Review

Function of meibomian gland: Contribution of proteins

M. Vimalin Jeyalatha, Yangluow Qu, Zhen Liu, Shangkun Ou, Xin He, Jinghua Bu, Sanming Li, Peter Sol Reinach, Zuguo Liu, Wei Li

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The meibomian gland is the major contributor to the tear film lipid layer. It is generally accepted that meibomian gland secretions, i.e. meibum, play a critical role in the homeostasis of the tear film. Lipid components of meibum and their structure, as well as functions were intensively studied. However, the proteins from meibum have not attracted enough attention. This review summarizes current knowledge about protein components of the meibum, particularly their function on tear film and ocular surface, and changes in the proteins during meibomian gland dysfunction (MGD).

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1. Introduction

Meibomian glands are modified holocrine sebaceous glands that are embedded in the tarsal plate of both the upper and the lower
eyelids (Jester et al., 1981). The meibomian gland secretions, i.e. meibum, consist of a complex mixture of various polar and nonpolar lipids containing cholesterol, wax esters, diesters, tricacylglycerol, free cholesterol, free fatty acids, and phospholipids (Green-Church et al., 2011). The meibum spreads onto the tear film and functions to reduce the evaporation of the aqueous component, smoothen the corneal surface, and form a barrier to protect the eye from microbial agents and organic matter such as dust and pollen (Wang et al., 2016a; Den et al., 2006; Holly and Lemp, 1977).

In the pathological stage, meibomian gland can undergo functional and structural changes, leading to meibomian gland dysfunction (MGD). Although the precise aetiology and pathophysiology of MGD remains elusive, in 2011 the International Workshop on MGD proposed a definition for MGD as “a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion. It may result in alteration of the tear film, symptoms of eye irritation, clinically apparent inflammation, and ocular surface disease” (Nichols et al., 2011).

It is well accepted that MGD is the most common cause of evaporative dry eye (Schaumberg et al., 2011). MGD is found even in situations previously considered to be the primary aqueous deficient dry eye (Lemp et al., 2015). Therefore, MGD may also have some association with this condition. It is commonly believed that lipid plays a critical role in the homeostasis of tear film. MGD related dry eye is due to the reduction of lipid secretion from meibomian glands which induce tear film instability, hyper-evaporation of aqueous tear and entry into the vicious circle of dry eye.

Recently, there are emerging evidences showing that rather than just lipid components, meibum also contains different proteins. How these proteins contribute to the homeostasis of tear film and the ocular surface is attracting more attention. In this review, we summarize our current knowledge about the protein components in meibomian gland secretion, analyze possible functions of these proteins on the homeostasis of tear film and ocular surface, and propose future research direction in this field.

2. Meibum protein components

The tear film is packed with numerous proteins whose major sources are corneal epithelial cells, goblet cells, lacrimal gland, meibomian gland, and blood vessels. Proteomics analysis of human tears in healthy subjects and dry eye patients has been intensively performed in recent years (Srinivasan et al., 2012) (Li et al., 2014; Aluru et al., 2012). However, there are very few studies describing the identity of proteins in meibum. Infrared spectrum and nuclear magnetic resonance spectroscopy indicated the presence of protein components in the meibum (Borchman et al., 2011, 2010, 2012). Till now, the only proteomic analysis of human meibomian gland secretions was done by Tsai et al. (2006). They identified more than 90 proteins in human meibum. Keratins (K1, 5, 6, 7, 9, 10, 13, 16), lactoferrin, lipophillins, lipocalins, phospholipid transfer proteins, surfactant proteins (SP-B,SP-C), proteoglycans, cytchrome c, farnesoid X receptor are the major protein components of meibum (Tsai et al., 2006; Glasgow et al., 1995; Jauhiainen et al., 2005). The major categories of meibum proteins are listed in Table 1.

