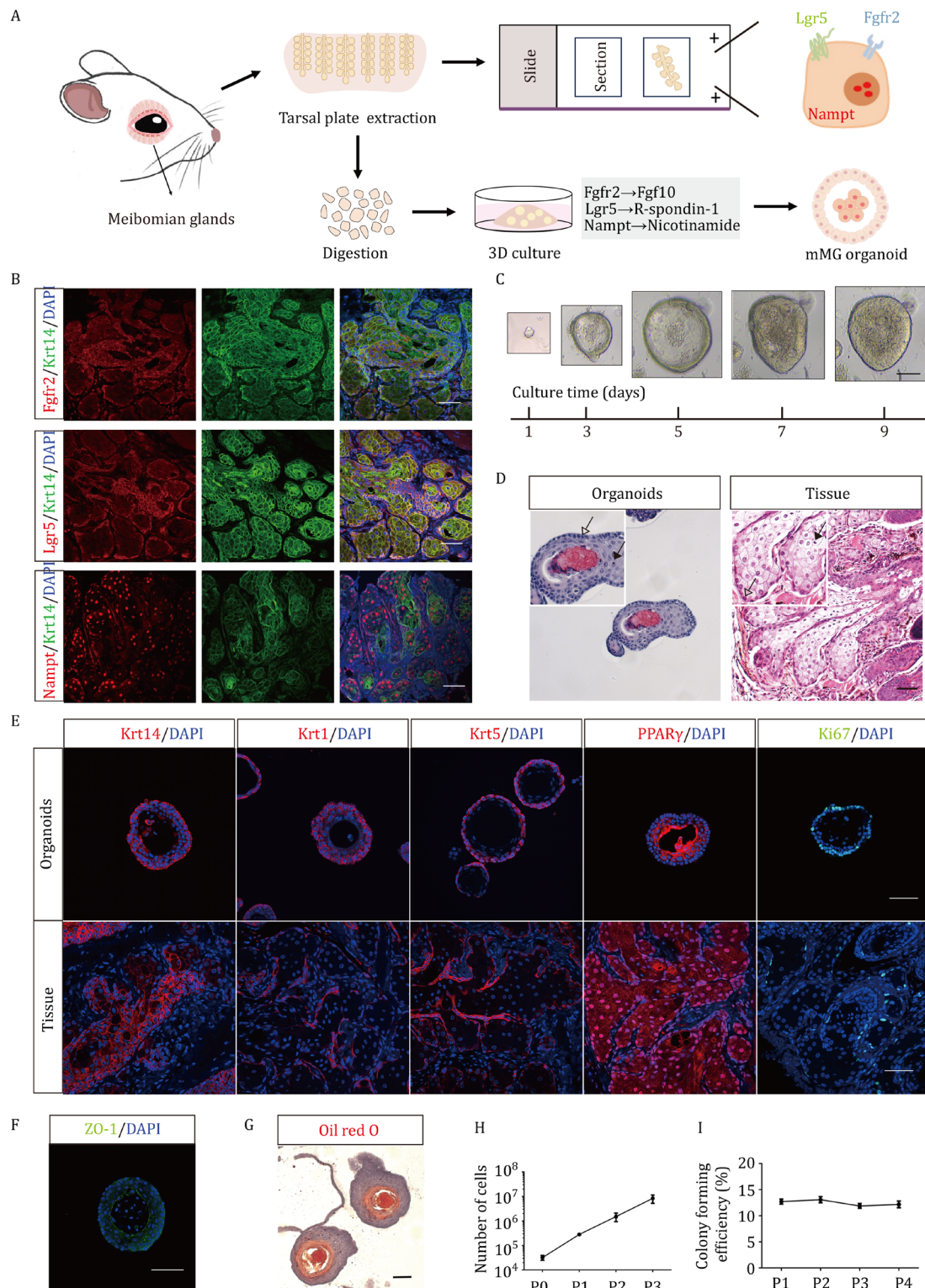


Figure 1. Generation of functional murine MG organoids (mMGOs). (A) Schematic diagram of organoid culture from murine MG tissue. (B) Immunofluorescent staining for Fgfr2, Lgr5, and Nampt in murine MG tissue (marked by Krt14). Scale bar, 50 μ m. (C) Representative bright-field images of the outgrowth of a single organoid during the 9-day culture period. Scale bar, 100 μ m. (D) H&E staining of passage 5, Day 9 organoid, and the murine MG tissue. White box indicates regions shown in higher magnifications ($\times 2$). Filled arrow indicates basal cells, and empty arrow indicates meibocytes with a pyknotic nucleus. Scale bar, 50 μ m. (E) Immunofluorescent staining for the MG epithelial cell marker (Krt14), ductal cell marker (Krt1), basal cell marker (Krt5), meibocyte marker (PPAR γ), and the proliferation marker Ki67 in organoids and in tissue. Scale bar, 50 μ m. (F) Immunofluorescent staining for ZO-1 in mMGs. Scale bar, 50 μ m. (G) Oil Red O staining of lipids in mMGs. Scale bar, 50 μ m. (H and I) The cell number (H) and colony forming efficiency (I) of mMGs during passage 0–4. See also Figs. S1 and S2.

and functional MGOs preserved the cell lineages, gene expression signatures, and lipid-secretion capability of MGs.

FGF10 sustained MG organoids' expansion and prevented MG dysfunction

The regeneration of MG depends on the self-renewal acinar basal cells, and the long-term expandable MGO culture system highlighted critical signaling pathways that boost basal cell amplification (Fig. 1E). We reasoned that the dominating signaling could be identified using an organoid culture system. After removing FGF10, Noggin, and R-spondin1 from culture medium individually, we found that MGOs were most sensitive to FGF10, rather than Noggin or R-spondin1 (Fig. 2A–C). FGF10 was a critical factor in regulating the development of the eyelid and the proliferation of immortalized human MG epithelial cells (Nuwormegbe et al., 2022; Tao et al., 2005), while its role in regeneration was less investigated. Inspired by the above, we then determined the function of FGF10 in MG dysfunction (MGD).

To establish a mouse MGD model, we optimized the previously described retinoic acid method by replacing 13-cis retinoic acid (13-cis-RA) with all-trans retinoic acid (ATRA) (Ibrahim and Elwan, 2017), since it is generally accepted that the effects of 13-cis-RA are mediated by isomerization to the more transcriptionally active ATRA form (Armstrong et al., 2005). Mice were treated with ATRA through gavage for a month to induce MG damage, and the FGF10 solution was dripped into the right eye (phosphate buffered saline, PBS for the left eye) three times/day as the therapeutic group (Fig. 2D). Ophthalmologic examinations, including ocular epithelial integrity, tear secretion and MG length, were conducted to score the MGD development and FGF10 treatment. Compared with the untreated group, the ATRA + PBS group showed pervaded corneal fluorescein staining (CFS), indicating compromised ocular epithelium. In addition, tear secretion ability and MG length were also reduced in the ATRA + PBS group (Fig. 2E–J). Histologically, ATRA + PBS also reduced the number and size of the acini in the MGs (Fig. 2K–M) and inhibited the proliferative cells in both the ductal (Krt6⁺ cells) and non-ductal cells (Krt6⁻ cells) of the MGs (Figs. 2N, 2O, S3A and S3B). Although no significant effects were found in the expression of PPAR γ , the lipid synthesis (oil-red) was inhibited in the ATRA + PBS group (Fig. S3C and S3D). The above data suggested that the ATRA-induced MGD model was successfully established. Interestingly, based on the MGD model, the ATRA + FGF10 group exhibited significant MG recovery in all aspects, compared with the ATRA + PBS group. Ideally, ATRA + FGF10 rescued ocular epithelium integrity, tear secretion, gland architecture, cell proliferation, and lipid synthesis.

To dive deeper into the role of FGF10 signaling during MGD, we performed phospho-p38 (downstream indicator

of FGF10 signaling) and KRT14 co-immunostaining, and found that ATRA treatment reduced the phospho-p38 signal and FGF10 application restores it (Fig. S4). These data demonstrated that FGF10 contributed to the MGs homeostasis, and the activation of FGF10 signaling alleviated the progression of MGD.

