

角膜上皮细胞代谢的研究进展

马佰凯 何昕 李炜

【摘要】角膜上皮层位于角膜表面,外邻泪膜,内与角膜前弹力层相连。角膜上皮细胞代谢所需营养及氧分主要通过泪膜、房水和角膜缘毛细血管运送。正常的角膜上皮细胞代谢是维持角膜上皮细胞正常增殖与分化状态的关键。角膜上皮细胞代谢异常可导致上皮损伤或变性,是多种角膜疾病的病理基础。本文就近年来关于角膜上皮细胞代谢相关的组织结构、营养来源、细胞增殖分化以及相关疾病的研究进展进行综述。

【关键词】角膜; 上皮细胞; 代谢

Research progress in the metabolism of corneal epithelial cells Ma Baikai, He Xin, Li Wei.

Medical College of Xiamen University, Xiamen 361102, China

Corresponding author: LI Wei, Email: wei1018@xmu.edu.cn

【Abstract】 Corneal epithelium is located in the surface layer of the cornea, with tear film outside and Bowman's layer inside. The nutrient and oxygen for the metabolism of corneal epithelial cells is transmitted by tear film, anterior aqueous humor and corneal limbal capillary. Metabolism of the corneal epithelial cells is tightly correlated with its proliferation and differentiation. The abnormal metabolism of corneal epithelial cells can cause epithelial damage or dystrophy, which is the pathological basis of many corneal diseases. This review summarizes recent research progress regarding corneal epithelial cell metabolism related tissue structure, origin of nutrient, cell proliferation and differentiation and diseases related to corneal epithelial cell metabolism.

【Key words】 Cornea; Epithelial cells; Metabolism

角膜上皮细胞位于角膜外表面,由4-5层非角化鳞状上皮细胞组成,在维持眼表稳态以及角膜透明度中发挥重要作用。正常的角膜上皮细胞代谢与其增殖分化有密切关系,很多疾病也与其代谢密切相关。本文总结角膜上皮细胞代谢的研究进展,旨在为角膜上皮代谢相关的研究提供参考与建议。

一、角膜上皮细胞的结构特点

角膜上皮的厚度约为55 μm,约占角膜总厚度的10%,包含三种细胞类型:鳞状细胞、翼状细胞和基底细胞。角膜上皮表面与泪膜相接触,表层上皮细胞表面具有微绒毛和微褶皱,已有研究表明微绒毛和微褶皱对角膜前泪膜的滞留、泪液内营养和代

谢物质的吸收和交换起重要作用^[1-2]。角膜基底层上皮细胞与基底膜、前弹力层的连接主要是借助于半桥粒等结构而实现的^[3-4],这些连接在维持角膜上皮的良好状态上起到了重要的作用^[5]。角膜上皮细胞之间通过高表达紧密连接蛋白 Claudin、ZO、Occludin 等参与构成角膜的屏障系统^[6-8]。它对溶质和大分子的运输也起重要的调节作用^[9],可通过水通道蛋白外排钠离子、氯离子以及水分子,从而维持角膜基质层的脱水状态,保持角膜透明性^[10]。结合角膜上皮无血管的特性,若能证明角膜上皮细胞间的紧密连接在物质扩散中起一定作用,将有助于我们深入了解角膜上皮细胞代谢的途径。相邻的上皮细胞间有10~20 μm的间隙,间隙中存留有由酸性黏多糖、蛋白质等形成的低电子密度物质,具有黏合作用,这类物质统称为桥粒结构。桥粒以闭锁小带的方式使两个上皮细胞相互黏连,桥粒结构中的一些蛋白如E-钙黏蛋白在信号通路中起调节作用^[11],但是这些连接蛋白及连接方式是否参与上皮细胞代谢的过程与调节尚不清楚。

DOI:10.3877/cma.j.issn.2095-1221.2014.01.011

基金项目:国家重大科学研究计划项目(2013CB967003);国家自然科学基金面上项目(81270977);国家卫生和计划生育委员会科研基金项目(WKJ-FJ-26);福建省高等学校新世纪优秀人才支持计划项目资助

作者单位:361102 厦门,厦门大学医学院(马佰凯,李炜); 皖南医学院(何昕);福建省眼科与视觉科学重点实验室 厦门大学眼科研究所(李炜)