Among the listed proteins detected in meibum, keratins are most commonly studied in the ocular surface. Keratin 10 was detected in both normal and MGD meibum and considered to be secreted from keratinized duct epithelial cells (Igor et al., 2014). Keratin7 and Keratin13 are expressed in the meibomian acinar cells. Due to non nuclear protein co-expression with keratin positive expression, the keratins in meibum were considered to be derived from dead/lysed cells (Ashraf et al., 2011). They most likely originate from the shedding of keratinized epithelial cells lining the meibomian gland ducts (Ong et al., 1991). It has been shown that the levels of keratin in meibum increase up to 10% in MGD patients. Keratin may have a role in obstructing secretion of meibum from the ducts due to hyperkeratinization (Ong et al., 1991). Recent studies observed that keratin mixes into the lipid layer of the tear film, and likely destabilizes the lipid layer in vitro (Borchman et al., 2010; Palaniappan et al., 2013). Thus, excess concentrations of keratin in patients with MGD may disrupt the normal structure of the meibomian lipid film, which may reduce tear break-up time (Igor et al., 2014). However, it is not known in vivo if the increased keratin affects changes in the function of the tear film lipid layer.

3. Function of meibum proteins

Although Tsai et al. described the functions of some proteins in the meibum (Tsai et al., 2006), the function of most of the proteins on the tear film and ocular surface was not well studied. In this review, we have updated the functions of these proteins and focused on their role in maintaining the ocular surface integrity, and have proposed their potential functions based on studies in other organs or tissues (Table 2). We classified these proteins into three major categories according to their function.

3.1. Proteins related to ocular surface epithelial protection

Basic proline rich protein 1 was mostly found in human saliva. It could provide protection against dietary tannins which could damage the gastrointestinal epithelium and mucosa (Cai, 2006; Shimada, 2006). Actotransferrin, also named as lactoferrin, is an iron-binding protein, which can reduce the availability of iron necessary for microbial growth and survival. It may stabilize the tear film, avoid excessive tear evaporation, ocular surface desiccation and protect eyes from oxidative stress (Pastori et al., 2015). It can promote wound healing and suppress inflammatory cytokine IL-1 (Pattamatta et al., 2013; Ashby et al., 2011). The amount of lactoferrin was found reduced in keratoconus tears (Balasubramanian et al., 2012). Cytochrome C is related to caspase-dependent apoptosis, it could have been released from mitochondria of the conjunctival cells which is evident in BAK induced dry eye model (Clouzeau et al., 2012).

Proteoglycan 4 (PRG4, lubricin) mRNA was detected in human meibomian gland epithelial cells and expressed in the full thickness of the corneal and conjunctival epithelium. PRG4 knockout mice demonstrated significant corneal fluorescein staining, suggesting increased corneal damage (Samsom et al., 2014). PRG4 could be a protectant by reducing friction at the human cornea-eyelid, cornea-conjunctiva and human cornea-polydimethylsiloxane (PDMS) interfaces. Conjunctival changes may have occurred as a result of PRG4 absence and limited the efficacy of lubricin (Schmidt et al., 2013). In clinical trials, conjunctival erythema was significantly reduced by application of lubricin eye drops to the biointerfaces. Loss or downregulation of lubricin likely increases shear stress at the ocular surface, which, in turn may lead to inflammation, stimulation of corneal nerves, and accumulation of inflammatory mediators (Morrison et al., 2012).

Forkhead related protein is involved in normal corneal development. Increase in its expression levels may alter corneal epithelial thickness (Lehmann et al., 2003). Farnesoid X activated receptor is also expressed in the basal cells of corneal epithelium (Higashiyama et al., 2008), it can accelerate epidermal barrier development. TRK-C tyrosine kinase (TRKc) was noted in the supra-basal layers of corneal and limbal epithelia (Touhami et al., 2012). Ellis Van Creveld Syndrome 2 protein (EVC2) maintains the stemness of the dental mesenchymal stem cells. Loss of EVC2 could lead
dental mesenchymal stem cells transition into hypomorphic enamel formation (Zhang et al., 2017). MSX2-interacting protein is the nuclear matrix protein that regulates the development of many tissues. Deficiency of this protein could cause declines in dental epithelial proliferation and influence tooth morphogenesis (Zhu et al., 2011). It is also an endogenous inhibitor of Notch regulation and regulates the thymocyte differentiation (Tsujii et al., 2007). Notch signaling pathway may play an important part in the differentiation and proliferation of ocular surface epithelium, so MSX2-interacting protein maybe a candidate factor for maintaining homeostasis of the ocular surface epithelium.

