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The human meibomian gland epithelial cell line as a model to study meibomian gland dysfunction

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ABSTRACT

The meibomian gland dysfunction (MGD) is the leading cause of dry eye disease (DED) throughout the world. The investigation of MGD lacks suitable *in vivo* and *in vitro* models. In 2010 a human meibomian gland epithelial cell line (HMGEC) was established, so far the only available meibomian gland cell line. The characterization of HMGEC is of major importance to clarify its suitability for studying the meibomian gland (patho)physiology *in vitro*. The current culture protocol and new concepts of HMGEC culture will be compared. Hormones are believed to be a key factor in meibomian gland dysfunction thus HMGEC responsiveness to hormone stimulation is crucial to elucidate the hormonal influence on the meibomian gland. This review will summarize current findings about HMGEC and discuss its role in the meibomian gland dysfunction research.

Contents

1. Introduction

Since the report of the TFOS Workshop on meibomian gland dysfunction in 2011 the meibomian gland dysfunction (MGD) received increased interest in dry eye research (Nichols et al., 2011). The report underlined that MGD is the main course of dry eye disease. Since then, more *in vitro* and *in vivo* models for studying MGD are available (Nichols et al., 2014; Nien et al., 2009).

In 2010, Sullivan and coworkers immortalized and established a human meibomian gland epithelial cell line (HMGEC) in which the (patho)physiology of MGD can be studied *in vitro* (Liu et al., 2010). Previous studies, including our own, showed functional impact of cultivation conditions, e.g. fetal calf serum, culture supplements such as omega 3 fatty acids or calcium supplemental to HMGEC (Hampel et al., 2015a; Schroder et al., 2016; Sullivan et al., 2014). Furthermore, several studies showed effect of MGD-associated molecules, like sex hormones, inflammatory and growth factors, neurotransmitter or azithromycin (Ding and Sullivan, 2014; Kam

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and Sullivan, 2011; Liu et al., 2014a; Sahin et al., 2012). This review summarizes the findings on HMGECs and discusses the role in MGD research.

2. Characterization of the human meibomian gland epithelial cell line

In 2010, Sullivan and coworkers established an immortalized human meibomian gland epithelial cell line (HMGEC) (Liu et al., 2010). Therefore, they obtained meibomian gland epithelial cells of a 58-year-old male donor from lid segments after surgery. These cells were immortalized by infection with retroviral human telomerase reverse transcriptase (hTERT) (Liu et al., 2010). *In vivo* acinar cells (meibocytes) of the meibomian glands differentiate and maturate from proliferating, non-lipid producing basal cells to non-proliferating, but lipid-accumulating differentiated mature cells. Meibocytes run through several stages that can be differentiated morphologically and termed basal, differentiating, mature and hypermature (Gorgas and Volkl, 1984). During the maturation process, meibocytes accumulate lipid vesicles and finally undergo cell death. After cell rupture, the whole cell content forms the meibum that is released into the meibomian gland duct and from there onto the ocular surface. This process is classified as holocrine secretion. A single meibomian gland consists of clusters of meibocytes which connect via smaller ductuli to a central excretory duct that opens at the free lid margin near the *mucocutaneus junction* (Fig. 1) (Paulsen and Garreis, 2014; Tektas et al., 2012). For *in vitro* analyses of meibocyte differentiation, this process must be simulated. According to the proposed culture protocol HMGECs are cultured in a low-calcium, serum-free keratinocyte basal medium with 5 ng/ml epidermal growth factor (EGF) and 50 μg/ml bovine pituitary extract (BPE). HMGECs then showed on the one side increased cell proliferation and a significant up-regulation of genes involved in cell cycle, DNA replication and protein translation. On the other side, genes linked to differentiation and lipid biosynthesis pathways are down-regulated (Liu et al., 2010, 2013). To induce differentiation in HMGEC, medium is switched to a high-calcium Dulbecco's modified Eagle's medium (DMEM) and Ham's F12, supplemented with 10% fetal bovine serum (FBS) and 10 ng/ml EGF. This induces lipid accumulation as well as an increased expression of genes associated with cell differentiation, epithelium development and cell components such as endoplasmic reticulum, Golgi apparatus, vesicles, and lysosomes (Liu et al., 2010, 2013). Furthermore, serum-induced differentiation altered the fatty acid content of polar and nonpolar lipids and downregulated genes related to cell cycle, mitochondria, ribosomes, and protein translation (Sullivan et al., 2014). Additional, serum treatment changed morphology dramatically. HMGECs start to form desmosomes and cytokeratin filaments (Hampel et al., 2015b). Electron microscopy analysis reveals the formation of lamellar bodies (Hampel et al., 2015b; Liu et al., 2014b). Lamellar bodies represent a special type of lysosome serving as lipid storage and secretory organelles (Schmitz and Muller, 1991). Lamellar bodies can not only be found in the pneumocytes of the lung, but also in many other tissues such as meibomian glands (Jester et al., 1981; Sirigu et al., 1992). Electron microscopy images as well as Sudan III lipid staining and lipidTOX neutral lipid staining are used to visualize lipid vesicles in HMGEC and show lipid accumulations under serum treatment (Hampel et al., 2015b; Liu et al., 2010; Sullivan et al., 2014) (Fig. 2). Sudan III lipid staining is a cheap and easy method to visualize