MG organoids mimicked the drug responses

Since the MGOs could recapitulate the physiological aspects of MGs, we reasoned that the MGOs might be used to discover the drugs that resolved dry eye disease. To demonstrate this, we selected drugs with known effects on MGs: ATRA and 13-cis-RA were confirmed to have a detrimental effect, resulting in cell death, atrophy, and altered gene expression (Samarawickrama et al., 2015). Azithromycin (AZM) can stimulate lipid synthesis in iHMGECs and is the prescription medication for the treatment of MGD (Liu et al., 2014; Tao and Tao, 2020). Rapamycin, the mTOR signaling pathway inhibitor, is a potential drug for the treatment of age-related disease (Selvarani et al., 2021). Dihydrotestosterone (DHT) is a major androgen that has been considered to regulate MG function by suppressing inflammation (Sahin et al., 2020) and enhancing lipid synthesis (Ibrahim and Elwan, 2017).

We exposed the MGOs to those drugs individually and followed by the morphologic and functional characterizations. Compared to the dimethyl sulfoxide (DMSO) group, treatment with 100 μ mol/L 13-cis RA, 10 μ mol/L Rapamycin, and 10 μ mol/L DHT resulted in a significant decrease in organoid size (Fig. 3A and 3B). The inner cavity of organoids became condensed after being treated with 10 μ mol/L AZM and 10 μ mol/L DHT, suggesting that AZM and DHT might induce the differentiation (Fig. 3A). Administration of 100 μ mol/L ATRA and 100 μ mol/L 13-cis-RA significantly inhibited the expression of proliferation and stemness genes (Xie et al., 2018), including *Krt5*, *Lrig1*, and *Pcna*, as well as the protein level of PCNA and Sox2 (Fig. 3C and 3G). Furthermore, the MG markers, *Krt10* and *Krt14*, decreased after the treatment with ATRA and 13-cis-RA, while the ductal cells marker gene *Krt6* was upregulated. The data suggested that the effects of the ATRA and 13-cis RA on the acinar and duct cells of the MGs might be different (Figs. 3G, S5A and S5B).

Regarding lipid synthesis, AZM, Rapamycin, and DHT treatments resulted in a significant increase in lipid production capacity, while ATRA and 13-cis RA led to a decrease, as indicated by the Oil Red O staining (Fig. 3D and 3E). Additionally, ATRA and 13-cis RA led to the reduction of lipid synthesis-related genes *Far1*, *Pparg*, and *Scd1* while promoting fatty acid synthase (*Fasn*) and Perilipin (*Plin2*) expression, suggesting that ATRA and 13-cis RA affected specific stages of differentiation (Figs. 3F, S5C and S5D). In summary, the MGOs could rapidly assess the drug effects and precisely simulate drug responses *ex vivo*.

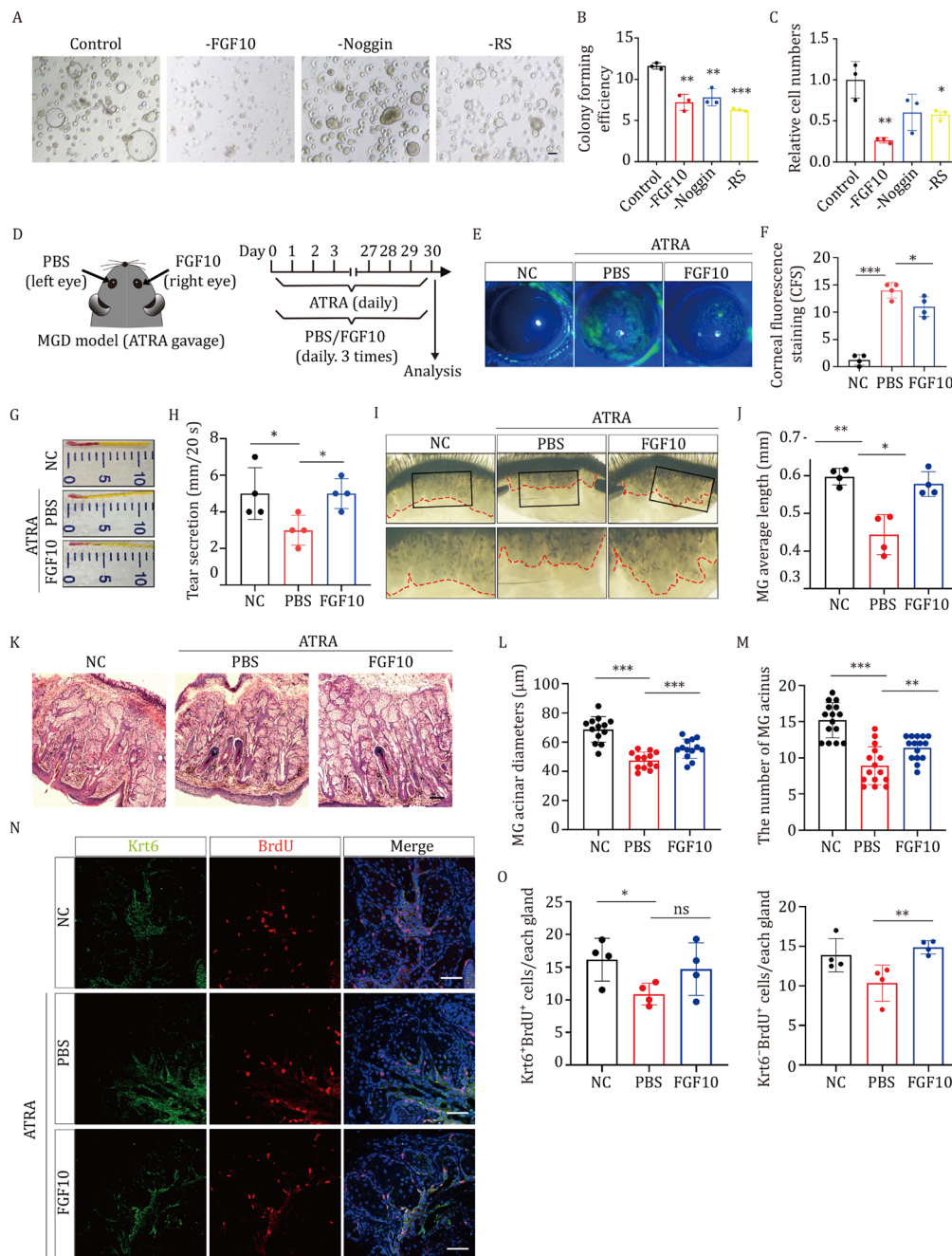


Figure 2. FGF10 is required for the organoid expansion and prevents MG dysfunction (MGD). (A) Representative bright-field images of the mMGOs in 4 conditions (removing the FGF10, Noggin, Rspo-1, and control). Scale bar, 100 μ m. (B and C) Quantification of colony-forming efficiency (B) and cell numbers (C) of organoids in the four indicated conditions. Data were represented as mean $\pm$ SEM in 3 independent experiments. Unpaired two-tailed Student's t-test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (D) Schematic of the workflow and the timeline for the ATRA-induced MGD model and FGF10 eye drops administration. (E and F) Corneal fluorescein staining (E) images and (F) scores of normal control mice and ATRA-induced MGD mice treated with PBS or FGF10 eye drops. NC: normal control group without any treatment; PBS: ATRA-induced MGD group treated with PBS eye drops; FGF10: ATRA-induced MGD group treated with FGF10 eye drops. Data were represented as mean $\pm$ SEM. $n=4$ mice for each group. Unpaired two-tailed Student's t-test: * $P < 0.05$, *** $P < 0.001$. (G and H) Representative images (G) and measurement (H) of tear secretion volume using the phenol red thread test of the indicated groups. Data were represented as mean $\pm$ SEM. $n=4$ mice for each group. Unpaired two-tailed Student's t-test: * $P < 0.05$. (I and J) Representative bright-field images of MGs (I) and quantification of MG length (J) of the indicated groups. Red dotted line indicates the margin of MGs. Lower panel: higher magnifications of the upper panel. Data were represented as mean $\pm$ SEM. $n=4$ mice for each group. Unpaired two-tailed Student's t-test: * $P < 0.05$, ** $P < 0.01$. (K–M) H&E staining (K) and quantification of size (L) and number (M) of MG acini of the indicated groups. Quantified data were determined in 12 randomly selected fields. Data were represented as mean $\pm$ SEM. Unpaired two-tailed Student's t-test: ** $P < 0.01$, *** $P < 0.001$. Scale bar, 100 μ m. (N) Immunofluorescent staining for Krt6 (ductal cell marker) and BrdU (proliferation marker) of the indicated groups. Scale bar, 50 μ m. (O) Quantification of the number of proliferative non-ductal cells (Krt6⁻/BrdU⁺ cells) and ductal cells (Krt6⁺/BrdU⁺ cells) in the indicated groups. Data were represented as mean $\pm$ SEM. $n=4$ mice for each group. Unpaired two-tailed Student's t-test: * $P < 0.05$, ** $P < 0.01$. See also Figs. S3 and S4.