3.2. Proteins related with lipid metabolism

Lipocalin 1 is referred to as the major protein in tears. It can remove fatty acids and phospholipids from the corneal surface (Gasymov et al., 2005). Its concentration was reduced in samples from patients with MGD and dry eye (Yamada et al., 2005). CCCL8, which is found in meibum, is related with lipid metabolism through the interferon-mediated immune response and mediates virus-induced susceptibility to bacterial superinfection. Its deficiency in mice may cause lung inflammation (Schliehe et al., 2015). Leukotriene B4 (LTB4) belongs to the leukotrienes (LTs) class which is the major mediator of LPS-induced inflammation. The meibomian gland cells secrete LTB4 when they are exposed to LPS (Sahin et al., 2011). Lipocalin prevents corneal desiccation by scavenging lipids, protein carrier for Vitamin E in tear; Removal of fatty acid and phospholipid from the corneal surface (Qi et al., 2016). Lctoferrin is a potent antibacterial, enhances cell mediated immunity (Koo et al., 2005) and regulates the thymocyte differentiation (Tsuji et al., 2007). Interferon regulatory factor 3 can be activated after viral infection and to protect the ocular surface (Hara et al., 2009; Broekhuyse, 1974). Among these, interferon regulatory factor 3 is also present in meibum and its levels are decreased in the dry eye patient tears at an early stage (Versura et al., 2013). Adenylate cyclase 7 belongs to the family of adenylyl cyclase isoforms (ACs) which can synthesize cyclic adenosine monophosphate (cAMP), and CAMP may inhibit TNFs induced inflammatory effects (Risoe et al., 2015; Duan et al., 2010). Setdb2 serves as a regulator for the interferon-mediated immune response and mediate virus-induced susceptibility to bacterial superinfection. Its deficiency in mice may cause lung inflammation (Schliehe et al., 2015). Leukotriene B4 belongs to the leukotrienes (LTs) class which is the major mediator of LPS-induced inflammation. The meibomian gland cells secrete LTB4 when they are exposed to LPS (Sahin et al., 2011).

3.3. Proteins related with anti-inflammatory and/or anti-microbial functions

Similar to the lacrimal gland, meibomian glands also secrete proteins with anti-inflammatory and/or anti-microbial functions. Among these, interferon regulatory factor 3 can be activated after viral infection and to protect the ocular surface (Hara et al., 2009; Conrady et al., 2012; Roy et al., 2014). Lysozyme C precursor is also present in meibum and its levels are decreased in the dry eye patient tears at an early stage (Versura et al., 2013). Adenylate cyclase 7 belongs to the family of adenylyl cyclase isoforms (ACs) which can synthesize cyclic adenosine monophosphate (cAMP), and CAMP may inhibit TNFs induced inflammatory effects (Risoe et al., 2015; Duan et al., 2010). Setdb2 serves as a regulator for the interferon-mediated immune response and mediate virus-induced susceptibility to bacterial superinfection. Its deficiency in mice may cause lung inflammation (Schliehe et al., 2015). Leukotriene B4 (LTB4) belongs to the leukotrienes (LTs) class which is the major mediator of LPS-induced inflammation. The meibomian gland cells secrete LTB4 when they are exposed to LPS (Sahin et al., 2011).
and et al., 2012). Till now, the function of these proteins on the ocular surface is not well known. Further study is needed to disclose the specific role of these proteins.

