Pathobiology of Meibomian Gland at Wakayama 2016

An ISER 2016 Satellite Meeting

Sep. 24 (Saturday) – 25 (Sunday), 2016

Venue:

Library, 3rd floor

Lifelong learning and Medical community Center

Wakayama Medical University, Wakayama, Japan

Access:

* **KIX airport → Shuttle bus for JR Wakayama Station (40 min*) → taxi (20 min*) → WMU**
* **KIX airport → taxi (1 hr*) → WMU**
* **JR Shin-Osaka Station → I hr → JR Wakayama station → taxi (20 min*) → WMU**

*approx.

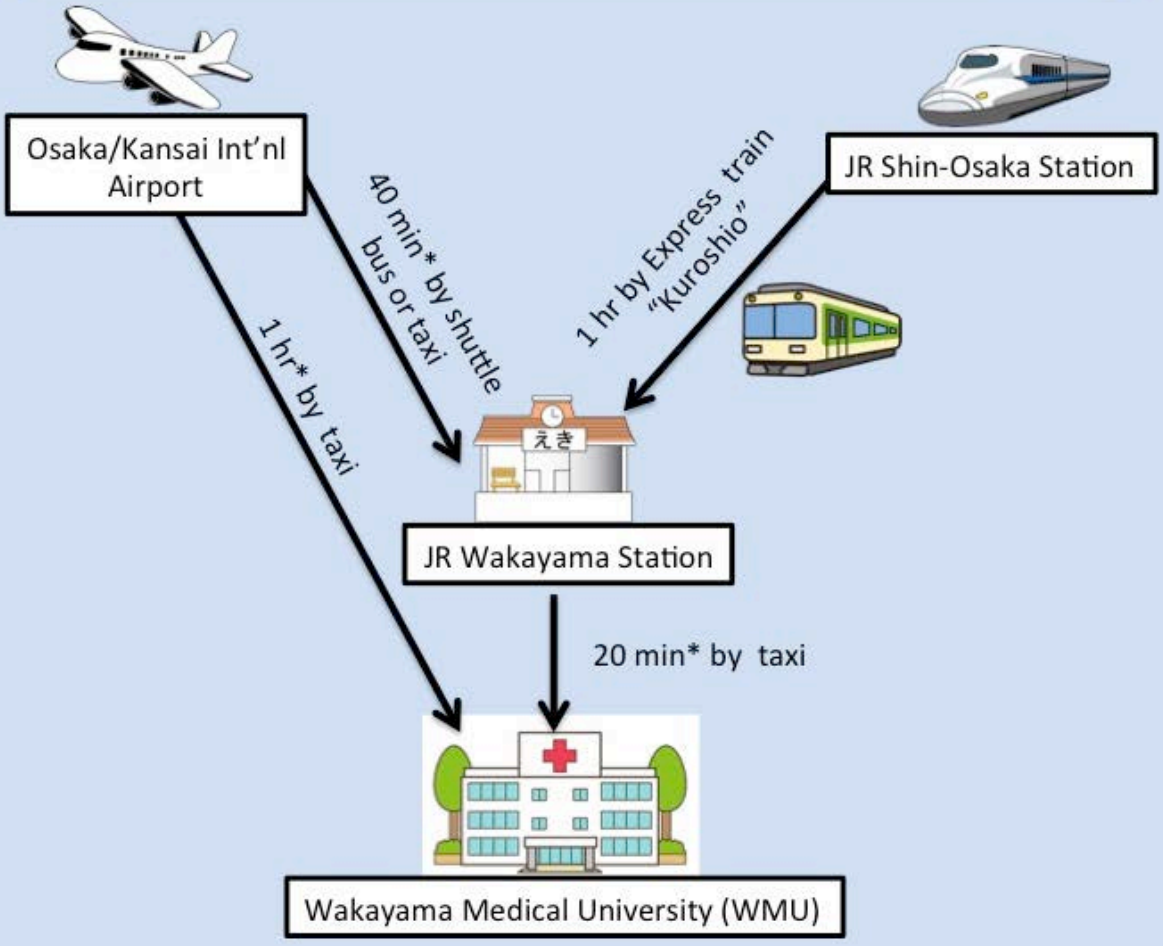

Illustration
http://illpop.com/png_season/may03_a15.htm
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Welcome to Wakayama

It's a great honor for us to host the conference entitled "*Pathobiology of Meibomian Gland at Wakayama 2016*", as an ISER 2016 Satellite meeting. We are very pleased to have distinguished speakers here upon our invitation, as well as poster presentations. The presentations will cover the various aspects of the current topics on Meibomian galnd with deep scientific value. It is not the first time for some of the speakers to come to Wakayama, but this time we will be able to much focus on the Pathobiology of Meibomian Gland.