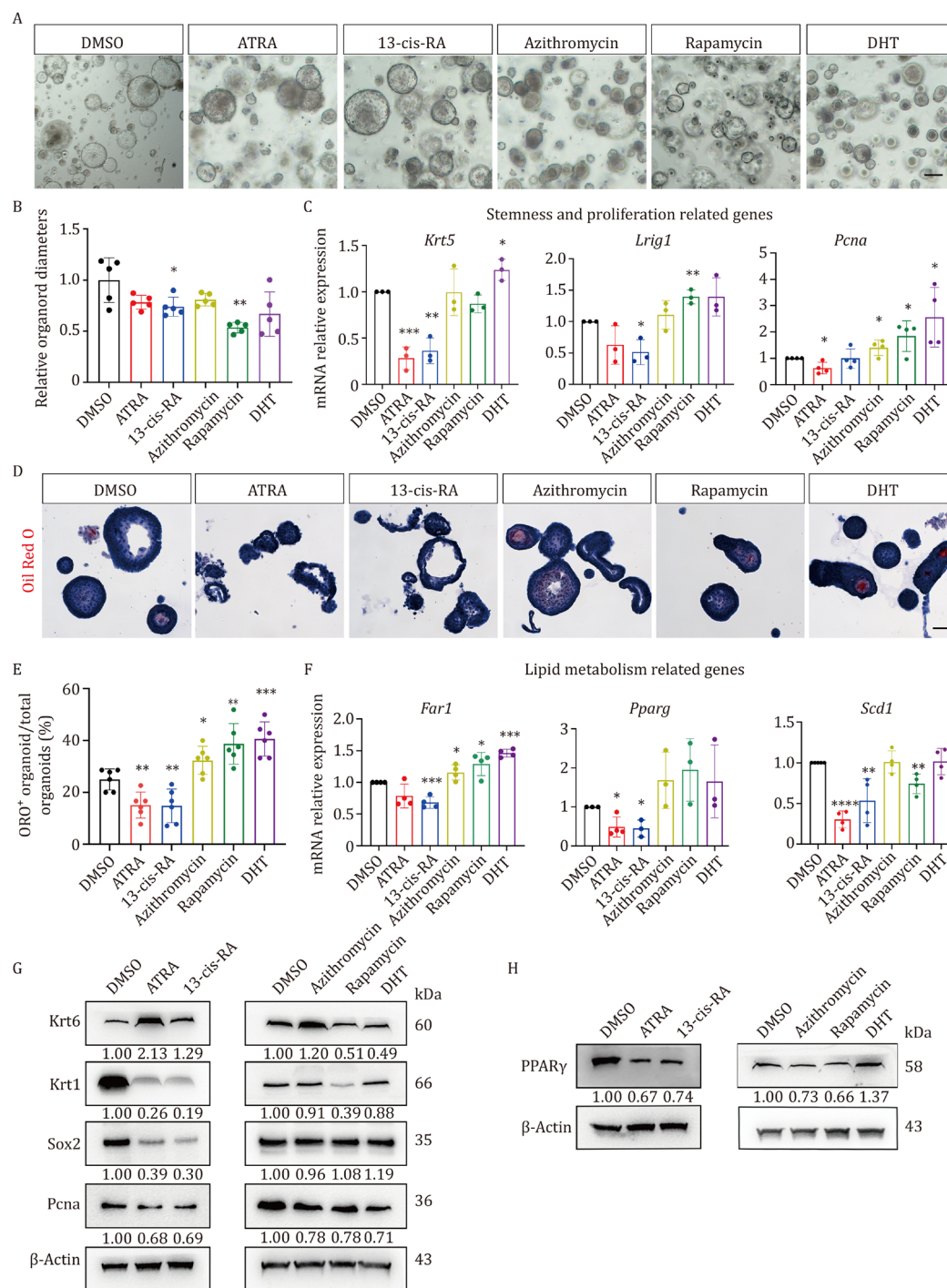


Figure 3. Organoids mimic the drug response of MGs. (A) Representative bright-field images of MGOs incubated with 0.1% DMSO, 100 $\mu\text{mol/L}$ ATRA, 100 $\mu\text{mol/L}$ 13-cis RA, 10 $\mu\text{mol/L}$ AZM, 10 $\mu\text{mol/L}$ Rapamycin, and 10 $\mu\text{mol/L}$ DHT for 3 days, respectively. Scale bar, 100 μm . (B) Quantification of the diameters of organoids of the indicated treatment. Quantified data were determined in five randomly selected fields. Data were represented as mean $\pm$ SEM. Unpaired two-tailed Student's t-test: * $P < 0.05$, ** $P < 0.01$. (C) Quantitative real-time PCR (qRT-PCR) analysis of the expression level of proliferative and stemness-related genes, including *Krt-5*, *Lrig1*, and *Pcnal*, of the organoids after indicated treatment. Data are represented as mean $\pm$ SEM in ≥ 3 independent experiments. Unpaired two-tailed Student's t-test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (D) Oil Red O staining of lipids in organoids with indicated treatment. Scale bar, 50 μm . (E) Quantification of the number of Oil Red O⁺ organoids divided by the total number of organoids with the indicated treatment. Quantified data were determined in six randomly selected fields. Data are represented as mean $\pm$ SEM. Unpaired two-tailed Student's t-test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (F) Quantitative real-time (RT)-PCR analysis of the expression level of lipid metabolism-related genes, including *Far1*, *Pparg*, and *Scd1*, with RNA extracted from the organoids after indicated treatment. Data were represented as mean $\pm$ SEM in ≥ 3 independent experiments. Unpaired two-tailed Student's t-test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (G and H) Western blot analysis of Krt6, Krt1, Sox2, Pcnal (stemness-related protein) (G), and PPAR γ (metabolism-related protein) (H) protein expression in organoids with the indicated treatment. β -Actin served as a loading control. The relative band intensity that was normalized to the loading control was marked under each band. $n = 3$ biological replicates. See also Fig. S5.

Establishment of human MG organoids for orthotopic transplantation

Next, we attempted to establish human MG organoids (hMGO). Refer to the murine organoid, we also mapped the expression pattern of FGFR2, LGR5, and NAMPT in human MG tissue (Fig. 4A). Unexpectedly, NAMPT was barely expressed in human MG tissue (Fig. 4B), so we suspected that its substance, nicotinamide, might not be required for the human organoid culture. Indeed, the mMGO expansion medium could not support hMGO expansion for more than 5 days. However, once nicotinamide was removed, the hMGO dramatically expanded, so we defined this formula as hMGO expansion medium (Fig. 4C). As expected, the hMGO medium could support long-term expansion of hMGO to 20 passages (P20), as well as nearly 10 times cell amplification per passage (Fig. 4D and 4E).

To further confirm the inhibitory effect of nicotinamide on hMGO growth, we added nicotinamide back to hMGO medium and found it significantly suppressed cell proliferation and lipid synthesis (Fig. S6A–E). Bulk RNA-seq revealed that nicotinamide supplement downregulated cell proliferation and lipid-producing-related genes, including PCNA, MKI67, PPARG, and SCD (Fig. S6F). Gene ontology (GO) term and Gene set enrichment analysis (GSEA) analysis further confirmed that nicotinamide inhibited cell proliferation and PPAR-related signaling pathways (Fig. S6G and S6H). Unexpectedly, nicotinamide also downregulated FGF10–FGFR2 signaling (Fig. S6H), implying the crosstalk between nicotinamide and FGF10 signaling. To further explore the mechanism, we investigated the phosphorylated ERK (pERK) level in organoids upon FGF10 and nicotinamide treatment. Different from mMGO, whose pERK level was not altered by nicotinamide, the hMGO's pERK was quite sensitive to nicotinamide and FGF10: FGF10 treatment significantly upregulated pERK, which was suppressed by nicotinamide subsequently (Fig. 4F). To ensure the necessity of other gradients in hMGO medium, we subtracted each component and evaluated the organoid growth, and confirmed the current hMGO medium is the minimal formula (Figs. S6I–K, S7A and S7B). The hMGO cultured with this medium also preserved the key makers (KRTs and PPAR γ) and lipid synthesis capacity, compared with parental tissues (Fig. S7C and S7D).