3.4. Meibum proteins contribute to colloidal osmotic pressure

Plasma colloidal osmotic pressure (COP) and hydrostatic pressure are critical in maintaining intravascular volume and preventing fluid leakage into the interstitium with the subsequent formation of interstitial edema (Weil et al., 1979). Osmotic pressure also plays an important role in maintaining ocular surface microenvironment. Tear film hyperosmolarity development can contribute to initiating ocular surface changes promoting the dry eye clinical symptoms. Earlier studies were limited in focusing on the role of tear film meibum protein in mediating tear film crystalloid osmotic pressure even though the colloidal osmolality of tears is twenty-folds less than that of the corneal stroma, which in turn is less than 1% of the total osmolality of an isotonic solution. These low levels may be the reason why their role were not further addressed by many researchers (Holly and Esquivel, 1985). However, as recent studies indicate that proteinaceous meibomian gland secretions that they have other functions, we propose that meibum proteins may also contribute to formation of tear film colloidal osmotic pressure maintaining ocular surface homeostasis. Some proteins such as actotransferrin, lipocalin 1, PRCA and lubricin are decreased in dry eye patients while some proteins such as cytosome care increased (Balasubramanian et al., 2012; Clouzeau et al., 2012; Yamada et al., 2005; Lambiase et al., 2017). There is no study yet describing either the amount or identity of proteins changed in MGD patients. Such changes coupled with possible protein pattern expression variability in different types of MGD patients could affect alterations in MGD patient tear film colloidal osmotic pressure. These questions warrant further study.

4. Lipid-protein interaction in meibum

With the development of the method of lipidomics, increased evidences support that proteins are a major part of the tear film lipid layer (Gasymov et al., 2005). Nevertheless scant attention has been paid to the importance of the mechanism of lipid-protein interaction. As we know, membrane proteins play a pivotal role in maintaining the lipid bilayer function (Gasymov et al., 2005). The lipid-protein interaction may be consequence of lipid binding with either transporter proteins or hydrolytic enzymes. In early 1978, Mateu et al. showed that proteins alter the lipid structure through a simple system involving a single type of protein (lysozyme or cytochrome C) and single type of lipid (phosphatidic acid) (Mateu et al., 1978). Tragogulas ST et al. used a Langmuir trough to measure and compare the surface activities of albumin, lipocalin, β-lactoglobulin, lactoferrin, lysozyme, secretory IgA, mucin, meibomian lipid, and tears. They proved that proteins are major contributors to the surface activity of the tear film (Gooden et al., 1982). At the same time, Miano et al. found a similar phenomenon by adopting a pendant drop to investigate the insertion of tear proteins into the tear meibomian lipid layer (Miano et al., 2005). The concept of proteins being a part of the lipid layer has been strengthened by the discovery of surfactant proteins B and C which play an important role in stabilizing the surface of lung surfactants (Brauer et al., 2007; Brauer et al., 2007).

Lysozyme, a major bactericolytic protein found in tears, is incapable of lipid scavenging, but studies show that it can interact with a phospholipid membrane and meibum layer (Glasgow et al., 1995), (Miano et al., 2005; Millar et al., 2006, 2009; Khanal et al., 2009). Mucins are essential proteins secreted by the conjunctiva involved in the maintenance of the ocular surface epithelial integrity. The mucins aid in the uniform and slow spread of meibomian lipids following each blink. Millar et al. examined mixtures of meibomian lipids and purified ocular mucins in vitro and suggested that mucins may be forming aggregates surrounded by lipids that occupy the surface of the tear film lipid layer (Millar et al., 2006). Niko et al. proved that phospholipid transfer protein (PLTP); a glycoprotein interacts with the lipids by acting as a phospholipid transporter and scavenges lipophiles from the mucin layer (Setala et al., 2010). The proteins can interact with lipids in the aqueous—lipid interfaces or lipid bilayers of bacterial cell wall, plasma membranes of epithelial cells, or with the lipid vesicles. Thus the proteins and lipids maintain a harmonious chemistry, either of them acting as a transporter or scavenger thereby maintaining the tri layered tear film stability. Yet in the meibomian secretion, the lipid layer harbors various anonymous proteins and lipids whose function is elusive and the field of tear lipid protein chemistry requires further study.