通讯作者:李炜, Email:wei1018@xmu.edu.cn

二、角膜上皮细胞的营养来源与代谢方式

角膜的营养物质有三个来源:角膜周围毛细血管、泪液和前房水^[12]。周边部角膜的代谢主要依靠角膜缘血管网,中央部角膜的营养物质则通过角膜上皮细胞或内皮细胞进入角膜内。葡萄糖和糖原是上皮细胞的产能物质,葡萄糖的直接来源主要是房水,约10%是从角膜缘和泪膜扩散得来。角膜上皮细胞具有一定的糖原储备^[13],葡萄糖也可由糖原分解产生。从内皮层至上皮层,乳酸含量增多而葡萄糖与碳酸氢盐含量降低^[14]。已有证据说明兔角膜上皮细胞中线粒体含量较少^[15],兔角膜中糖代谢的主要方式是无氧糖酵解,占85%,剩余为有氧氧化^[16-17],由于有氧氧化释放的ATP很多,且不产生代谢废物,所以在角膜上皮细胞代谢中仍发挥重要作用。然而,人角膜上皮中糖酵解与有氧氧化的比例仍不清楚。糖酵解途径可以产生大量乳酸,正常泪膜中含有乳酸脱氢酶^[18],由此推测乳酸可能被转运至泪膜中进行分解,具体机制还有待进一步研究。

有研究发现角膜上皮选择性高表达脂肪氧化酶15^[19-20],提示脂类有可能作为角膜上皮细胞的能量来源。脂质通过脂肪动员形成甘油和脂肪酸,甘油可以通过糖异生转变为葡萄糖,脂肪酸可以通过 β -氧化产物进入三羧酸循环,如能证明角膜上皮细胞表达其中关键酶或相关产物,则可以证明这条能量来源途径的可行性。

三、角膜上皮细胞的氧气来源与代谢

大气供应角膜上皮有氧代谢所需的大部分氧气并且能抑制缺氧性肿胀^[14],角膜缘血管网和房水也能提供少量氧需求。睁眼时,氧气自角膜向房水内渗透,睡眠时,由结膜血管和房水提供氧气。正常代谢情况下,睁眼时中央角膜上皮氧分压约为105~155 mmHg,闭眼时其值约为22~61.5 mmHg^[14,21],角膜缘上皮细胞也有低水平的缺氧诱导因子-1(hypoxia inducible factor-1,HIF-1 α)的mRNA表达^[22],对缺氧情况下角膜上皮细胞的代谢有一定作用。正常情况下葡萄糖代谢过程中糖酵解尚且比有氧氧化占更大的比例,在夜晚闭眼或者佩戴接触镜的情况下,角膜上皮细胞缺氧会更严重,糖酵解所占据的比例更大,从而产生更多的乳酸,糖原也会大量消耗,屏障系统受损,角膜易出现炎症或感染^[23-24]。由于睡眠是间断性、生理性的,所以机体具有一定的调节机制,睁眼情况下得到改善,具体的生理机制还有待研究。长期佩戴接触镜可能使角膜上皮细胞始终处于缺氧状态,从而导致

各种缺氧并发症。

四、泪液在角膜上皮细胞代谢中的作用

1. 泪膜的组成:泪膜可分为三层,包括最表面的脂质层,中间的水性泪液层,以及最内层的黏液层。脂质层的厚度为20~160nm^[25],由蜡酯、胆固醇以及脂肪酯酸等构成,包括与外界空气接触的较厚的非极性脂肪层,和靠近水性泪液层较薄的极性脂肪层^[26]。水性泪液层主要包含泪腺及副泪腺分泌的水、蛋白质和电解质成分,其中水的含量占泪液总量的98%以上。泪液的蛋白质和电解质与血清均有差异,泪液中氯离子和钾离子浓度要高于血清,但葡萄糖浓度要低于血清。正常泪液渗透压为300~305 mOsm/l。正常泪液的蛋白质含量大约为6~10 mg/ml,总蛋白质种类超过1500种^[27]。黏液层由结膜杯状细胞以及结膜、角膜上皮细胞分泌,主要成分为黏蛋白、硫黏蛋白、cyalomucin等,现在发现的眼表面黏蛋白有7种,包括分泌型的黏蛋白2(mucin2,MUC2)、MUC5AC、MUC5B、MUC7和膜结合型的MUC1、MUC4、MUC16^[28-29]。