Dry eye syndrome caused by MGD is a chronic disease that cannot be completely cured theoretically, so we are wondering if the transplanted hMGO could generate functional MGs. We transplanted hMGO into the MG sites of immunodeficient nonobese diabetic (NOD) severe combined immunodeficiency (SCID) gamma (NSG) mice (Fig. 4G). After 1 month, the recipient MGs were collected for analysis. The injected organoids could be observed in the MG tissue (Fig. 4H). The human-derived organoids

were confirmed by the staining of human NuMA and KRT14 in the engraftment organoids (Fig. 4I and 4K). Through LipidTOX detecting, the secreted lipids were detected in the center of the engrafted organoids (Fig. 4I). Furthermore, a similar level of lipid production was observed in the engrafted organoids and the host MGs, confirming that the engrafted organoids acquired functional maturity *in vivo* (Fig. 4I). Additionally, the engrafted cells retained the expression of functional MG biomarkers, including KRT1, KRT14, PPAR γ , and P63 α , corresponding with the host tissues (Fig. 4J and 4K). Taken together, the long-term expandable hMGOs can be engrafted into mice and exhibit features of functional maturation, including lipid production and lineage-specific marker expression.

Dissection of single-cell atlas in human MGs and organoids

To dissect the cellular composition and heterogeneity in human MGs and organoids, we performed parallel single-cell RNA sequencing (scRNA-seq). The human MG tissues were digested into single cells and subjected to 10 $\times$ genomics-based scRNA-seq platform (Fig. 5A). After quality control, a total of 23,323 cells were obtained for further analysis, and six cell types were identified (Fig. S8A and S8B). Focusing on the epithelial homeostasis, we selected KRT14 $^{+}$ MG epithelial cells ($n=13,684$) and further identified nine subclusters according to the established cell markers (Fig. 5B and S8C–G). Based on the high expression of the mature lipid-synthetic marker genes, we identified two types of differentiated acinar cells. Type 1 acinar cells (FAR2 $^{+}$) are featured by lipid metabolic genes, including PPARG, SCD, FAR2, and MSMD1. While type 2 acinar cells (LYZ $^{+}$) are featured by secretive genes, including LYZ, SCGB3A1, PLA2GA2, and PIP. Thus, those two types of acinar cells were identified as mature acinar cells (Fig. 5C and S8G). We also identified other cell lineages of MGs, including MG basal cells (KRT15 $^{+}$ /SOCS3 $^{+}$; ID4 $^{+}$ /CCN4 $^{+}$; UBE2C $^{+}$ /TOP2A $^{+}$), transitional cells (CREB5 $^{+}$ /LAMA4 $^{+}$; MMP10 $^{+}$ /FST $^{+}$), and ductal cells (PIGR $^{+}$ /OPRN $^{+}$; KRT6A $^{+}$ /SBSN $^{+}$; Fig. S8D–F).

In parallel, we also sequenced 22,077 cells in MGOs. After aligning with human MG tissues (KRT14 $^{+}$), the total cells could be aligned and regrouped into five different clusters, namely acinar cells (type 1 and 2), ductal cells (type 1 and 2), transitional cells, and basal cells (Fig. 5D–G). Notably, the type 1 acinar cells (FAR2 $^{+}$) were absent in the organoids, while another type of acinar cells (LYZ $^{+}$) had a much lower abundance in the organoids (Fig. 5E–G). This indicated that hMGOs retained a relatively immature status. Besides, the cell component proportion analysis also revealed an increase in the ductal cells and basal cells in organoids and a decrease in transitional cells (Fig. 5H), indicating hMGOs preserved more stemness-related cells than tissue. Collectively, the conducted single-cell atlas

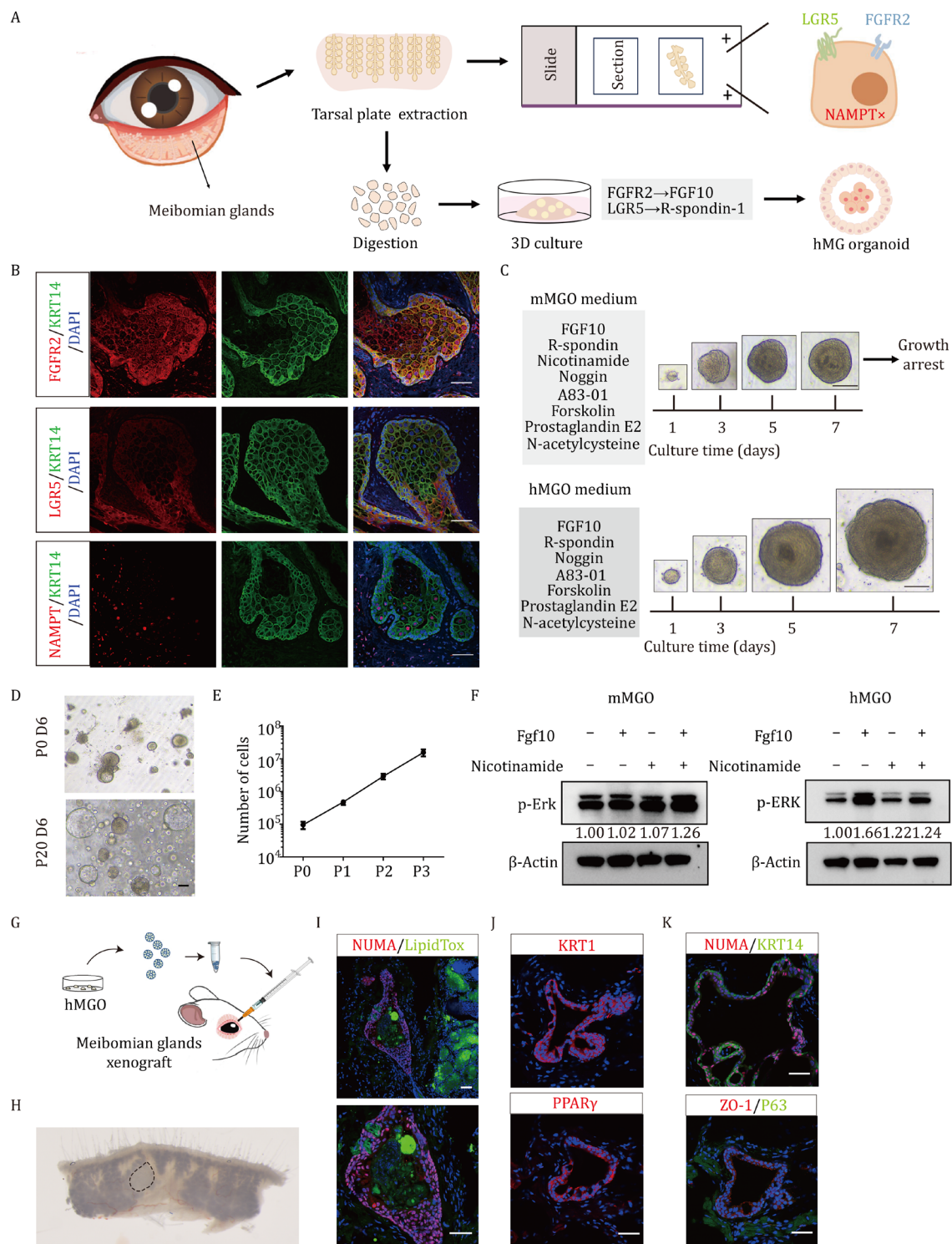


Figure 4. Establishment of human MG organoids (hMGs) for orthotopic transplantation. (A) Schematic diagram of organoid culture from human MG tissue. (B) Immunofluorescent staining for FGFR2, LGR5, and NAMPT in human MG tissue (marked by KRT14). Scale bar, 50 μ m. (C) Representative bright-field image of hMGs with mMGO medium and hMGO medium at the indicated days. Scale bar, 50 μ m. (D) Representative images of hMGs outgrowth from primary organoids to passage 20. Scale bar, 100 μ m. (E) The cell number of hMGs during passage 0–3. (F) Western blot analysis of phospho-ERK for mMGOs organoids and hMGs cultured under the indicated treatment. β -Actin served as a loading control. The relative band intensity that was normalized to the loading control was marked under each band. $n=2$ biological replicates. (G) Schematic diagram of hMGs orthotopic transplantation in NSG mice. (H) Representative image of hMGs orthotopic transplantation. Black dashed box indicates the region of the xenograft. (I) Immunofluorescence staining for human NuMA and LipidTox staining of human engrafted organoids. Lower panel: higher magnifications of the upper panel. Scale bar, 50 μ m. (J and K) Immunofluorescence staining for KRT1, PPAR γ (J), human NuMA, human KRT14 (K), ZO-1, and P63 (L) in engrafted MG organoids. Scale bar: 50 μ m. See also Figs. S6 and S7.