Lipocalin secreted by the lacrimal gland and meibomian gland is a major tear protein (33%) (Tsai et al., 2006; Fullard, 1988). Tear lipocalin deficiency is associated with dry eye (Yamada et al., 2005). It has been reported that tear lipocalin can bind a variety of lipophilic compounds, including fatty acids, fatty alcohols, phospholipids, glycolipids, retinol, arachidonic acid, and cholesterol (Glasgow et al., 1995). Among various lipocalins, human lipocalin-1 has affinity for insoluble long-chain fatty acids and phospholipids.

Tear lipocalin can bind to a wide range of ligands because of its structure, which exhibits considerable flexibility (Breustedt et al., 2005). There are two possible ways that tear lipocalin could bind to a wide range of lipids. Its first potential function is scavenging lipids from the corneal surface to maintain the wettability and integrity of the ocular surface (Gasymov et al., 2005). However, such a role in vivo has not yet been demonstrated because the native tear film was not present in any of the studies. The second potential function is that tear lipocalin could scavenge lipids from the tear film to increase tear viscosity (Mudgil and Miller, 2008). Based on these studies, it seems likely that tear lipid–lipocalin interactions change the physical properties of tear. To date, it is still not clear whether tear lipocalin release these lipids into the lipid layer of the tear film either, or drain the lipid through the tear drainage system. These are important questions in need of resolution to reduce dry eye and MGD symptoms in future studies.

5. Differentially expressed proteins in MGD and dry eye

New emerging proteomics technologies such as the mass spectrometric technology, Tags for Relative and Absolute Quantitation (TIRQA) and label-free quantification (LFQ) has enabled researchers to use dry eye tear proteomics to compare changes with normal tears and thereby elucidate candidate proteins involved in the pathology of this disease. Proteomics study of the tear samples has revealed the presence of 491 proteins among which only a few (approximately 80) play a critical role in the pathogenesis of dry eye (de Souza et al., 2006; Perumal et al., 2016). Studies focused on quantifying the levels of differentially expressed proteins in the tears of dry eye patients with those in the controls are needed to better understand the pathology of MGD, dry eye and to identify a needed diagnostic tool. Proteomic analysis by Soria et al. validated the overexpression of S100A6, S100A9, S100A8, S100A4, glutathione S-transferase P (GSTP1) annexin A1 (ANXA1) in both dry eye and the MGD group are common and may help explain the underlying pathophysiologies of these diseases (Soria et al., 2013). The data also supports the usage of the proposed alternative term “latent MGD” for dry eye. Proline rich protein (PRP3 & PRP4), prolactin-inducible protein (PIP), lipocalin-1 (LCN1), lactoferrin (LTF) and lysozyme are the proteins which were proven to be down regulated consistently in all the proteomic analyses (Grus et al.,
2005; Boehm et al., 2013). The lacunae in the proteomic analysis of the tears of the dry eye and the MGD are: the post-translational modification, protein complex formation, transient interaction between the differentially expressed proteins, and series of signal transductions. We listed differentially expressed proteins in dry eye and MGD from various studies in Table 3. Different techniques used in different studies may also have an impact on the variation of the data. In the future, proteomic analysis of meibum in MGD may help to differentiate whether the tear protein changes in dry eye originated from meibum or secondary to dry eye changes.