We sincerely hope the meeting to be finalized with a great success with active discussion and reunion /our friendship of all the attendees, speakers and audience.

Shizuya Saika, MD, PhD

Program

Sep 24th, Saturday (13:30 - 17:45)

13:30 Opening remarks & Business announcement

Ssssion #1 (Clinical Science)

13:45 Invited talk 1 (Dr Ulrike Hampel) /Chiar: Dr Peter Reinach
14:15 Invited talk 2 (Dr James V. Jester) /Chiar: Dr Roger W. Beuerman
14:45 Invited talk 3 (Dr Igor Butovich) /Chair: Dr James V. Jester

15:15 Coffee break & Poster viewing

Session #2 (Basic Science)

15:30 Invited talk 4 (Dr Chia-Yang Liu) /Chair: Dr Wei Li
16:00 Invited talk 5 (Dr Wei Li) /Chair Dr Chia-Yang Liu

16:30 Coffee break & Poster viewing

Session #3 (Clinical Science)

16:45 Invited talk 6 (Dr Roger W. Beuerman) /Chair: Dr Erich Knop
17:15 Invited talk 7 (Dr Kyoung Yul Seo; LIME awardee) /Chair: Dr Igor Butovich

17:45 Business announcement

19:00 Banquet for all the attendees

Marina City Hotel
http://www.marinacity.com/hotel/english/

Sep 25th Sunday (8:55 - 13:00)

8:55 Good morning

Session #4 (Clinical Science)

09:00 Invited talk 8 (Dr Choun-Ki Joo) /Chair: Dr Winston W.-Y. Kao
09:30 Invited talk 9 (Dr Erich Knop; LIME awardee) /Chair: Dr Reiko Arita

10:00 Coffee break & Poster viewing

Session #6 (Clinical Science)

10:15 Invited talk 10 (Dr Reiko Arita) /Chair: Dr Choun-Ki Joo
10:45 Invited talk 11 (Dr Kyung-Sun Na) / Chair: Dr Ulrike Hampel

11:15 Coffee break & Poster viewing

Session #7 (Basic Science)

11:30 Invited talk 12 (Dr Shin Mizoguchi) /Chair: Dr Kyung-Sun Na
12:00 Invited talk 13 (Dr Winston W.-Y. Kao) /Chair: Dr Shizuya Saika

12:30 Closing remarks & Business announcement

12:45 Lunch and leaving for Tokyo

Presentations

What do we know about the human meibomian gland epithelial cell line?

Dr. med. habil. Ulrike Hampel

Universitatsstr 18, Erlangen, Germany.

The investigation of the meibomian gland dysfunction lacks suitable models *in vivo* and *in vitro*. 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. Furthermore, the meibomian gland is the main source of tear film lipids. Emerging questions arise from newly available eye drops and capsules containing lipids such as omega-3 fatty acids. This review will summarize current findings about HMGEC and discuss its role in the meibomian gland dysfunction research.

Characterization of Meibocyte Differentiation in Human and Mouse Meibomian Glands.

James V. Jester, Yilu Xie, Geraint J. Parfitt, Donald J. Brown

Department of Ophthalmology Research, University of California, Irvine, CA, USA.

While the cellular and molecular mechanisms underlying meibocyte differentiation and holocrine secretion are poorly understood, recent RNA Seq analysis of young and old mouse meibomian glands point to several genes that may play important roles in this process. The purpose of this study was to validate the protein expression of these identified genes in human and mouse meibomian glands and begin to characterize their potential role in meibocyte differentiation in culture. Mouse and post-surgical human eyelid tissue were obtained with approval from the UCI IACUC and IRB and immunostained for 1) age (DKKL1 and Caspase 14), 2) apoptosis (Caspase 3a, 9) and 3) autophagy (BECN1 and ULK1) related genes. In addition, human telomerized meibomian gland epithelial cells (hTMGE) cultures were stimulated by the PPARγ agonist, rosiglitazone (10 - 50 μM), and evaluated for lipogenesis and expression of meibocyte specific proteins. DKKL1 and Caspase 14 both showed strong meibocyte immunostaining, with anti-DKKL1 showing staining of the ductal epithelium while anti-Caspase 14 was specific for the suprabasal acinar meibocytes. Caspase 3a and 9, also showed strong staining specific for suprabasal meibocytes; however, TUNEL labeling was limited to the ductal epithelium and the very distal, disintegrating meibocytes. BECN1 and ULK1 were localized to suprabasal meibocytes. hTMGE cells showed a significant, dose dependent increase in lipid synthesis following treatment with rosiglitazone combined with expression of all meibocyte specific genes. Additionally, protein extracts showed hyper-phosphorylation of ULK1 in rosiglitazone treated cells suggesting induction of autophagy. These results indicate proteins associated with age, apoptosis and autophagy are expressed in human and mouse meibomian glands. Both age-related proteins are known to be involved in epithelial differentiation suggesting that atrophy of meibomian glands may involve loss of meibocyte differentiation potential. The finding that ULK1 is hyper-phosphorylated following stimulation with rosiglitazone suggests that autophagy may play an important role in meibocyte degeneration and disintegration.

