The Role of Decorin in Corneal Fibrotic Pathways

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Purpose: The ocular surface (OcS) environment has evolved to limit inflammation in order to maintain optical clarity through mechanisms delivered by a highly specialised and rapidly self-renewing ocular mucosal barrier (conjunctiva, corneoscleral limbus, cornea). The OcS is vulnerable to infections which can be devastating leading to rapid loss of sight. Corneal scarring is a leading cause of preventable worldwide blindness. The mechanisms underpinning corneal fibrosis are complex but are linked to the Transforming growth factor-β (TGF-β) and induction of toll-like receptors (TLRs). Decorin, a small leucine rich repeat protein, has been shown to attenuate the activity of TGF-β in various systemic fibrotic processes. In this study we examined the effect of decorin in suppressing TGF-β/TLR mediated pro-fibrotic activity in human corneal cells.

Methods: Primary cultures of human corneal fibroblasts (PHCF) were incubated with TGF-β1/2, TLR3/4, decorin and/or dexamethasone to investigate cellular migration using scratch assays, myofibroblastic (α-SMA) transformation using immunofluorescence and collagen production using Sirius-red assay. Whole human tearfilm decorin was quantified by ELISA.

Results: TGF-β1/2 significantly increased PHCF migration (p<0.001), myofibroblast transformation (p<0.01) and collagen production (p<0.01), while TLR3 stimulation reduced collagen production (p<0.05). Decorin significantly inhibited TGF-β1/2 mediated cellular migration (p<0.001), collagen production and myofibroblastic (α-SMA) transformation. Dexamethasone antagonised the inhibitory effects of decorin in respect to collagen production and myofibroblast transformation, however this effect did not reach statistical significance. Decorin was detected in healthy human tears (1.62±0.18 ng/ml, mean n=3, range 2.57 - 0.54 ng/ml).

Conclusions: This study has shown the presence of decorin in human tears and is capable of inhibiting multiple TGF-β1/2 mediated pro-fibrotic processes. Decorin may have a protective anti-scarring role in physiological states and could provide a novel therapeutic modality to attenuate scarring during wound healing. Further work is required to characterise the interaction between glucocorticoid receptor agonists and the use of decorin.

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**Cholinergic Stimulation Modulates Conjunctival GC Antigen Passages**

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**Purpose:** This study evaluated effects of cholinergic stimulation on mucin binding and antigen migration through conjunctival goblet cells (GCs).

**Methods:** Experiments were performed without and with pre-topical stimulation of mucin secretion by the cholinergic agonist carbachol in female B6 mice. OVA Alexafluor-594 (45kDA) or labeled Dextran (10kDA or 70kDA) was applied topically and cornea and fornix conjunctiva were harvested after 30 minutes and stained for either MUC2 or MUC5AC with an immunofluorescent technique. Whole epithelial and stroma thickness digital confocal images were captured with a laser scanning confocal microscope using the Z-stack option to evaluate OVA and Dextran distribution on the ocular surface and stroma and mucin binding.

**Results:** During homeostatic conditions both antigens diffused into the stroma, but the pattern was different. Dextran was found in clusters while OVA was diffusely distributed. Goblet cells mucins bound topically applied antigens and goblet cells provided conduits for antigen passage into the stroma (Figure). There was greater migration of 10kDA dextran through goblet cells than 70kDA dextran. Pre-cholinergic stimulation with carbachol 20 minutes prior to topically applying antigens increased mucin binding on the ocular surface and decreased diffusion into the stroma at 30 minutes.

**Conclusions:** This study confirmed that goblet cell mucins bind topically applied antigens and goblet cells serve as conduits for antigen passage to the conjunctival stroma. Cholinergic stimulation results in greater antigen trapping by mucins on the ocular surface and limits diffusion into the stroma.

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**PTGER3 SNPs associated with Cold Medicine–Related Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis with Severe Ocular complications**

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**Purpose:** Stevens-Johnson syndrome (SJS) and its severe form, toxic epidermal necrolysis (TEN), are acute inflammatory vesiculobullous reactions of the skin and mucous membranes including the ocular surface, oral cavity, and genitals. These reactions are very rare but are often associated with inciting drugs and infectious agents. We previously reported that a genome-wide association study showed associations between six SNPs in the prostaglandin E receptor 3 (EP3) gene (PTGER3) and SJS/TEN with severe ocular complications (SOC). Moreover, we analyzed totally 38 SNPs of PTGER3 and found 20 SNPs of PTGER3 associated with SJS/TEN with SOC. We also reported that about 80% of our SJS/TEN patients had taken cold medicines such as multi-ingredient cold medications and NSAIDs within several days before disease onset; they were classified as
CM-SJS/TEN patients. In this study, we focused on CM-SJS/TEN with SOC, and analyzed the PTGER3 SNPs.

