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厦门大学

博 士 学 位 论 文

睑板腺功能障碍引起的眼表病变及其分子机制研究

The pathophysiology of meibomian gland dysfunction related ocular surface diseases and the molecular mechanism

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摘 要

第一部分：睑板腺功能障碍诱导的小鼠干眼动物模型的建立

目的：睑板腺功能障碍（MGD）是引起蒸发过强型干眼最常见的病因，但是 MGD 引起的眼表面病变的病理生理过程目前还知之甚少。本研究的主要目的是建立蒸发过强型干眼的动物模型，并观察该类型干眼的眼表病理变化过程。

方法：用裂隙灯显微镜观察外异蛋白基因（Ectodysplasin A , EDA)突变鼠（Tabby 鼠）的眼表病变过程，用荧光素钠染色方法检测 Tabby 鼠的角膜上皮缺损状况，用酚红棉线检测小鼠的水性泪液分泌，对角膜和眼睑进行 H&E 染色和油红染色。用扫描电镜观察眼表面上皮细胞的微绒毛变化。用蛋白质电泳（Western blot）和/或实时定量 PCR（qRT-PCR）方法检测眼表上皮 K12、K10、SPRR1B、MUC5AC、MUC1、MUC4、MUC13 和 MUC15 等基因或蛋白的表达情况。

结果：8 周龄至 16 周龄 Tabby 鼠的眼表相继出现角膜上皮缺损，角膜基质混浊，角膜新生血管长入以及角膜血管翳形成等病变。Tabby 鼠的水性泪液分泌没有减少，但体外泪液蒸发时间显著变短。Tabby 鼠角膜上皮细胞表面微绒毛明显减少、缩短。Tabby 鼠的结膜杯状细胞分布密度没有变化，但其 MUC5AC 和 MUC5B 表达显著增加。Tabby 鼠角膜上皮 K10 和 SPRR1B 的表达于出生后第 4 周开始上升，并随时间逐渐增强，这说明 Tabby 鼠角膜出现鳞状上皮化生。

结论：Tabby 鼠的眼表病变与蒸发过强型干眼的眼表病变相似，可以作为睑板腺功能障碍诱导的干眼小鼠动物模型。对 Tabby 鼠眼表病变的研究将有助于我们更深入地了解睑板腺在维持眼表健康中的作用，为今后开发筛选新药物治疗这类疾病提供帮助。

关键词：睑板腺功能障碍；干眼；外异蛋白

第二部分：外异蛋白（Eda）促进角膜上皮细胞增殖

目的：EDA 基因编码 Ectodysplasin A (Eda)蛋白，该基因突变会导致 X 染色体连锁外胚层发育不良综合征(X-linked anhidrotic-hypohidrotic ectodermal dysplasia, XLHED)。XLHED 患者可出现睑板腺发育障碍，但 Eda 蛋白在眼表的作用并不清楚。

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方法：裂隙灯显微镜下观察 EDA 基因突变鼠（Tabby 鼠）的眼表病变。采用 ELISA 方法检测人泪液、血浆中的 Eda 浓度。用蛋白质电泳（Western blot）和/或免疫荧光（IF）以及实时定量 PCR（qRT-PCR）检测 Eda、Eda 受体 Edar、Ki67、EGFR、磷酸化 EGF（p-EGFR）、磷酸化 ERK1/2（p-ERK）的表达。用划痕实验检测 Eda 蛋白对人角膜上皮细胞系 HCE 细胞的迁移作用，用 CCK8 试剂检测 Eda 蛋白对 HCE 细胞的促增殖作用。

结果：Eda 蛋白高表达于小鼠睑板腺腺泡上，人泪液中含有 Eda 蛋白，但血浆中未检测出 Eda 蛋白。在 4-8 周龄 Tabby 鼠，角膜上皮存在缺损，角膜上皮厚度较薄。Tabby 鼠角膜上皮的增殖细胞较少，角膜上皮损伤愈合速度较慢，在体外培养眼球中发现 Eda 蛋白促进角膜上皮损伤愈合。Tabby 鼠角膜上皮 EGFR、p-EGFR、p-ERK1/2 的表达水平较同龄野生型小鼠低，体外细胞培养显示 Eda 蛋白促进 HCE 细胞 Ki67、EGFR、p-EGFR 和 p-ERK 1/2 的表达，且变化趋势与 Eda 蛋白浓度正相关。

结论：睑板腺分泌 Eda 蛋白进入泪液，并促进角膜上皮细胞增殖。Eda 重组蛋白有望在临床上用于治疗角膜上皮损伤。

关键词：外异蛋白；细胞增殖；角膜上皮

第三部分：外异蛋白（Eda）通过激活 SHH 信号通路调控上皮屏障

目的：探讨 Eda 蛋白对角膜和皮肤上皮屏障功能的作用。

方法：裂隙灯显微镜下用荧光素钠染色观察 Tabby 鼠的眼表病变。用羧基荧光素（carboxy fluorescein）做角膜渗透性实验，用蛋白质电泳（Western blot）和/或实时定量 PCR（qRT-PCR），以及免疫荧光（IF）方法检测角膜、皮肤，以及人角膜上皮细胞系 HCE 细胞的 ZO-1、Claudin-1、Gli-1、Shh 的表达。用 LB 固体培养基进行角膜组织匀浆的细菌培养。