Supported in part by NIH Grant EY021510, the Skirball Program in Molecular Ophthalmology, and Research to Prevent Blindness, Inc. Unrestricted Grant.

Molecular Mechanisms of the Lipid Biosynthesis in Meibomian Glands

Igor A. Butovich, Ph.D.

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Phone: (214)-538-7557, Email: igor.butovich@gmail.com

The main function of holocrine Meibomian glands is to produce a lipid-enriched secretion (called meibum) and secrete it onto the ocular surface through a system of ducts and orifices. The secretion itself is an extremely complex mixture of hundreds of lipid species of various classes. Meibum is formed in mature, differentiated meibocytes, in a complex, poorly understood metabolic process that we call *meibogenesis*. Our long-term goal is to characterize molecular mechanisms of meibogenesis. We have characterized the major lipid classes, and their individual constituents, of human meibum using chromatographic, mass-spectrometric, and nuclear magnetic resonance approaches.

These experiments resulted in identification of key structural features of major meibomian lipids, such as: 1) extreme lengths (with fatty acid and fatty alcohol residues approaching, in many cases, C_{34}-C_{36}); 2) extensive *iso*- and *anteiso*-branching of fatty chains; 3) relatively low degree of unsaturation of major lipid components (typically, from zero to three double bonds per fatty acid or fatty alcohol residue); 4) measurable presence of various ω-hydroxylated and α,ω-dihydroxylated lipids, such as extremely long chain ω-hydroxy-fatty acids and alcohols, which are esterified to other fatty acids, fatty alcohols, and cholesterol, through one or several ester bonds.

These features require a rather unique set of enzymes to be expressed in meibocytes. To determine which enzymes may be involved in meibogenesis, we have analyzed gene expression patterns in tarsal plates of four humans using mRNA microarrays, and conducted immunohistochemical analyses of tarsal plate tissue sections to identify selected proteins. Among highly expressed genes were found those responsible for: 1) fatty acid elongation (such as *ELOVL*s, in the following order *ELOVL4*>*ELOVL3*>*ELOVL6*>*ELOVL1*>*ELOVL5*>*ELOVL2*); 2) reduction of fatty acids into alcohols (*FAR2*>>*FAR1*); 3) fatty acid branching (*BCKDH*, *DBT*); 4) biosynthesis of cholesterol and cholesteryl esters (*HMGR*, *SQLE*, *Δ7-DHCR*, *ACAT*/*SOAT*); 5) biosynthesis of waxes (*AWAT2*/*WES*); 6) ω-hydroxylation of fatty acids (various *CYP4*s; 7) fatty acid desaturation (*SCD1*>>*SCD5*) and other related genes that are putatively involved in meibogenesis and its regulation. All evaluated genes were expressed almost identically across all study samples. This set of genes effectively covers the key proposed steps in meibogenesis and in its regulation. Next, we performed histochemical and immunohistochemical evaluation of human tarsal plates using lipid-specific stains and antibodies against selected representative proteins and their expected cellular loci. The experiments confirmed a high abundance of BCKDHA, DBT, ELOV3, ELOVL4, and other proteins of interest in the proper intracellular compartments of human meibocytes.

The results obtained for human tarsal plates were compared with the data for other human tissues, and distinctive differences between the former and the later were noted.

A detailed biosynthetic scheme that connects our observations and literature data is proposed, and its implications for Meibomian gland and the ocular surface physiology are discussed.

Canonical Jagged-1-Notch1 signaling axis is required for meibocyte differentiation during Meibomian gland morphogenesis

Chia-Yang Liu[1,2], Yujin Zhang[1,2], Simi Goins[2], Mindy Call[2], Winston Kao[2]

[1] School of Optometry, Indiana University, Bloomington IN 47405
[2] Department of Ophthalmology College of Medicine University of Cincinnati, Cincinnati OH 46267, USA.