2. 泪膜的功能:泪膜的功能可归纳如下:(1)形成并维持角膜光滑的折射表面;(2)维持角膜和结膜上皮细胞的湿润环境;(3)有杀菌作用;(4)润滑眼睑;(5)在上皮层和实质浅层之间输送代谢产物(主要是氧和二氧化碳);(6)在损伤病理途径中提供白细胞通路;(7)稀释及清除有害刺激物,包括上皮碎屑、细菌、异物等。

3. 泪膜在角膜上皮细胞代谢中的作用:角膜上皮细胞与泪膜紧密接触。泪膜在角膜上皮细胞代谢中可能发挥的作用正逐渐引起人们的重视。泪膜中葡萄糖含量很少,但富含脂质,而且角膜上皮细胞具有脂性屏障,脂溶性和非极性物质易通过,表明角膜上皮细胞有可能从泪膜中摄取脂质来获得代谢所必需的营养来源。泪膜的脂质层还可以减少泪水的挥发,协助维持湿润的眼表,同时保护眼睛免受微生物或者其他异物如灰尘、花粉的伤害^[30],从而为正常的角膜上皮细胞代谢提供良好的微环境。

泪膜中其它成分在角膜上皮细胞代谢中也发挥重要作用。黏蛋白作为黏液层的主要成分,可以通过包裹外来物质发挥眼表防御作用^[31],高浓度的溶菌酶可溶解细菌细胞壁而保持眼表正常微环境^[32-33]。乳铁蛋白除抗菌外还可结合自由基从而抑制脂质过氧化^[34]。免疫球蛋白A构成宿主眼表的第一道防线。这些泪膜成分一起构成了角膜上皮细胞代谢的正常微环境。

当泪液生成减少或蒸发过量时会造成泪液的缺失,引起干眼。睑板腺功能障碍会造成泪膜的改变、脂酶分泌增加和细菌的繁殖,进而引起眼表的炎症反应或损伤。不稳定的泪膜本身也会引起干眼症状^[35]。当泪液发生功能障碍时,其组成成分的质与量发生改变,会影响角膜从泪液中汲取氧气及营养,同时也会引起角膜、结膜的炎性损伤。当泪液减少时,眼表面的乳酸脱氢酶可能减少,可导致乳酸清除障碍。至于干眼时角膜上皮细胞如何发生代谢异常进而影响上皮细胞的增殖与凋亡,破坏角膜上皮的完整性都还有待研究。

五、角膜上皮细胞代谢与细胞增殖、分化的关系

1. 正常情况下角膜上皮细胞的增殖与分化:成熟角膜上皮细胞由角膜缘基底部干细胞增殖分化而来^[36]。在正常情况下,角膜上皮干细胞表现较低的增殖状态,分裂时产生短暂扩充细胞(transient amplifying cell, TAC), TAC增殖数次后脱离基底膜,向上皮表面和角膜中央迁移并继续增殖,逐步分化为终末分化细胞,即成熟角膜上皮细胞,使其持续更新以维持生理功能^[37]。

2. 角膜上皮干细胞的代谢特点:角膜上皮干细胞具有某些独特的特征,包括较长的生存时间,强大的自我更新能力,细胞周期长, S 期持续时间短,无错增殖,不分化等^[38]。相比中央角膜,角膜缘上皮基底细胞中含有高浓度的代谢相关酶类,如细胞色素氧化酶^[39]、钠-钾-ATP 酶^[40]和碳酸酐酶^[41],而醛脱氢酶^[42]与酮基转移酶^[43]浓度则比中央角膜上皮小很多,参与糖酵解的 α -烯醇化酶在角膜缘上皮细胞含量也较多^[44],说明角膜缘上皮干细胞代谢更活跃。

3. 创伤情况下角膜上皮细胞的增殖、分化与代谢:角膜上皮细胞位于角膜最外层,最易受到伤害,当角膜上皮细胞受到物理、化学或生物损伤时,细胞代谢发生紊乱,为及时修复其机能,细胞启动自身修复机制。受损区周边上皮细胞参与其中,角膜上皮受损约1 h后,未受损区的上皮细胞扩大变平,伸出伪足,以阿米巴的形式迁移至受损区并增殖,迁移的能量来源于上皮细胞中储存的糖原^[45],并发生有丝分裂,大约6周后上皮与基底膜贴紧。毗邻受伤部位可见基质细胞凋亡,远离受伤部位则可见角膜基质细胞转变为激活的纤维母细胞并迁移至受伤部位^[46]。同时,角膜缘干细胞加速增殖分化为角膜上皮细胞。