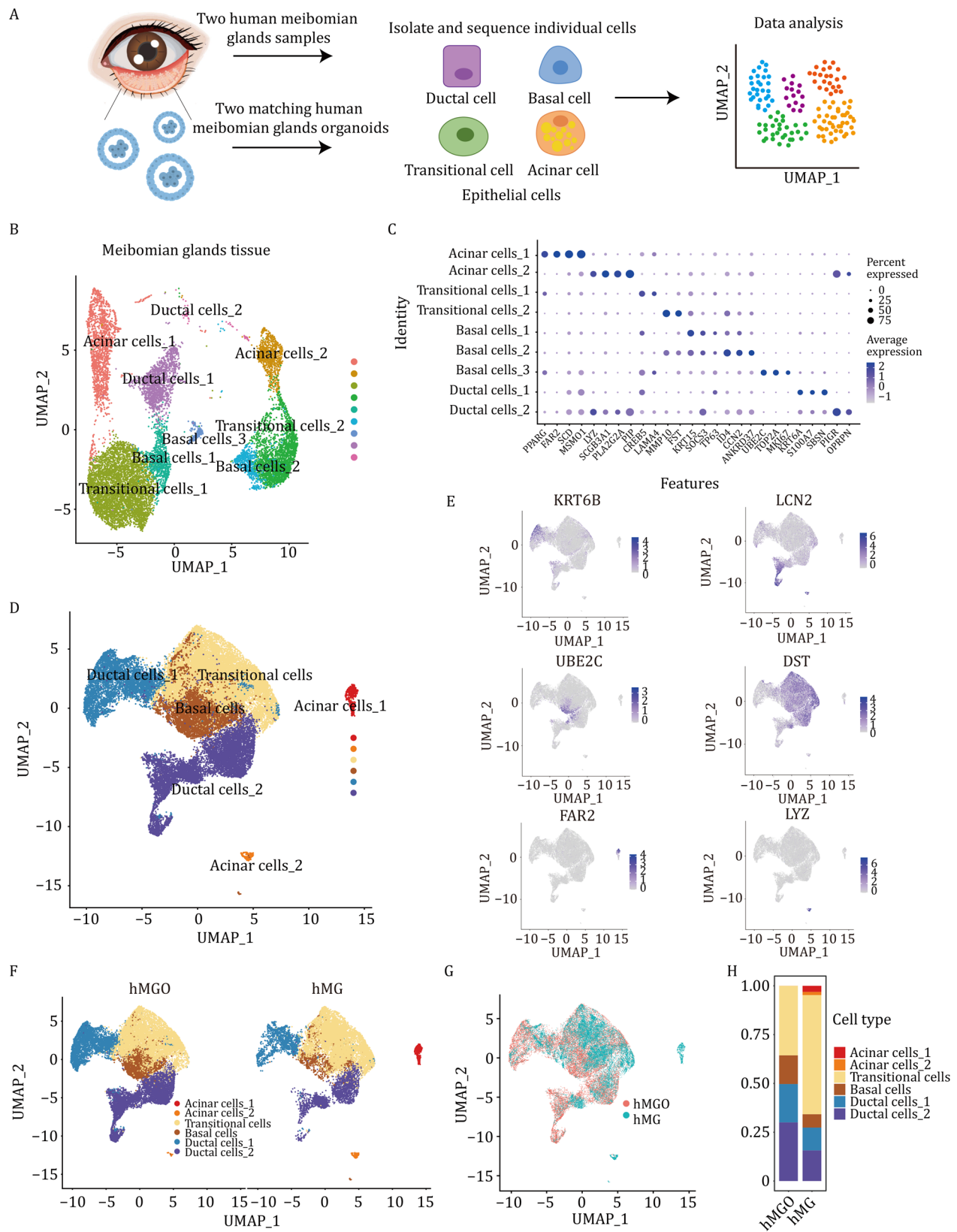


Figure 5. Mapping the single-cell atlas for human MG tissue and organoids. (A) The schematic diagram of single-cell mRNA sequencing for 2 human MG tissue samples and matching organoids. (B) UMAP representation of 9 clusters of human MG epithelial cells ($n=12,707$). (C) The expression level of MG cell markers and cluster-specific genes in different clusters of human MG tissue. (D) Cell clusters were visualized for hMGs and tissue cells. (E) The expression of cluster-specific genes in organoids and tissue cells. (F) UMAP plot showing that the parallel cell clusters were identified in organoid and tissue cells. (G) UMAP of human MG cells from organoids (tomato dots) and tissue (cyan dots). (H) The proportion of diverse cell types in hMGs and tissue. See also Fig. S8.

described parallel heterogeneous cell types between the organoids and MGs, and pinpointed the maturation gap between the organoid and tissue.

Human MG organoids generated featured lipidome

Since we have proved that hMGs could reproduce the capacity of lipid synthesis (Fig. S7D), we wonder whether the lipid contents of organoids would resemble those of tissues. In order to depict a broader landscape of lipidome, we initially performed non-targeting lipidomic analysis: using human SGs as control, the lipid samples of human MGs and organoids were extracted and coupled with an electrospray ionization high-resolution mass spectrum (MS) analysis. Then, the normalized intensity profiles of the identified lipid compounds were generated. On principal component analysis (PCA) score plot, MGs and organoids are distinctly separated from the SGs sample (Fig. 6A), and hierarchical clustering grouped MG tissue and organoids together, confirming organoids shared a similar lipidome with MG tissue (Fig. 6B). Then, we probed the content of subclass lipids in hMGs normal MG tissue, and SG tissue. Distinguished with SG, MG tissue and organoids shared a similar level of sphingomyelins (SM), phosphatidylglycerols (PG), and phosphatidylserines (PS). While diacylglycerols (DAG), the lipid content mainly secreted in SG, were much lower in MG tissue and organoids (Figs. 6C, S9A and S9B). Apart from the similarities, we also noticed the 240 differential lipids between hMG and hMGO (Fig. S9C). Among those, FAHFA and TAG, which were detected in human meibum (Butovich et al., 2021; Riecan et al., 2022), were downregulated in hMGO (Fig. S9D).

In summary, consistent with scRNA-seq data above, the lipidomic comparison between MG tissue and organoids not only displayed the overall consistency, but also suggested the maturation gaps, such as terminal differentiated cell types and featured lipids.

MAPK signaling inhibition boosted human MG organoids' maturation

Since the hMGs are less mature than MG tissues, we reasoned that the underlying signals in MG tissues could enhance organoid maturation. To unveil the mechanism of acinar cell maturation, lineage trajectory analysis was performed based on the scRNA-seq data of MG tissues. On the pseudotime plot, basal cells, transitional cells, and acinar cells of MGs are distributed along the pseudotime trajectory. The type 1 acinar cells (FAR2⁺), which were identified as the mature MG cells, were located at the right-most of the trajectory (Fig. 6D and 6E), suggesting that the differentiation path of basal cells–transitional cells–acinar cells. Then we analyzed the signaling pathway enriched in acinar and transitional cells, in order to find key signaling pathways guiding acinar cell maturation. In acinar cells, multiple lipid synthesis-related signaling pathways

were activated (Fig. 6F), while the main signaling pathways enriched in transitional cells were inflammation-related pathways such as the IL-17, TNF, NOD-like receptor (NLR), FoxO, and MAPK signaling pathways (Fig. 6G). Interestingly, MGD patients were generally associated with increased tear cytokines, such as TNF- α , IL-17A, and IL-1 β (Yu et al., 2022). Reducing the inflammatory cytokines could relieve both the signs and the symptoms of MGD (Li et al., 2021; Yu et al., 2022). Thus, we exposed hMGs to the inhibitors of the IL-17, TNF, NLR, FOX, and MAPK signaling pathways and attempted to enhance the lipid production capacity of hMGs. Notably, a selective p38MAPK signaling pathway inhibitor, SB202190, showed the potential to facilitate MGO maturation.