Purpose: To demonstrate that Jagged1/Notch1→N1-ICD/Rbp-jκ/MAML1→Klf4/5 axis is critical for meibocyte differentiation during Meibomian gland morphogenesis.
Methods: Compound transgenic mice consisting of *K14-rtTA/tetO-Cre* and floxed *Jagged1*, *Dll1*, *Notch1*, *Notch2*, *Rbp-j*κ, and *Rosa26-dnMAML1* alleles, respectively, were used to ablate the gene of interest in a spatial-temporal specific manner from embryonic (E) day 14, before meibocyte cell differentiation. Phenotypic analyses of the resultant ocular surface were compared to the wild-type littermates at various stages. Oil red O (ORO) staining and antibodies specific against elongation of very long chain fatty acids protein 4 (ELOL4) were performed to identify lipid synthesis. Antibodies against the Krüppel-like family of transcription factor (KLF)-4 and -5 were performed to identify meibocyte differentiation.
Results: Ablation of Dll1 or Noth2 from K14-positive cells did not reveal any phenotypical abnormality. However, loss of *Jagged1*, *Notch1*, *Rbp-j*κ expression, and gain of *dnMAML1*expression in K14-positive cells all manifested severe ocular surface malformation including corneal ulceration and conjunctivitis sicca. Immunohistological analysis revealed that both lipid synthesis and expression of ELOVL4, Klf-4 and -5 were diminished.
Conclusion: Our data indicate that ligand Jagged-1 but not Dll1 and receptor Notch1 but not Notch2 are adopted to execute Notch juxtacrine signaling in ocular surface epithelia, which is of paramount importance for meibocyte differentiation in Meibomian gland morphogenesis.

Acknowledgment: NIH/RO1 EY-21501, EY-23086

Meibomian Gland Absence Related Dry Eye in Ectodysplasin A Mutant Mice

Wei Li

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ABSTRACT

Meibomian gland dysfunction (MGD) is the most frequent cause of evaporative dry eye, yet its underlying pathophysiology is unknown. To gain insight into this pathophysiology, we characterized the time dependent tear film and ocular surface changes occurring in X-linked anhidrotic-hypohidrotic ectodermal dysplasia mice (Tabby), which are absence of meibomian gland. These mice sequentially developed corneal epithelial defects, central corneal stromal edema, neovascularization, and pannus 8 to 16 weeks after birth. Aqueous tear secretion was normal, while tear break-up time and ex vivo tear evaporation times were all shortened. Corneal epithelial microvilli were less numerous, conjunctival goblet cell density was unaffected, and MUC5AC and MUC5B gene expression was increased. Markers of squamous metaplasia (cytokeratin 10 and SPRR1B) were noticed in the corneal epithelium of Tabby mice as early as the 4th week. Taken together, the Tabby mouse is a relevant MGD related dry eye model that may lead to a better understanding how of meibomian glands are related to ocular surface health. It may also help with discovering novel drug options for treating evaporative dry eye.

Title: Innervation of the Meibomian Gland in Aging

Author: RW Beuerman
Singapore Eye Research Institute
Duke-NUS

Introduction: The specially modified sebaceous glands of the eye-lids have a critical role in supporting the visual function of the cornea. Appropriately the Meibomian Glands, MG, are implicated in dry eye disease, DED. As with other sebaceous glands their function changes with age. Neural sources complement the MGs although their function is not clear. RNA and protein levels for neuropeptide Y (NPY) receptor, vasoactive intestinal peptide (VIP) receptor, substance P (SP) receptor (also known as NK1 receptor) and muscarinic receptor (MR) subtypes m1–m5 in the mouse Meibomian glands. Therapeutic methods for MG secretions are not successful and a goal of this research is to understand how to increase MG function in DED.
Methods: Meibomian gland ductal and acinar cells were isolated from frozen sections of eyelids of 4-6week Balb/c mice using laser capture microdissection (LCM). Real-time PCR, immunofluorescent staining and western blot analyses for SP receptor, VIP receptor, NPY receptor and m1–m5 were performed on meibomian gland ductal and acinar cells. Results: Expression of NPY1 receptor, VIP receptor 1, SP receptor and all five MR subtypes was found in all meibomian gland ductal and acinar cells analyzed by immunofluorescent staining. M1 and SP receptor transcripts were undetectable in meibomian gland ductal cells by real-time PCR. Immunofluorescent staining and western blot analysis confirmed the presence of NPY1 receptor, VIP receptor 1, SP receptor and all five MR subtypes in the Meibomian gland. Conclusions: VIP receptor 1, SP receptor, NPY1 receptor and all five MR subtypes may mediate VIP, SP, NP and all five muscarinic acetylcholine subtypes action are involved in regulating synthesis of meibomian gland secretion. These data are considered to be the molecular basis for neuropeptide receptor targeting of dry eye diseases. Using this information the next step is to understand how the neural receptors affect function in aging.