6. Alteration of proteins in MGD and the significance of their changes

6.1. Protein-lipid ratio change in MGD

Lipogenesis is being widely studied considering the quantity and the quality of lipid changes in MGD. Peroxisome Proliferator-Activated Receptor-gamma (PPAR-g) is a ligand-activated transcription factor which is considered to be involved in the lipogenesis and regulates the differentiation of meibocytes (Jester and Brown, 2012; Nien et al., 2010). Chen et al. reported that the PPAR-g expression was down regulated in the conjunctiva of the dry eye mice model with dysregulated increases in proinflammatory cytokines, TNF-α and IL-1β (Li et al., 2014). Over expression of α-enolase reveals the externalization of the enzyme into the tears. Glycolytic enzyme externalization is a well known feature exhibited by the apoptotic cells and the externalized proteins are incapable of performing their innate function (Ucker et al., 2012). Lipogenesis requires abundant energy which is generated by the glycolytic pathway. Thus the over expressed non-functional α-enolase can be correlated with the decline of lipogenesis in MGD. Cystatins are inhibitors of cysteine proteinases involved in preventing uncontrolled proteolysis and tissue damage. Down regulation of the protease inhibitors in MGD underlie the mechanism accounting for increased matrix degradation and apoptosis of the meibocytes. Suhalim et al. showed that the ratio of protein-lipid varies across the meibomian gland i.e. the ratio decreased from the acinus proceeding to the central duct (Suhalim et al., 2014). It has been shown that increases in meibium viscosity are associated with increases in the protein ratio (Ashraf et al., 2011). This finding also explains how the lipid order and packing change in the meibium, i.e, overexpression of protein and under expression of the lipid moieties affect changes in the meibum viscosity. Taken together, differences in the protein-lipid interactions between MGD and evaporative dry eye help explain why their pathogenic mechanisms are different from one another.

6.2. Down regulation of lipid affinity protein network

Lipocalins, lipophillins are an important group of lipid affinity proteins in tears. Lipocalins are transporter proteins of hydrophobic molecules like lipids (Gasymov et al., 2005). Lipocalins are known to exhibit multiple protective functions among which lipid transport and the scavenging of lipid peroxidation products are considered important in the rehabilitation of the ocular surface (Stopkova et al., 2009). Meibomian secretion of the MGD patient is characterized by depletion of lipocalins (Zhou et al., 2009; Versura et al., 2010; Yamada et al., 2005) which eventually leads to the accumulation of toxic byproducts of lipid peroxidation, increased oxidative stress and lack of lipid transport. Disrupted lipid transport results in the aggregation of lipids, increased lipid-lipid interaction resulting in a highly viscous meibium. Free fatty acids are capable of inducing steatosis and mitochondrial-induced cytochrome c release leading to hepatocyte apoptosis (Malhi et al., 2006). A similar hypothesis can be applicable to explain how dysfunctional free lipids in the meibomian gland adhere and contaminate the cornea creating a hydrophobic unwettable surface leading to apoptosis of the ocular surface epithelial cells.

Lipophillins as the name suggests are “lipid loving” secretary proteins found in higher concentrations in the glandular secretions and they also possess an anti-inflammatory property (Acea et al., 2011). Lipophillins are named instead proteolipid proteins when they are expressed in the myelin sheath. As high level of cholesterol are required for normal myelination, the proteolipid proteins using their molecular association towards lipids efficiently co-transport...
the cholesterol moiety (Werner et al., 2013). Thus it is evident that lipophillin downregulation impairs lipid transport. Lipophillins are often found to be co-expressed with mammaglobin in the mammary gland secretions. Lipophillins and mammaglobins form a complex and it is down regulated in breast cancer but the exact role of this complex remains elusive (Sjödin et al., 2008). The proteomics of MGD secretions showed that there is under expression of the lipophillin and the mammaglobins (Srinivasan et al., 2012). Yet the studies have not focused on whether the lipophillin mammaglobin complex is formed in the tears and if the complex has a role to play in lipid transport and metabolism.