细胞代谢中的中间物与酶在损伤修复中的作用历来为人们研究的焦点。Notch1 信号通过调控

维生素 A 代谢中的细胞视黄醇结合蛋白-1 (cellular retinol-binding protein1, CRBP1) 在角膜上皮细胞损伤修复的过程中起重要作用^[47], 维生素 D3 抑制绿脓假单胞菌入侵过程中白介素-1 β (IL-1 β)、IL-6 和 IL-8 炎症因子与趋化因子的表达^[48], 乳酸脱氢酶作为角膜上皮细胞无氧代谢中转化乳酸^[49-50]和提供能量^[45]的重要因子,同时被证明可以作为角膜上皮细胞分化的标志^[51]。在家兔体内还可以诱导12-脂氧酶(12-lipoxygenase, 12-LOX)并合成12(S)羟基二十碳四烯酸(12(S)-hydroxy-eicosatetraenoic acid, 12(S)-HETE)加快细胞增殖以修复损伤^[52]。醛脱氢酶3A1在角膜上皮细胞中高表达,通过其自身的氧化作用与延长细胞周期的功能可以保护角膜上皮细胞免受氧化性损伤^[53]。在离体培养眼球过程中,角膜受到划伤或碱侵蚀时,角膜上皮细胞中糖酵解途径增多,蛋白结合型还原型辅酶II (triphosphopyridine nucleotide, NADPH) 转变为游离 NADPH 增多^[54]。很多脂质在角膜上皮细胞损伤修复中起重要作用,如眼表炎症时,花生四烯酸、12[S]-HETE (12(S)-hydroxy-eicosatetraenoic acid)、15[S]-HETE (15(S)-hydroxy-eicosatetraenoic acid) 作为第二信使促进细胞增殖,一些脂酸合酶的衍生物可参与抑制炎症^[55],花生四烯酸还可以转化为前列腺素、白三烯和碳四烯酸抑制炎症损伤^[56]。

细胞代谢的调节因子也在修复损伤中发挥重要作用。促炎因子 IL-1 β 与肿瘤坏死因子- α (TNF- α) 上调基质金属蛋白酶9 (matrix metalloproteinase-9, MMP-9) 在角膜上皮细胞中的表达,对伤口的愈合与角膜基质的降解起重要作用^[57]。角膜损伤状态下高表达的热休克蛋白27(heat shock protein-27, HSP-27) 被证明是不同类型细胞中抗细胞凋亡的介导因子^[58-59]。乙酰胆碱可促进受损角膜上皮的修复^[60]。表皮生长因子可通过诱导活性氧生成^[61]和通过上调细胞周期蛋白D1(cyclinD1)和细胞周期蛋白依赖性激酶4 (cyclin-dependent kinase4, CDK4)、下调 p27^[62]促进角膜上皮细胞增殖和损伤修复。人类角膜上皮细胞储存、释放神经生长因子 (nerve growth factor, NGF), 在角膜受损后一过性升高,可加速愈合^[63]。肝细胞生长因子 (hepatocyte growth factor, HGF) 在伤口愈合中促进角膜上皮细胞的增殖与迁移^[64-65], 并可以保护细胞免受低氧诱导的细胞凋亡^[66-67]。胸腺素 β 4 通过降低中性粒细胞的浸润和炎症细胞因子、趋化因子的 mRNA 水平来促进角膜的损伤修复^[68]。

基膜聚糖在角膜上皮出现受损时可调节细胞的黏附、迁移,有助于角膜的损伤修复^[69]。透明质酸和白细胞分化抗原分化群 44 (cluster of differentiation 44,CD44) 相互支持促进角膜上皮细胞的迁移,从而促进受损角膜的修复^[70]。