LIME Awardee

Intense Pulsed Light therapy for Meibomian Gland Dysfunction

Kyoung Yul Seo

Department of Ophthalmology, Severance Hospital, Yonsei University College of Medicine, Seoul, South Korea.

Meibomian gland dysfunction (MGD) is a common cause of evaporative dry eye and is a prevalent condition, affecting more than 60% of the Asian population. Current treatment options include self-administered management of lid hygiene, meibum expression, lubricants, oral tetracycline derivatives, and anti-inflammatory therapy.

Intense pulsed light (IPL) has been widely used to treat dermatologic conditions such as telangiectasia, benign venous malformations, and pigmented lesions. Concurrent improvement of ocular surface conditions observed in patients treated for rosacea led to potential application of IPL for the treatment of MGD.

Multiple homogenously sculpted light pulses are delivered to the periocular skin inferior and lateral to the eye, and is repeated for a total of two passes on each side. After the IPL treatment, the meibomian glands are manually expressed. Most patients undergo four treatments of approximately 1 month interval.

Previous studies have reported favorable outcome on the therapeutic effect of IPL on MGD. A prospective paired eye study by Craig et al. found improvement in lipid layer grade, tear break-up time, and symptom scores in the treated eye. In this talk, I would like to review the latest updates on this emerging treatment modality for MGD, and share my experience with IPL.

Three Dimensional Meibography for Diagnosis of Dry Eye Associated with Meibomian Gland Dysfunction

Choun-Ki Joo, MD, PhD

Department of Ophthalmology and Visual Science, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, South Korea.

Determination of the changes of the acini in the meibomian glands is important for the diagnosis and management of Meibomian Gland Dysfunction (MGD) in the clinical field. Infrared (IR) meibography is commonly used in the clinical setting in various devices as a direct method for diagnosis, and enables the two-dimensional imaging of the meibomian glands. Using this technique, the definite abnormal structure of the meibomian glands, such as a dropout, can be confirmed; however, the detailed anatomic structure of the acini or ducts in the meibomian glands cannot be elucidated. Furthermore, two-dimensional IR imaging does not yield depth information, and may lead to varying results depending on the amount of light from the external source.

Optical Coherent Tomography (OCT) images provided the detailed information for acina and duct on dropout lesions at the meibomian glands than IR images. The loss of meibomian glands, as observed on IR imaging, should be carefully interpreted, and OCT images may be useful to confirm the anatomic details of meibomian glands.

LIME Awardee

Title:

Glandulae tarsales Meibom – the glands that make all the difference at the ocular surface

Authors: **Erich KNOP** (MD, PHD) **and NADJA KNOP** (MD, PHD)
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(***250 words***)

The Meibomian glands were first investigated and described in more detail by the German professor for medicine, history and poetry Heinrich Meibom in 1666 - 600 years after William the conqueror investigated Britain. Whereas the latter achievement of William had great influence on history, the simultaneous achievement of Heinrich was not so influential until recently. This is probably not due to the years Heinrich spent as a travelling scholar but rather due to a tradition to underestimate the importance of lipids for the ocular surface.
Since the TFOS Workshop Report on *M*eibomian *G*land *D*ysfunction (MGD) in 2011 (see www.tearfilm.org) it has become obvious that a quantitative or qualitative deficiency of Meibomian lipids due to mainly obstructive dysfunction of the glands represents the underlying causative factor in the vast majority of patients with dry eye disease. Anatomical, physiological and pathophysiological characteristics of the Meibomian glands such as their delicate structure, cell turnover, epithelial maturation and regulation that are related to their dysfunction will be explained and discussed. This enlightenment in ocular surface research and clinics has since then resulted in a change of paradigms for dry eye disease that had practically long been focused on a mere lack of water as seemed to be indicated by the apparently compelling logics of the term “dry eye”. Meanwhile we have achieved deeper insights into the anatomy, biology and pathophysiology of the MG and several new approaches, techniques and devices for diagnostics and therapy of MGD have been developed and introduced into clinical practice.

Visualization of invisible findings on meibomian glands using non-invasive meibography and tear interferometry

Reiko Arita

Itoh Clinic, LIME working group, Japan.

Meibomian gland secrets lipids (meibum) into the tear film and prevent excessive evaporation from the tearfilm. We developed “Non-invasive Meibography”, which enables us to observe meibomian gland without any invasive manner and discomfot sensation. Based on the obtained images of meibomian glands, the lost area of the meibomian gland was semi-quantitatively evaluated as meiboscore. Partial or complete loss of meibomian glands was scored for each eyelids from grade 0 to grade 3. Since we established the observation method of meibomian gland and the evaluation method, we investigated the alternation of the meibomian gland by aging, meibomian gland dysfunction, aqueous deficient dry eye, contact lens wear, allergic conjunctivitis or anti-glaucomatous eye drops use. Especially, we found that the decreased temperature of tarsal conjunctiva in patients with meibomian gland dysfunction was detected, where the area was coincided to the lesion of lost area of meibomian glands by meibography. In addition to the semi-quantitative evaluation for meibomian gland, we developed the automatic quantification program of meibomian gland area. This method enabled us to evaluate the efficacy of the treatment for meibomian gland dysfunction.