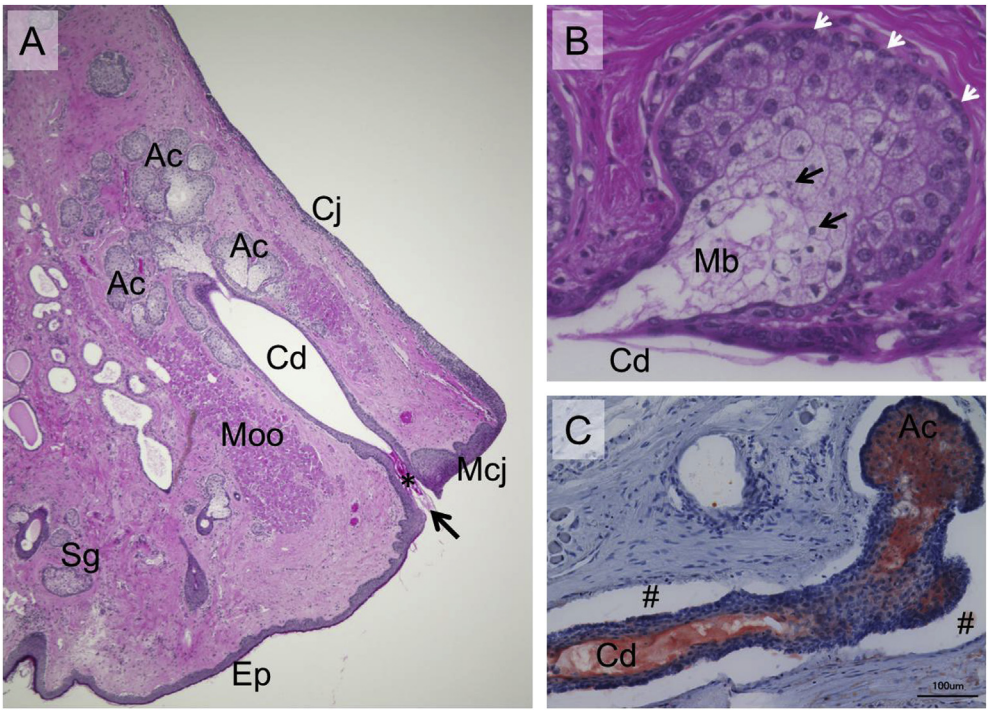

Fig. 1. Histology and anatomy of human meibomian gland. **(A)** Overview of a human eye lid with acini (Ac), central duct (cd) and orifice (*arrow*) of a meibomian gland at the lid margin anterior to the mucocutaneous junction (Mcj). The glandular orifice contains keratinized epithelial lamellae (*asterisk*). Epidermis (Ep) with hair-associated sebaceous glands (Sg), conjunctiva (Cj), *musculus orbicularis oculi* (Moo) **(B)** A higher magnification of a single holocrine acinus of human meibomian gland. The differentiation and maturation process of acini cells (meibocytes) starts from no lipid containing basal cells (*white arrows*). Toward the center of the acinus, meibocytes accumulate lipid vesicles (non-colored, frothy vesicles) and undergo apoptosis. (Hyper)mature meibocytes are identified by pyknotic cell nucleus (*black arrows*). Whole meibocytes form the secretory product termed meibum (Mb). The meibum flows through a short connecting ductule into a central duct (Cd). **(C)** Sudan III staining shows lipid-producing acinar cells (Ac) and meibum passaging through a central duct (Cd) of a meibomian gland. Meibum lipids are stained in red. Hash tags (#) document artificial shrinking artifact.

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