六、角膜上皮细胞代谢相关性疾病

1. 角膜上皮病变对代谢的影响:角膜上皮细胞在遭受物理、化学或者生物方面的损伤时,会出现相应的结构改变、成分失调以及代谢紊乱,导致上皮细胞凋亡、坏死或异常分化。

一些角膜上皮变性性疾病也与上皮细胞代谢有关,如 Terrien 角膜边缘变性推测继发于营养障碍性疾病、泪液成分异常等情况,会造成角膜缘新生血管生成和脂质浸润^[71]。Meessmann 角膜营养不良和角膜上皮基底膜营养不良会造成角膜上皮的反复糜烂,形成角膜瘢痕,影响视力,这些细胞内有糖原染色阳性的不明沉积物存在^[72]。

2. 全身性代谢性疾病在眼部的表现:很多全身性疾病在眼部也有病变表现,如糖尿病会造成眼部的鳞状化生,杯状细胞的缺失,从而致使泪膜的质与量失调,造成新陈代谢紊乱并发生周围神经病变^[73]。糖尿病也会影响泪腺的功能,造成泪液产生减少^[74],引起干眼症。同时也会造成角膜水、离子物质的通透性增加,影响角膜的代谢^[75]。糖尿病患者泪膜脂质层的非均一性,角膜的敏感性,泪膜的破裂时间均遭到破坏,泪膜脂质层与糖尿病性角膜病有密切关系^[76]。角膜上皮表面的微绒毛、微皱褶也会随着病程逐渐减少^[77],进一步影响角膜上皮细胞代谢。

3. 角膜上皮细胞内环境改变引起的病变:角膜上皮所处内环境的变化也会引起相应病变,如高渗泪液被证实为干眼症中角膜上皮相应病变的起始因素之一^[78],同时可以通过谷氨酰基转移酶-2 (transglutaminase-2, TGM-2) 致线粒体损伤,导致细胞代谢紊乱^[79],缺氧环境下细菌性角膜炎的危险性提高^[80]。角膜上皮发生病变后会导致代谢异常,无法正常吸收利用营养物质,细胞内信号通路受损,增殖分化异常,生理功能损伤或丧失。

4. 佩戴接触镜对角膜上皮代谢的影响:长期佩戴接触镜会造成角膜低氧、高碳酸血症,会引起角膜上皮、基质、内皮的显著变化,这些变化也会随着睡眠周期而变化,上皮的变化包括代谢速率的下降,形态学的改变,出现滤泡,交界完整性改变,角膜知觉减退,血管翳形成等^[81]。

七、角膜上皮细胞代谢研究的展望

角膜作为眼表最重要的光学结构,历来为眼表研究的焦点。角膜上皮由于特殊的位置、结构及周边环境,其代谢具有不同于其它组织的特点。如泪膜脂质在角膜上皮细胞代谢中的作用,角膜上皮不同部位氧气代谢的特点,角膜上皮细胞代谢产物的排出机制等等,针对这些问题的研究将有助于揭示角膜上皮细胞增殖与分化的机制,有助于深入认识角膜上皮病变的病理生理过程。同时,眼部其他部位病变以及全身性疾病对角膜上皮细胞代谢的影响也受到越来越多的关注,也将是今后研究的热点。

参 考 文 献

- 1 Beuerman RW, Pedroza L. Ultrastructure of the human cornea[J]. *Microsc Res Tech*, 1996, 33(4):320-335.
- 2 Ojeda JL, Ventosa JA, Piedra S. The three-dimensional microanatomy of the rabbit and human cornea. A chemical and mechanical microdissection-SEM approach[J]. *J Anat*, 2001, 199(5):567-576.
- 3 Buck RC. Hemidesmosomes of normal and regenerating mouse corneal epithelium[J]. *Virchows Arch B Cell Pathol Incl Mol Pathol*, 1983, 42(1):1-16.
- 4 Gipson IK. Adhesive mechanisms of the corneal epithelium[J]. *Acta Ophthalmol Suppl*, 1992, 70(202):13-17.
- 5 Kaufman HE. The cornea[M]. Butterworth-Heinemann, 1988.
- 6 Wang Y, Chen M, Wolosin JM. ZO-1 In Corneal Epithelium; Stratal Distribution and Synthesis Induction by Outer Cell Removal[J]. *Exp Eye Res*, 1993, 57(3): 283-292.
- 7 Sugrue SP, Zieske JD. ZO1 in Corneal Epithelium: Association to the Zonula Occludens and Adherens Junctions[J]. *Exp Eye Res*, 1997, 64(1): 11-20.
- 8 Yi XJ, Wang Y, Yu FS. Corneal Epithelial Tight Junctions and Their Response to Lipopolysaccharide Challenge[J]. *Invest Ophthalmol Vis Sci*, 2000, 41(13): 4093-4100.
- 9 Fischbarg J. Mechanism of fluid transport across corneal endothelium and other epithelial layers: a possible explanation based on cyclic cell volume regulatory changes[J]. *Br J Ophthalmol*, 1997, 81(1):85-89.
- 10 Thiagarajah JR, Verkman A. Aquaporin deletion in mice reduces corneal water permeability and delays restoration of transparency after swelling[J]. *J Biol Chem* 2002, 277(21):19139-19144.