Tear interferometry has been applied as a noninvasive method for visualization of the lucent lipid layer at the surface of the tear film. Tear interference images associated with surface phenomena of the tear film have thus been obtained based on a principle first described by Newton. This approach has been adopted to study tear dynamics in individuals with Sjögren syndrome or dry eye, including MGD, as well as in contact lens wearers. Tear interferometry thus provides information on both the quality and quantity of the lipid layer, yielding insight into the function of the entire tear film. We investigated whether the tear interferometric pattern was able to identify differences in tear film kinetics among clinical subtypes of dry eye. Our results indicate that the interferometric color and fringe patterns associated with tear film stability reflect the balance between the aqueous and lipid layers of the tear film, and they suggest that these patterns are able to identify subtypes of dry eye.

In this talk, I am going to review the series of investigations in non-invasive meibography, and present the latest studies regarding the function of meibomian gland dysfunction using tear interferometry.

Longitudinal Changes of Meibomian Glands in Immune-mediated Dry Eye Disease

Kyung-Sun Na, MD, PhD

Department of Ophthalmology and Visual Science, Yeouido St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea.

Meibomian gland dysfunction (MGD) has been defined as a chronic, diffuse abnormality of the meibomian glands commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion. Risk factors hypothesized to correlated with MGD are aging, androgen deficiency, atopy, menopause, Sjogren's syndrome, hematopoietic stem cell transplantation, and contact lens wearhe proposed pathogenesis of MGD is that qualitative alterations in the composition of the meibum lead to hyperkeratinization of the ductal epithelium and increased viscosity of the meibum which result, either alone or in combination, in obstruction of the duct and orifice. At the same time, obstruction leads to a stasis of meibum inside the meibomian gland with increased pressure and resulting dilatation of the ducts and in atrophy of the acini with rarefaction of the secretory meibocytes and gland dropout. Currently, infrared meibography is available to image the actual dropout of the meibomian glands from upper and lower lids. Dropout areas, which appear as dark lesions on the images of the lid, are considered to missing or truncated meibomian glands.

Little is known regarding longitudinal changes of MGD changes in human subjects, because the changes are very slow to detect. Relative faster changes of meibomain gland and ocular surface in immune-mediated dry eye disease enable authors to show the morphological changes of meibomiand lands via infrared camera. The purpose of this talk is to analyze the longitudinal changes of meibomian gland morphology and tear dynamics changes in immune-mediated dry eye disease.

Effects of alkali burn of the ocular surface on meibomian gland structure

Shin Mizoguchi [1], Yuka Okada [1], Rika Shirakawa [2], Reiko Arita [3], Geraint J Parfitt [4] Yilu Xie [4], James V Jester [4], Shizuya Saika [1]

[1]Ophthalmology, Wakayama Medical University, Wakayama, Japan, [2]Ophthalmology, Tokyo University, Tokyo, Japan, [3]Itoh Clinic, Saitama, Japan, [4]Gavin Herbert Eye Institute, University of California, Irvine, CA, USA

Abstract

Purpose: To examine effects of alkali injury of the ocular surface on Meibomian gland pathology in mice. We observed abnormal meibomian gland structure in patients following ocular surfcae alkali burn. However, detailed analysis on the effects of ocular burn on meibimian glands remain to be investigated.

Merhods: Three μL of 1 N NaOH were applied under general anesthesia to the right eye of 10 week-old BALB/c (n = 44) mice to produce a total ocular surface alkali burn. The meibomian gland morphology was examined at each stage (day 1, 2, 5, 10, and 20) by stereomicroscopy with light emitting diode. Mice were then killed and eyelid was processed for histology with Hematoxilin-Eosin (HE). Serial paraffin sections were stained with DAPI. Localization of nuclei was captured and 3D image was reconstructed by 3D reconstruction software amira®. Another sets of specimens were processed for cryosectioning and Oil red O staining or immunohistochemistry for PPARγ, and for paraffin sections for immunohistochemistry for MPO, or ELOVL4, as well as TUNEL staining.