结果：饲养在普通级环境的 Tabby 鼠的角膜血管翳形成和尾部皮肤溃疡的发病率高于 SPF 级饲养环境。Tabby 鼠的角膜和皮肤上皮紧密连接蛋白 ZO-1 和 Claudin-1 表达水平明显低于同龄野生型小鼠。Eda 蛋白可以显著改善 Tabby 鼠的角膜上皮屏障破坏。体外皮肤培养以及 HCE 细胞培养实验证实 Eda 蛋白可以通过激活 Shh 信号通路来调节 ZO-1 和 Claudin-1 的表达。

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结论：Eda 蛋白调控表皮细胞间紧密连接的形成，对表皮屏障至关重要。我们的发现不仅揭示了 EDA 基因新的功能，也对 XLHED 相关疾病的发病机制提供新的视角。

关键词：外异蛋白；上皮屏障；紧密连接

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Abstract

Part I. A mouse dry eye model induced by meibomian gland dysfunction

Purpose: Meibomian gland dysfunction (MGD) is the most frequent cause of evaporative dry eye. However, its underlying pathophysiology is largely unknown. Our objective was to gain insight into the ocular surface change induced by MGD.

Methods: Ocular surface changes of Ectodysplasin A（EDA） gene mutant Tabby mice were observed under a slit-lamp microscope and corneal epithelia defect was evaluated by sodium fluorescein staining. Corneal sections and eyelids were stained by H&E and oil red. Aqueous tear secretion was measured with phenol red thread tear secretion test. The corneal surface epithelial microvilli change was observed under scanning electron microscope (SEM). K12, K10, SPRR1B, MUC5AC, MUC1, MUC4，MUC13, MUC15 expression were evaluated by immunostaining and/or quantitative real-time PCR (qRT-PCR).

Results: Tabby 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 ex vivo tear evaporation time were shortened. Corneal epithelial microvilli were less numerous whereas conjunctival goblet cell density was unaffected, but MUC5AC and MUC5B gene expression instead increased. Cytokeratin 10 and 12, SPRR1B gene expression was identified in the corneal epithelium of Tabby mice as early as the 4th week, which is indicative of squamous metaplasia.

Conclusions: Tabby mice are a relevant model of MGD related dry eye. They may lead to better delineate the function of meibomian gland in maintaining ocular surface health, and to identify novel drug options to treat evaporative dry eye disease.

Keywords: meibomian gland dysfunction; dry eye; Ectodysplasin A

Part II. Ectodysplasin A promotes corneal epithelial cell prolifertion

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Purpose: The EDA gene encodes ectodysplasin A (Eda) which if mutated causes X-linked Hypohidrotic Ectodermal Dysplasia (XLHED) disease in humans. Meibomian gland absent in XLHED patients. Our objective was to identify the effect of Eda protein on the corneal epithelium.

Methods: Ocular surface changes of EDA gene mutant Tabby mice were observed under a slit-lamp microscope. ELISA measured Eda concentration in human serum and tears. Eda, Eda receptor (Edar), Ki67, epidermal growth factor receptor (EGFR), phosphorylated EGFR (p-EGFR), and phosphorylated ERK1/2 (p-ERK) expression were evaluated by immunostaining and/or quantitative real-time PCR (qRT-PCR) and/or Western blot analysis. Eda effects on human corneal epithelial cell line (HCE) proliferation and migration were determined using the cell counting kit-8 (CCK8) assay and scratch wound healing assay, respectively.

Results: Eda was highly expressed in meibomian glands, and it was detected in human tears but not serum. Corneal epithelial integrity was defective and the thickness was reduced in the early postnatal stage of Tabby mice. Corneal epithelial cell proliferation decreased and the epithelial wound healing was delayed in Tabby mice. Eda exposure promoted corneal epithelial wound healing during organ culture. EGFR, p-EGFR and p-ERK were down-regulated in Tabby mice corneal epithelium. Eda treatment up-regulated the expression of Ki67, EGFR, p-EGFR and p-ERK in HCE cells in a dose dependent manner.

Conclusions: Eda protein can be secreted from meibomian glands and promotes corneal epithelial cell proliferation. Recombinant Eda may be used in the treatment of corneal epithelial wounding in a clinical setting.

Keywords: Ectodysplasin A; cell proliferation; corneal epithelium

Part III. Ectodysplasin A regulates epithelial barrier function though activating SHH signaling pathway

Purpose: To investigate the role of Ectodysplasin A（EDA）on the skin and corneal epithelial barrier function.

Methods: The ocular surface of Tabby mice was observed under slit lamp microscope with fluorescein staining. Corneal epithelial barrier function was evaluated with the permeability to carboxy fluorescein. ZO-1, Claudin-1, Gli-1 and Shh expression in the corneal and skin epithelia and HCE cells were performed by

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immunostaining and/or Western blot and/or quantitative real-time PCR assay. The cornea bacterial infection was examed by LB solid medium.

Results: Compromise of tight junction is associated with corneal pannus and tail ulcer in Tabby mice raised in conventional facilities. Tight junction proteins ZO-1 and Claudin-1 were dramatically decreased in skin and corneal epithelia of the Tabby mice. Topical application of recombinant Eda protein could rescue corneal epithelial barrier dysfunction to a large extent in Tabby mice. Moreover, the ex-vivo skin tissue culture and corneal epithelial cell culture indicated that the EDA regulates tight junction formation through activation of sonic hedgehog signaling pathway.

Conclusions: EDA contributes to the barrier function of surface epithelial cells. Our findings revealed new function of EDA signal and shed new light on the pathophysiology and treatment of XLHED related diseases.

Keywords: Ectodysplasin A; epithelial barrier function; tight junction

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