As previously reported, the p38MAPK signaling pathway contributes to cell apoptosis and the autophagy of adipogenic cells and plays a crucial role in inflammation-induced MGD (Liu et al., 2022; Sui et al., 2014). Treatment with SB202190 could significantly upregulate the expression of PPAR γ in organoids, and it is more effective than DHT in this regard (Fig. 6H). Using Oil Red O staining, we observed that upon SB202190 stimulation, the produced lipids, which were originally in the cytoplasm, were released into the cavity of the organoids (Fig. 6I). Although the ATRA treatment decreased lipid secretion, SB202190 mitigated the inhibiting effect of ATRA (Fig. 6I). These results confirmed that SB202190, a selective inhibitor of the p38MAPK signaling pathway, could induce the maturation of MGOs.

The mature meibum is featured by ultra-long chain lipids with fatty acid and/or fatty alcohol chains up to C34–C36, including cholesteryl esters (CE), (O)-acylated ω -hydroxy fatty acids (OAHFA), and wax esters (WE) (Butovich, 2017; Butovich and Wilkerson, 2022). However, the non-targeting lipidomic analysis could not robustly detect those major components, especially for the WE. To depict the maturity of human meibomian organoids, targeting lipidomic analysis was performed to evaluate the content of key lipids of meibum, including CE, OAHFA, and WE. Overall, hMGO resembled the lipidomic features of human MG tissue (hMG) in the PCA plot, compared with human periocular skin tissue (hST) (Fig. S10A). Then, we used heatmaps to delineate the similarity between hMG and hMGO on CE, OAHFA, and WE. Compared with the negative control hST, the average content of those featured lipids in hMG and hMGO is higher than hST (Fig. S10B–D). However, we also observed the variant lipidomic performance of hMGO, which showed less mature phenotype (such as hMGO1), suggesting the improving space for hMGO culture system.

Discussion

Efforts have been made to establish a 3D meibomian culture model, including a slice culture model and explant culture methods (Rötzer et al., 2019; Wang et al., 2023; Xu

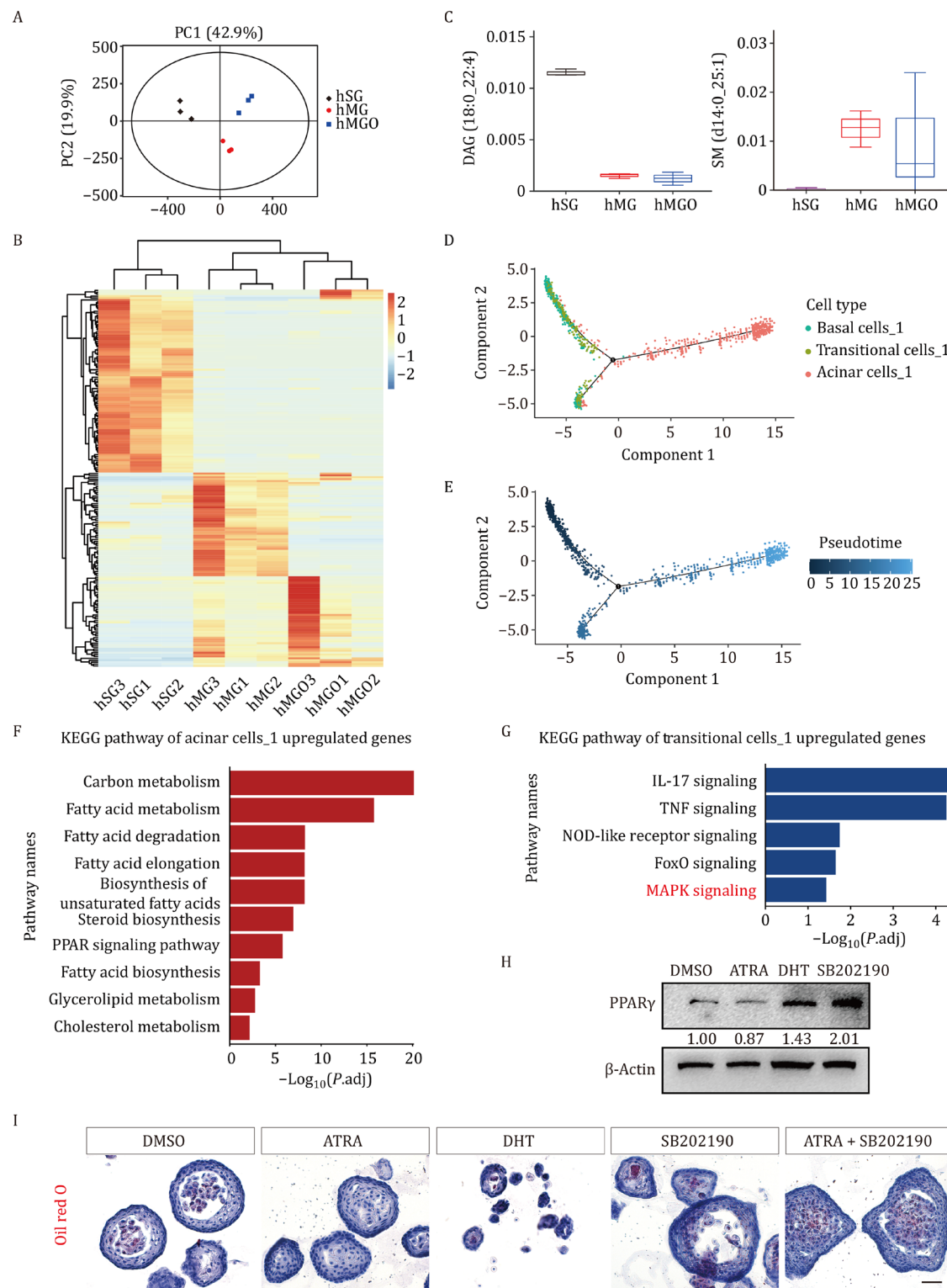


Figure 6. MAPK signaling inhibition promotes the maturation of hMGs. (A) PCA plot of human sebaceous gland (hSG), meibomian gland (hMG), and meibomian gland organoids (hMGO) in lipidomics analysis. 3 independent donors in each group. (B) Heatmap of differential lipid species among hSG, hMG, and hMGO. (C) Relative value of DAG (18:0_22:4) and SM (d14:0_25:1) in hSG, hMG, and hMGO. (D) Position of the MG cells in different cell clusters along the pseudotemporal trajectory. (E) Ordering of MG tissue cells along a pseudotemporal trajectory. MG cells are colored by pseudotime. (F and G) KEGG analysis of upregulated genes (F) and downregulated genes (G) that were differentially expressed in MG acinar cells, compared with MG transitional cells. (H) Western blot analysis of PPAR γ for hMGs cultured under the indicated treatments: 1% DMSO, 100 μ mol/L ATRA, 10 μ mol/L DHT, and 0.5 μ mol/L SB202190. β -Actin served as a loading control. The relative band intensity that was normalized to the loading control was marked under each band. $n=2$ biological replicates. (I) The Oil Red O staining of hMGs cultured under the treatment of ATRA, DHT, and SB202190. Scale bar: 50 μ m. See also Figs. S9 and S10.

et al., 2020; Zahn et al., 2022, 2025). Although these models preserved the tissue architectures and functions, such as lipid production, they could not sustain for more than 7 days *in vitro*. In this study, we achieved long-term expansion of murine and human MGOs, which retained all cell lineages and the capacity for lipid synthesis. Thus, the MGOs enable precise drug response and functional engraftment. In addition, MGOs also assist in untangling the underlying mechanism of tissue homeostasis and dysfunction. We found that FGF10 signaling is crucial for organoid expansion and MGs regeneration; NAMPT was barely expressed in human MG tissue, and nicotinamide specifically hindered hMGOs expansion, indicating the distinct NAD metabolism between mouse and human; Single-cell atlas of human MG epithelial cells indicated MAPK signaling inhibition is required for acinar cell differentiation, thus treated with p38MAPK signaling pathway inhibitor, SB202190, promote organoid maturation.