Results: Post-alkali burn deletion or marked dilation of meibomian gland duct was well observed. Oil red O staining showed the substance in the dilated duct was meibum. Such finding was more marked in the glands of the superior eyelid, while the loss of gland acini was predominant in the lower eyelid. PPARγ staining was markedly reduced in alkali-burned tissue as compared with an uninjured meibomian gland, although they were labeled for ELOVL4. Conjunctiva and Meibomian gland are labeled for MPO and TUNEL at day 1.

Conclusions: The meibomian glands were damaged after alkali burn besides ocular surface destruction. Post-alkali burn wound healing of the ocular surface might be further affected by dysfunction of meibomian glands.

Role of EGF Receptor Signaling on Morphogenesis and Maintenance of the Eyelid during Development

Fei Dong[1], Mindy Call[1], Ying Xia[1,2] and Winston W-Y Kao[1]

Departments of Ophthalmology[1] and Environmental Health[2], College of Medicine University of Cincinnati, Cincinnati, Ohio, USA

Abstract :

The epidermal growth factor receptor (EGFR) signaling pathway has a pivotal role in regulating morphogenesis during development and maintaining homeostasis in the adult eyelid and its Adnexa. During embryogenesis, targeted disruption of the EGFR signaling pathway results in a failure of prenatal eyelid fusion, which leads to malformation of the eyelids and its Adnexa, e.g., lacrimal glands, Meibomian glands, conjunctiva and orbicularis muscles, etc. Interestingly, unnatural activation of the EGFR signaling pathway either in the epidermis or in the eyelid stroma also results in eyelid anomalies such as precocious postnatal eye opening and Meibomian gland malformation. Studies have demonstrated that the EGFR signaling pathway is responsible for epithelial cell migration and actin stress fiber formation at the eyelid tip regulated by JNK and c-Jun. At the same time EGFR signaling also plays an important role in eyelid mesenchyme development and therefore regulates eyelid and meibomian gland morphogenesis through epithelial-mesenchymal interactions.

Posters

Poster 1

Change in the tear film lipid layer thickness after 3% diquafosol ophthalmic solution instillation in normal human eyes

Shima Fukuoka[1,3,4], Reiko Arita[2-4]

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Abstract

Purpose: Diquafosol is a $P2Y_2$ purinergic receptor agonist, and increased meibomian gland area by repeated application for months in MGD patients. However, little has been shown about the ability of diquafosol to increase lipid production in healthy human. The purpose of this study was to compare the efficacy of 3% diquafosol ophthalmic solution (DQS) with artificial tear (AT) on tear film lipid layer thickness (LLT) in normal human eyes.

Methods: One hundred eyes of 50 normal subjects (mean age 42.0 y-o) randomly received a drop of DQS in one eye and AT in the other. LLT of each eye was quantified by the tear interferometry before (pre) and 15, 30, 60 min after instillation. Change in meibomian gland area (meiboscore) were evaluated.

Results: Significant increases were seen in LLT 15, 30, 60 min after DQS instillation compared with pre LLT ($p < 0.0003$, <0.0003, $=0.003$, respectively, Wilcoxon signed-rank test, Bonferroni correction). AT did not increase LLT after instillation. Spearman's rank correlation coefficient revealed that maximum difference between pre LLT and LLT after DQS instillation as well as meiboscore of lower eyelids were correlated with pre LLT (ρ=0.69 and -0.33, $p<0.0001$ and 0.019, respectively). Age, temperature and relative humidity of the room was not correlated with LLT values.
Conclusion: Topical instillation of DQS increased LLT in normal human eyes.

Poster 2

MMP (matrix metalloproteinase)-9 expressions in patients with obstructive meibomian gland dysfunction using InflammaDry® - Multicenter study -

Tohru Sakimoto[1,2], Naoyuki Morishige[1,3], Reiko Arita[1,4]

1) Lid and Meibomian Gland Working Group, Japan.
2) Nihon University, Tokyo, Japan.
3) Yamaguchi University, Ymaguchi, Japan.
4) Itoh Clinic, Japan.

Purpose: Recently, MMP (matrix metalloproteinase)-9 detection kit (InflammaDry®, Rapid Pathogen Screening, Inc.) was approved as a diagnosis kit of dry eye disease in United States. We applied this kit for patients with obstructive meibomian gland dysfunction (MGD).
Methods: Twenty one eyes of 21 patients (3 men and 18 female) with obstructive MGD were studied. After dabbing the sample collector on lower palpebral conjunctiva, the test cassette was immersed to buffer vial. Positive result of MMP-9 was determined by the appearance of result line. We also examined slit-lamp findings, tear break-up time, Schirmer test, and meiboscore determined by non-invasive meibography.
Results: MMP-9 was positive in 15 eyes (15/21, 71.4%). Meiboscore had the tendency to be higher in MMP-9 positive group (positive group: 4.1±1.1, negative group: 2.8±1.1, p=0.06. There was no significant difference in other studied parameters.
Conclusions: It is well known that inflammation is related to the pathophysiology of obstructive MGD, and some reports indicated the MMP-9 expression in tears of patients with obstructive MGD. Our results were consistent with those previous reports, and the positive results of InflammaDry® have the possibility to be referred to anti-inflammatory therapies of MGD.