6.3. Denaturation of essential meibum proteins

There is a limited understanding of the factors causing changes in MGD proteomic displays because such studies have focused on the quantities of the protein and not the quality and the conformational change of the proteins. Protein conformation is determined by the unique amino acid sequences and their interactions. Protein conformation is maintained at their isoelectric pH. The proteins lose their positive charge and attain a net negative charge at higher pHs. Charge repulsion results in alteration of the protein conformation leading to protein denaturation and dysfunction (Jaenicke and Závodszky, 1990). Though protein denaturation is reversible, exposure to extreme conditions like high temperature, pH, reactive oxygen species, hyperosmolarity results in irreversible lysis of the peptide bonds between the amino acids, which is evident in MGD. Molecular chaperone are protein systems which can assist in the remodeling of the denatured, misfolded proteins and in regaining their function. Hsp60, Hsp70, Hsp90, Hsp100 and the small Hsps are the common chaperone systems present in humans (Hendrick and Hartl, 1995). Downregulation of the molecular chaperone system may eventually lead to the uncontrolled denaturation of the proteins. Yet our literature survey indicates that studies have not focused on any relationship between the chaperone system and dry eye. It is crucial that additional attention should be paid to understanding if drug-induced changes in the chaperon system may be of therapeutic benefit in dry eye management.

6.4. Unfolded protein response in MGD

The endoplasmic reticulum (ER) plays a major role in determining protein conformation as they undergo folding and maturation during their ductal transit and eventual release into the ER lumen. The ER protein folding machinery is under an imposed high stress especially in the secretory cellular system as it continuously synthesizes and secretes the essential proteins. An inefficiency of the ER in the protein folding process results in activation of the unfolded protein response (UPR) pathway. This response is induced by three signaling pathways PRKR-like ER kinase (PERK)—eukaryotic translation initiation factor 2α (eIF2α), inositol-requiring protein 1α (IRE1α)—X-box binding protein 1 (XBP1) and activating transcription factor 6α (ATF6α). UPR pathway activation leads to declines in protein synthesis to prevent further aggregation of unfolded proteins, enhancing synthesis of chaperone systems, synthesis of ER-associated degradation (ERAD) machinery to reduce the burden of ER stress, expansion of the ER membrane to accommodate the unfolded protein aggregates. If these compensatory pathways are successfully executed the cell survives. However, if the UPR is prolonged, the apoptotic signaling pathway is activated (Hetz, 2012). As discussed earlier, increases in tear film osmotic pressure is a characteristic of dry eye irrespective of the type of osmotic pressure increase. Wang et al. in their in vitro study has shown that hyperosmolarity leads to ER stress and production of ROS (Wang et al., 2016b). Relationship between UPR activation and dry eye development has not been described yet. Nevertheless, various studies did show that there is an association between hyperosmolarity and excessive ROS generation. However, neither the underlying molecular mechanisms nor the consequence has been characterized in detail. Focusing on the roles of tear film proteins may provide new insight into the underlying pathophysiology of MGD related dry eye.

7. Prospective of future research

Our knowledge about the meibum protein components and their function on the tear film as well as ocular surface is still in its infancy. In an attempt to gain insight into this question, we propose possible functions of these proteins. By doing this, we may help realize how they may: 1) stabilize the lipid and their integration into the lipid layer after being secreted onto the ocular surface; 2) regulate the lipid metabolism during or after the meibum secretion; 3) contribute to the colloid osmotic pressure of the tear film; 4) play an anti-inflammatory and/or an anti-microbial function; 5) be an active component of immune system of the ocular surface; 6) have specific function on the ocular surface epithelial cells. Addressing the validity of these suggested functions requires dealing with a large number of unknowns and uncertainties. They include resolving how protein components contribute to the meibum lipid profile and their intrinsic characteristics. Another issue concerns delineating how these proteins get modified during MGD or other meibomian gland related diseases. Answering these questions will enable us to better understand the enigma regarding how changes in meibomian function in health and disease affect changes in the meibum makeup and expression.

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