MGD is the leading cause of dry eye disease. Clinically, the main treatments for MGD include cleaning the eyelids through hot compresses and massages, instilling artificial tears containing lipid components, and using antibiotic drugs to reduce inflammation. Currently, the development of artificial tears is prevailing. In order to make the artificial tears as similar as possible to the composition of tears, various substances are added, including mineral oil and phospholipids (Jones et al., 2017). We identified that FGF10 supported both murine and human MGO expansion. And FGF10 eye drops also rescued the ATRA-induced MGD (Figs. 1 and 2). Our data suggested that the FGF10 signaling is not only required for MG development and homeostasis (Reneker et al., 2017; Tao et al., 2005; Tsau et al., 2011), but also prevents the progression of MGD. Therefore, FGF10 eye drops are a promising reagent for human dry eye disease, which needs more human-relevant tests for its application. In addition to those physiological restoration, FGF10 treatment also increased the proliferation of acinar cells, instead of ductal cells (Fig. 2N). Consistent with our observation, FGFR2 deletion severely affected the proliferation and differentiation of MG acinar cells but affected MG ductal cells to a lesser extent (Reneker et al., 2017). We also found that the proliferating cells in acini are mainly located at the edges (Fig. S3A), which were likely basal cells. Consistent with these data, the MGOs also showed proliferative Krt5⁺ basal cells under the FGF10-containing medium (Fig. 1E). Hence, it is conceived that basal cells are the major proliferative cells that respond to FGF10 signals.

NAMPT generates nicotinamide mononucleotide from nicotinamide and 5'-phosphoribosyl-1-pyrophosphate, thereby catalyzing the rate-limiting step in the mammalian NAD salvage pathway (Garten et al., 2015). Therefore, the bonus of nicotinamide largely depends on the activity of NAMPT. We found the distinct expression level of NAMPT in MGs between mouse and human (Fig. 4B). And

the addition of nicotinamide into human organoid culture medium suppressed the organoid growth (Fig. 4C). Previous studies also revealed that high doses of nicotinamide may exert adverse effects, such as DNA damage, increased intracellular ROS, spindle defects, and mitochondria dysfunction (Oikawa et al., 1980; Utakoji et al., 1979; Zhang et al., 2016). In this study, we found that nicotinamide inhibited the activity of FGF10 signaling through decreasing the pERK level in human organoids. But how nicotinamide crosstalk with the upstream of FGF10 signaling need further investigation. It was reported that the prevalence of dry eye disease was higher in female than male (Kim et al., 2023). Considering that nicotinamide is frequently used in cosmetics, it's likely that excess nicotinamide exposure is also an emerging risk factor for MGD.

MAPK signaling participates in various cellular programs like proliferation, differentiation, transformation, and apoptosis (Zhang and Liu, 2002). Leveraging scRNA-seq analysis, we found that the MAPK signaling pathway was significantly enriched in transitional cells (immature acinar cells), compared to mature acinar cells (Fig. 6G). And identified SB202190, a selective p38MAPK inhibitor, promoted lipid production in MGOs (Fig. 6I), suggesting that MAPK signaling pathway inhibition is required for the differentiation of mature acinar cells. Similar to adipocytes, the p38MAPK signaling was more active in preadipocytes than adipocytes (Aouadi et al., 2006). Consistently, another selective p38MAPK inhibitor, SB203580, could block IL-1 β -induced MGD and restore its functions in rats (Qu et al., 2023). These findings indicate that MAPK signaling inhibition enhances the functions of terminally differentiated cells and could be considered as potential targets for MGD. However, whether MAPK inhibition affects the self-renewal of basal cells remains to be further investigated.

MGOs provide the opportunity for cell therapy for MG atrophy. Given that MG atrophy is the terminal stage of MGD, when MG stem cells cannot regenerate the whole MGs, the orthotopic transplantation might be the only choice for those patients. Successfully engrafting MGOs is the first step for potential regenerative therapies (Bannier-Hélaouët et al., 2021). The established MGO culture and differentiation protocol would provide the "seed cells" for bioengineered MG grafts. Efforts should be made to improve the orthotopic transplantation efficiency and reduce the immune rejection effects caused by xenotransplantation. In conclusion, we established functional, long-term cultured MGO cultures for MG homeostasis and diseases research, which provides a useful platform for the development of associated medical interventions and regenerative medicines.

In MGs, the acini are connected to the main duct by small ducts, so that the lipid produced by the meibocytes can be transported through the ducts to the eyelid. Although our organoid model contained all cell types of

MGs, the ductal cells resided with basal cells to form the outer layer of the organoid, losing their function to drain out the meibum. Additional optimization of MGO establishment is required to develop ducts-acinus structure.

In addition, we showed that the hMGOs engrafted upon orthotopic transplantation, and the transplanted organoids could produce comparable lipids to their neighboring tissue. However, whether the MGO transplantation could rescue the MG dysfunction remains to be further investigated.

Author contributions

C.Y., X.W., J.W., J.H., and B.Z. conceived the study; C.Y., X.W., J.W., Z.X., X.W., X.W., Y.M., Y.Z., X.Z., Q.L., J.X., C.Z., X.S., and X.Z. performed the experiments; J.H. and B.Z. supervised the work; and C.Y., X.W., J.W., J.H., and B.Z. wrote the manuscript.

Supplementary data

Supplementary data is available at *Protein & Cell* online <https://doi.org/10.1093/procel/pwaf095>.

Conflict of interest

The authors declare that they have no conflicts of interest.

Funding

This study is sponsored by bioGenous BIOTECH. J.H. was supported by the National Science Fund for Distinguished Young Scholars (82425015), the National Natural Science Foundation of China (82171102), the National Key Research and Development Program of China (2023YFA0915003), the Shanghai Medical Innovation Research Program (22Y21900900), the “Dawn” Program of Shanghai Municipal Education Commission China (24SG11), the Shanghai Science and Technology Innovation Action Plan for Cell and Gene Therapy (24J22800500), and the Shanghai Science and Technology Innovation Action Plan for Advanced Materials (24CL2900802).

Data availability

Single-cell and bulk RNA-seq data have been deposited at the NCBI SRA database and are publicly available with the accession number: SRP497138. Lipidomics raw data have been deposited to the MetaboLights with the accession number: MTBLS9799, and can be fast downloaded from Google Drive with the link: drive.google.com/drive/folders/1mb84LM6ZEaX-naEBJ24hQx9nLBJ7K7Ix?usp=sharing. Any additional information required to reanalyze the data reported is available from the lead contact upon request.

Code availability

This paper does not report original code.

Ethics approval

The animal study was approved by the Laboratory Animal Center, School of Life Sciences, Fudan University and was performed in accordance with the statement of the Association for Research in Vision and Ophthalmology (ARVO) entitled “Use of Animals in Ophthalmic and Vision Research” (EENT-IRB20220301). The research was conducted in accordance with the Declaration of Helsinki, and the use of human tissues for this study was approved by the Ethics Committee of EENT Hospital of Fudan University (EENT-IRB20220222).

Consent to participate

All authors give their consent to participate.

Consent for publication

All authors give their consent to publication.

References

- Aouadi M, Laurent K, Prot M *et al*. Inhibition of p38MAPK increases adipogenesis from embryonic to adult stages. *Diabetes* 2006;**55**:281–289.
- Armstrong JL, Redfern CP, Veal GJ. 13-cis retinoic acid and isomerisation in paediatric oncology—is changing shape the key to success? *Biochem Pharmacol* 2005;**69**:1299–1306.
- Bannier-Hélaouët M, Post Y, Korving J *et al*. Exploring the human lacrimal gland using organoids and single-cell sequencing. *Cell Stem Cell* 2021;**28**:1221–1232.e7.
- Bu J, Wu Y, Cai X *et al*. Hyperlipidemia induces meibomian gland dysfunction. *Ocul Surf* 2019;**17**:777–786.
- Bu J, Zhang M, Wu Y *et al*. High-fat diet induces inflammation of meibomian gland. *Invest Ophthalmol Vis Sci* 2021;**62**:13.
- Butovich IA. Meibomian glands, meibum, and meibogenesis. *Exp Eye Res* 2017;**163**:2–16.
- Butovich IA, Wilkerson A. Dynamic changes in the gene expression patterns and lipid profiles in the developing and maturing meibomian glands. *Int J Mol Sci* 2022;**23**.
- Butovich IA, Wilkerson A, Yuksel S. Differential effects of dietary cholesterol and triglycerides on the lipid homeostasis in meibomian glands. *J Steroid Biochem Mol Biol* 2021;**211**:105894.
- Caffery BE, Josephson JE. Ocular side effects of isotretinoin therapy. *J Am Optom Assoc* 1988;**59**:221–224.
- Clevers H. Modeling development and disease with organoids. *Cell* 2016;**165**:1586–1597.
- Garten A, Schuster S, Penke M *et al*. Physiological and pathophysiological roles of NAMPT and NAD metabolism. *Nat Rev Endocrinol* 2015;**11**:535–546.