Poster 3

Evaluation for the morphology and function of Meibomian gland and the tear film parameters in Junior high school students

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Purpose: To evaluate the morphological changes, function of meibomian glands and tear film parameters in Junior high school students (15 years old).
Methods: One hundred eleven students in 15 years old (56 men, 55 women) were recruited at physical examination at Junior high school. Examinations were performed sequentially as follows. 1) ocular symptom score (0-14). 2) questionnaires regarding study and visual display terminals (VDTs) (study hour). 3) lid margin abnormality score (0-4). 4) conjunctival and corneal staining score (0-9)(fluorescein score). 5) tear film break-up time (TBUT). 6) meibosucore (0-6) using non-invasive meibograohy. 7) meibum score (0-3). 8) lipid layer thickness (LLT) using LipiView (TearScience Inc, Morrisvill, NC). 9) tear-film production by Schirmer's test without anesthetic. The correlations among the parameters were calculated using Pearson correlation coefficient analysis.
Results: The mean values (standard deviation) were ocular symptom score; 3.7(2.3), lid margin score; 0.1(0.3), fluorescein score; 1.1(1.4), TBUT; 8.6(7.2), meiboscore; 2.8(1.2), meibum score; 1.8(1.2) and Schirmer's test; 20.2(11.5). There was a significant correlation between study hour and meibum score (r=0.20, p=0.038), study hour and symptom score (r=0.33, p=0.0004), meibosocore and meibum score (r=0.30, p=0.0015), and meiboscore and LLT (0.27,p=0.004). Also, there was a significant correlation between TBUT and meibum score (r=-0.33, p=0.0004), and TBUT and LLT (r=0.32,p=0.0005).
Conclusion: Our data indicated that changes of morphology and function of meibomian glands might be developed even in 15 years old.

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Poster 4

The non-contact meibography findings of 2 cases of sebaceous adenoma of eyelid

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【Purpose】Sebaceous adenoma of eyelid is benign tumor arising from meibomian gland. There is no report of meibography findings of sebaceous adenoma. The purpose of this study is to detect the characteristic meibography findings of sebaceous adenoma.
【Cases】(Case 1) Fifty seven years old woman. She had been suffered from the tumor in her left lower eyelid slowly getting larger in 4 years. The size of tumor was 3mm, and its color was white at the first administration. The tumor showed low reflection with shaggy appearance in high reflection area on meibography. The tumor was surgically removed, and the histopathological diagnosis was sebaceous adenoma. (Case 2) Seventy eight years old woman. She had been suffered from the tumor in her right lower eyelid gradually getting larger in 4 years. The size of the tumor was 4mm, and its color was white at the first administration. The tumor showed low reflection with shaggy appearance in high reflection area on meibography. The tumor was surgically removed, and the histopathological diagnosis was sebaceous adenoma.
【Conclusion】Sebaceous adenoma shows low reflection with shaggy appearance in high reflection area on meibography.

Poster 5

Impairment of eyelid morphogenesis in Spinster2-null mice, in relation to reduction in sphingosine-1-phosphate level; A preliminary study

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Abstract

Introduction: Sphingosine-1-phosphate (S1P) is metabolite of sphingolipid with a multi-faceted biological activity. It is closely involved in inflammation and cancer as a lipid mediator that act on various cells. A mouse line that lacks either of the receptors, S1P2 or S1P3, does not exhibit eyelid abnormalities in my Lab. However, corneal opacity by blepharoplasty failure was reported in a mouse line with double knockout of two receptors, SIP2 and S1P3. The report promoted us to hypothesise that the tissue level of S1P might modulate eyelid morphogenesis. We therefore investigate the eyelid morphogenesis of the mouse line that lacks Spinster 2 (Spns2), a transporter of S1P. Spns2 pumps out S1P from inside the cells.

Methods: Both appearance and histology were employed. Each of the mice was sacrificed after intraperitoneal anesthesia using a Somnopentyl . We examined the eye and eyelid by histology and immunohistochemistry

Results: Hematoxylin-Eosin (HE) staining as well as immunohistochemistry for ELOVEL4 and PPAR gamma showed no meibomian glands of Spns2- KO mouse as compared with a WT mouse.

Conclusions: Gene ablation of Spns2 impairs eyelid morphogenesis and leads to the loss of meibomian gland.

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