- 11 Delva E, Tucker DK, Kowalczyk AP. The Desmosome [J]. *Cold Spring Harb Perspect Biol*, 2009, 1(2):a002543.
- 12 李凤鸣. 中华眼科学 [M]. 北京: 人民卫生出版社, 2005: 232-233.
- 13 Thoft RA, Friend J. Biochemical transformation of regenerating ocular surface epithelium[J]. *Invest Ophthalmol Vis Sci*, 1977, 16(1):14-20.
- 14 Chhabra M, Prausnitz JM, Radke CJ. Modeling corneal metabolism and oxygen transport during contact lens wear[J]. *Optom Vis Sci*, 2009, 86(5):454-466.
- 15 Kaye GI, Pappas GD. Studies on the cornea I. The fine structure of the rabbit cornea and the uptake and transport of colloidal particles by the cornea in vivo[J]. *J Cell Biol*, 1962, 12(3):457-479.
- 16 Riley M. Glucose and oxygen utilization by the rabbit cornea[J]. *Exp Eye Res*, 1969, 8(2):193-200.
- 17 Freeman RD. Oxygen consumption by the component layers of the cornea [J]. *J Physiol*, 1972, 225(1):15-32.
- 18 Fleiszig SM, Kwong MS, Evans DJ. Modification of *Pseudomonas aeruginosa* interactions with corneal epithelial cells by human tear fluid[J]. *Infect Immun*, 2003, 71(7):3866-3874.
- 19 Liminga M, Hörnsten L, Sprecher HW, et al. Arachidonate 15-lipoxygenase in human corneal epithelium and 12- and 15-lipoxygenases in bovine corneal epithelium: Comparison with other bovine 12-lipoxygenase[J]. *Biochim Biophys Acta*, 1994, 1210(3):288-296.
- 20 Hurst J, Balazy M, Bazan H, et al. The epithelium, endothelium, and stroma of the rabbit cornea generate (12S)-hydroxyeicosatetraenoic acid as the main lipoxygenase metabolite in response to injury[J]. *J Biol Chem*, 1991, 266(11):6726-6730.
- 21 Leung B, Bonanno J, Radke C. Oxygen-deficient metabolism and corneal edema[J]. *Prog Retin Eye Res*, 2011, 30(6):471-492.
- 22 Li C, Yin T, Dong N, et al. Oxygen tension affects terminal differentiation of corneal limbal epithelial cells[J]. *J Cell Physiol*, 2011, 226(9):2429-2437.
- 23 Kimura K, Teranishi S, Kawamoto K, et al. Protection of human corneal epithelial cells from hypoxia-induced disruption of barrier function by hepatocyte growth factor[J]. *Exp Eye Res*, 2010, 90(2):337-343.
- 24 Yamamoto N, Yamamoto N, Jester J V, et al. Prolonged hypoxia induces lipid raft formation and increases *Pseudomonas* internalization in vivo after contact lens wear and lid closure[J]. *Eye Contact Lens*, 2006, 32(3):114-120.
- 25 King-Smith PE, Hinel EA, Nichols JJ. Application of a novel interferometric method to investigate the relation between lipid layer thickness and tear film thinning[J]. *Invest Ophthalmol Vis Sci*, 2010, 51(5):2418-2423.
- 26 Wolff E. *The Anatomy of the Eye and Orbit* [M]. New York and Toronto, 1954, 207.
- 27 Zhou L, Beuerman RW. Tear analysis in ocular surface diseases[J]. *Prog Retin Eye Res*, 2012, 31(6):527-550.
- 28 Yasueda S-i, Yamakawa K, Nakanishi Y, et al. Decreased mucin concentrations in tear fluids of contact lens wearers[J]. *J Pharm Biomed Anal*, 2005, 39(1):187-195.
- 29 Liu W, Li H, Lu D, et al. The tear fluid mucin 5AC change of primary angle-closure glaucoma patients after short-term medications and phacotrabeculectomy[J]. *Mol Vis*, 2010, 16:2342-2346.
- 30 Holly F, Lemp M. Tear physiology and dry eyes[J]. *Surv Ophthalmol*, 1977, 22(2):69-87.
- 31 Ohashi Y, Dogru M, Tsubota K. Laboratory findings in tear fluid analysis[J]. *Clin Chim Acta*, 2006, 369(1):17-28.
- 32 Milder B. *The lacrimal apparatus* [M]. *Adlers Physiology of the eye*, 8th ed. St. Louis: CV Mosby, 1987, 15-35.
- 33 Mackie I, Seal D. Quantitative tear lysozyme assay in units of activity per microlitre[J]. *Bri J Ophthalmol*, 1976, 60(1):70-74.
- 34 Gutteridge JM, Paterson SK, Segal AW, et al. Inhibition of lipid peroxidation by the iron-binding protein lactoferrin[J]. *Biochem J*, 1981, 199(1):259-261.
- 35 Shimazaki-Den S, Dogru M, Higa K, et al. Symptoms, visual function, and mucin expression of eyes with tear film instability[J]. *Cornea*, 2013, 32(9):1211-1218.
- 36 Cotsarelis G, Cheng S-Z, Dong G, et al. Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells[J]. *Cell*, 1989, 57(2):201-209.
- 37 Li W, Hayashida Y, Chen YT, et al. Niche regulation of corneal epithelial stem cells at the limbus[J]. *Cell Res*, 2007, 17(1):26-36.
- 38 Dua HS, Azuara-Blanco A. Limbal stem cells of the corneal epithelium[J]. *Surv Ophthalmol*, 2000, 44(5):415-425.
- 39 Hayashi K, Kenyon KR. Increased cytochrome oxidase activity in alkali-burned corneas[J]. *Curr Eye Res*, 1988, 7(2):131-138.
- 40 Lütjen-Drecoll E, Steuhl P, Arnold W. Morphologische Besonderheiten der conjunctiva bulbi [M]. *Chronische Conjunctivitis—Trockenes Auge*, Springer, 1982:25-34.
- 41 Steuhl KP, Thiel HJ. Histochemical and morphological study of the regenerating corneal epithelium after limbus-to-limbus denudation[J]. *Graefes Arch Clin Exp*