- Hom MM, Martinson JR, Knapp LL et al. Prevalence of meibomian gland dysfunction. *Optom Vis Sci* 1990;**67**:710–712.
- Hwang HS, Parfitt GJ, Brown DJ et al. Meibocyte differentiation and renewal: insights into novel mechanisms of meibomian gland dysfunction (MGD). *Exp Eye Res* 2017;**163**:37–45.
- Ibrahim MAA, Elwan WM. Role of topical dehydroepiandrosterone in ameliorating isotretinoin-induced meibomian gland dysfunction in adult male albino rat. *Ann Anat* 2017;**211**:78–87.
- Jones L, Downie LE, Korb D et al. TFOS DEWS II management and therapy report. *Ocul Surf* 2017;**15**:575–628.
- Kim EJ, Jung HS, Lu P. Pleiotropic functions of fibroblast growth factor signaling in embryonic mammary gland development. *J Mammary Gland Biol Neoplasia* 2013;**18**:139–142.
- Kim H, An Y, Hwang WJ. Gender differences in dry eye disease symptoms associated with psychological health indicators among adults using mobile mental health apps. *PLoS One* 2023;**18**:e0278921.
- Li Q, Liu J, Liu C et al. Effects of intense pulsed light treatment on tear cytokines and clinical outcomes in meibomian gland dysfunction. *PLoS One* 2021;**16**:e0256533.
- Liu R, Li J, Xu Y et al. Melatonin attenuates LPS-induced proinflammatory cytokine response and lipogenesis in human meibomian gland epithelial cells via MAPK/NF- κ B pathway. *Invest Ophthalmol Vis Sci* 2022;**63**:6.
- Liu S, Hatton MP, Khandelwal P et al. Culture, immortalization, and characterization of human meibomian gland epithelial cells. *Invest Ophthalmol Vis Sci* 2010;**51**:3993–4005.
- Liu Y, Kam WR, Ding J et al. Effect of azithromycin on lipid accumulation in immortalized human meibomian gland epithelial cells. *JAMA Ophthalmol* 2014;**132**:226–228.
- Liu Y, Knop E, Knop N et al. Growth hormone influence on the morphology and size of the mouse meibomian gland. *J Ophthalmol* 2016;**2016**:5728071.
- Mailleux AA, Spencer-Dene B, Dillon C et al. Role of FGF10/FGFR2b signaling during mammary gland development in the mouse embryo. *Development* 2002;**129**:53–60.
- Mathers W. Evaporation from the ocular surface. *Exp Eye Res* 2004;**78**:389–394.
- Nien CJ, Massei S, Lin G et al. Effects of age and dysfunction on human meibomian glands. *Arch Ophthalmol* 2011;**129**:462–469.
- Nusse R, Clevers H. Wnt/beta-Catenin signaling, disease, and emerging therapeutic modalities. *Cell* 2017;**169**:985–999.
- Nuwormegbe S, Park NY, Park HJ et al. Induction of meibocyte differentiation by three-dimensional, matrigel culture of immortalized human meibomian gland epithelial cells to form acinar organoids. *Ocul Surf* 2022;**26**:271–282.
- Oikawa A, Tohda H, Kanai M et al. Inhibitors of poly(adenosine diphosphate ribose) polymerase induce sister chromatid exchanges. *Biochem Biophys Res Commun* 1980;**97**:1311–1316.
- Qu JY, Xie HT, Xiao YT et al. The inhibition of p38 MAPK blocked inflammation to restore the functions of rat meibomian gland epithelial cells. *Exp Eye Res* 2023;**231**:109470.
- Reneker LW, Wang L, Irlmeier RT et al. Fibroblast growth factor receptor 2 (FGFR2) is required for meibomian gland homeostasis in the adult mouse. *Invest Ophthalmol Vis Sci* 2017;**58**:2638–2646.
- Riecan M, Paluchova V, Lopes M et al. Branched and linear fatty acid esters of hydroxy fatty acids (FAHFA) relevant to human health. *Pharmacol Ther* 2022;**231**:107972.
- Rötzer V, Melega F, Garreis F et al. E-Cadherin is important for meibomian gland function as revealed by a new human ex vivo slice culture model. *Am J Pathol* 2019;**189**:1559–1568.
- Sahin A, Liu Y, Kam WR et al. Dihydrotestosterone suppression of proinflammatory gene expression in human meibomian gland epithelial cells. *Ocul Surf* 2020;**18**:199–205.
- Samarawickrama C, Chew S, Watson S. Retinoic acid and the ocular surface. *Surv Ophthalmol* 2015;**60**:183–195.
- Selvarani R, Mohammed S, Richardson A. Effect of rapamycin on aging and age-related diseases-past and future. *Geroscience* 2021;**43**:1135–1158.
- Sui X, Kong N, Ye L et al. p38 and JNK MAPK pathways control the balance of apoptosis and autophagy in response to chemotherapeutic agents. *Cancer Lett* 2014;**344**:174–179.
- Sullivan DA, Sullivan BD, Ullman MD et al. Androgen influence on the meibomian gland. *Invest Ophthalmol Vis Sci* 2000;**41**:3732–3742.
- Sullivan DA, Yamagami H, Liu M et al. Sex steroids, the meibomian gland and evaporative dry eye. *Adv Exp Med Biol* 2002;**506**:389–399.
- Sun M, Moreno IY, Dang M et al. Meibomian gland dysfunction: what have animal models taught us? *Int J Mol Sci* 2020;**21**:8822.
- Tao H, Shimizu M, Kusumoto R et al. A dual role of FGF10 in proliferation and coordinated migration of epithelial leading edge cells during mouse eyelid development. *Development* 2005;**132**:3217–3230.
- Tao T, Tao L. Systematic review and meta-analysis of treating meibomian gland dysfunction with azithromycin. *Eye* 2020;**34**:1797–1808.
- Tsai C, Ito M, Gromova A et al. Barx2 and Fgf10 regulate ocular glands branching morphogenesis by controlling extracellular matrix remodeling. *Development* 2011;**138**:3307–3317.
- Utakoji T, Hosoda K, Umezawa K et al. Induction of sister chromatid exchanges by nicotinamide in Chinese hamster lung fibroblasts and human lymphoblastoid cells. *Biochem Biophys Res Commun* 1979;**90**:1147–1152.
- Wang G, Xu L, Shi R et al. Organotypic culture model of mouse meibomian gland as a screening platform for risk factors related to meibomian gland dysfunction. *Ocul Surf* 2023;**30**:73–84.
- Xie HT, Sullivan DA, Chen D et al. Biomarkers for progenitor and differentiated epithelial cells in the human meibomian gland. *Stem Cells Transl Med* 2018;**7**:887–892.
- Xu KK, Huang YK, Liu X et al. Organotypic culture of mouse meibomian gland: a novel model to study meibomian gland dysfunction in vitro. *Invest Ophthalmol Vis Sci* 2020;**61**:30.
- Yu H, Zeng W, Zhao G et al. Response of tear cytokines following intense pulsed light combined with meibomian gland

- expression for treating meibomian gland dysfunction-related dry eye. *Front Endocrinol* 2022;**13**:973962.
- Zahn I, Garreis F, Schicht M *et al*. A new organotypic 3D slice culture of mouse meibomian glands reveals impact of melanocortins. *Int J Mol Sci* 2022;**23**:14947.
- Zahn I, Socher E, Bergua A *et al*. Alpha- and beta-melanocyte stimulating hormone positively impact lipogenesis of meibomian gland cells *in vitro* and *ex vivo*. *Biomed Pharmacother* 2025;**185**:117937.
- Zhang T, Zhou Y, Li L *et al*. SIRT1, 2, 3 protect mouse oocytes from postovulatory aging. *Aging* 2016;**8**:685–696.
- Zhang W, Liu HT. MAPK signal pathways in the regulation of cell proliferation in mammalian cells. *Cell Res* 2002;**12**:9–18.