Methods: We analyzed the 18 SNPs for which we could get functional TaqMan probes of 20 PTGER3 SNPs, which we reported to be associated with SJS/TEN with SOC, using Japanese 132 CM-SJS/TEN with SMI cases and 218 healthy controls by TaqMan SNP genotyping assay.

Results: In Japanese, 7 of the 18 SNPs were significantly associated with CM-SJS/TEN with SOC after Bonferroni correction. Especially, one PTGER3 SNP was strongly associated with CM-SJS/TEN with SOC (rs13277464 (G vs A), OR = 0.23, p = 7.9 x 10^{-10}).

Conclusions: PTGER3 gene polymorphisms might be one of the predisposition to CM-SJS/TEN with SOC.

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Quantification of 16S Bacteria and Torque Teno Virus in Dry Eye Syndrome and Clinical Correlations

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Purpose: Dry eye syndrome (DES) causes significant ocular morbidity world-wide. The role of the ocular surface microbiome in DES has not been established. This study investigated the 16S-bacterial and viral load of torque teno virus (TTV) in patients with DES and their clinical correlations.

Methods: Patients with clinical diagnosis of DES and controls were recruited from the University of Miami and Miami Veterans Affairs Healthcare. All underwent full exam including corneal fluorescein staining, Schirmer, tear break-up time (TBUT), and ocular surface disease index (OSDI) questionnaire. Conjunctival swabs were analyzed by qualitative and quantitative PCR. Analyses were performed with Wilcoxon signed rank and Spearman correlation tests. Multivariate regression and logistic regression models were used to analyze the contribution of each variable to the total 16S load and the status of DES.

Results: A total of 71 eyes of 37 patients (22 with DES and 15 controls) were included. Mean age was 63.6 in the DES group and 47.7 in the control group (p-value 0.0005). There were 20 males (91%) in the DES group and 12 (80%) in the control group. Between eye correlation was measured using the intraclass correlation coefficient (ICC=0.520). Median level of 16S bacteria (normalized to human actin copies) were 0.034 in the DES patients and 0.120 in the controls (p-value 0.0005). There was a negative correlation between the total 16S bacteria and the OSDI score (rho -0.526, p-value=0.0001). Similar negative correlation was observed between the total 16S bacterial load and the staining score (rho -0.373, p-value 0.003). Both Schirmer score and TBUT positively correlated with 16S load (rho 0.464, p-value 0.0002 and rho 0.402, p-value 0.002, respectively). The multivariate regression model that included age, OSDI, TBUT, corneal stain, and Schirmer was significant (p-value 0.001, adjusted R^2=0.28) in predicting total 16S. The most significant variable was the OSDI. The logistic regression model that included age, gender, TTV, and 16S showed that both TTV and 16 loads negatively correlated with DES status (OR: 0.025, 0.002, respectively).

Conclusions: This study highlights the disruption of the normal ocular surface microbiome and potentially infectious role in DES. Decreased level of 16S bacterial load and TTV correlated significantly with DES. Whether this relationship is correlative or causative remains to be determined.

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PlexinD1 is required for Vascular Patterning in the pericellular region and Establishment of Corneal Avascularity

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Purpose: Signaling of the plexinD1 receptor and Semaphorin3 (sema3) ligands underlies various neurovascular-patterning events during embryonic development. Previously we showed that PlexinD1 mRNA is expressed by angioblasts and blood vessels during ocular vasculogenesis in patterns that suggest its involvement with Sema3 ligands that are concurrently expressed in the anterior eye. In this study, we determine the role of PlexinD1 during vascular patterning in the anterior eye.

Methods: RNA and protein analysis were used to establish late-stage vascular patterning events in transgenic Tie1:H2B-eYFP quail embryos. Gene knock down using viral constructs containing mCherry reporter and either scrambled shRNA or PlexinD1-shRNA was used to study PlexinD1 function. Vascular patterning was analyzed by immunohistochemistry in whole-mount or sectioned eyes.

Results: During late stages of ocular development, YFP-positive vasculature formed in the pericorneal region and iris. All the blood vessels in the anterior eye showed robust expression of PlexinD1 mRNA. Eyes expressing mCherry-PlexinD1-shRNA exhibited two phenotypes by embryonic day (E)12. First, ectopic vascularization was observed in the periphery of the cornea, which is never vascularized during development. Neovascularization of the cornea coincided with the formation of the limbal vasculature in control embryos. Neither phenotype was observed in control embryos expressing scrambled-shRNA.

Conclusions: The expression of PlexinD1 by blood vessels during ocular development and the defects resulting from its knock down indicate that it plays a critical role during vascular patterning in the limbus and iris, and it is also essential for corneal avascularity. PlexinD1 may be involved in vascular response to anti-angiogenic Sema3 signaling that guide the iris and limbal blood vessels. Therefore PlexinD1 may provide a target for treatment of neovascularization of the cornea or iris and other vascular defects such as hemorrhaging or hyphema of the anterior chamber.

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