- Ophthalmol, 1987, 225(1):53-58.
- 42 Kays WT, Piatigorsky J. Aldehyde dehydrogenase class 3 expression: identification of a cornea-preferred gene promoter in transgenic mice[J]. Proc Natl Acad Sci USA, 1997, 94(25):13594-13599.
- 43 Guo J, Sax CM, Piatigorsky J, et al. Heterogeneous expression of transketolase in ocular tissues[J]. Curr Eye Res, 1997, 16(5):467-474.
- 44 Chen Z, de Paiva CS, Luo L, et al. Characterization of putative stem cell phenotype in human limbal epithelia[J]. Stem Cells, 2004, 22(3):355-366.
- 45 Kuwabara T, Perkins DG, Cogan DG. Sliding of the epithelium in experimental corneal wounds[J]. Invest Ophthalmol, 1976, 15(1):4-14.
- 46 Lim M, Goldstein MH, Tuli S, et al. Growth factor, cytokine and protease interactions during corneal wound healing[J]. Ocul Surf, 2003, 1(2):53-65.
- 47 Vauclair S, Majo F, Durham AD, et al. Corneal epithelial cell fate is maintained during repair by Notch1 signaling via the regulation of vitamin A metabolism[J]. Dev Cell, 2007, 13(2):242-253.
- 48 Xue ML, Zhu H, Thakur A, et al. $1\alpha, 25$ -Dihydroxyvitamin D3 inhibits pro-inflammatory cytokine and chemokine expression in human corneal epithelial cells colonized with *Pseudomonas aeruginosa*[J]. Immunol Cell Biol, 2002, 80(4):340-345.
- 49 Chen CH, Chen S C. Lactate transport and glycolytic activity in the freshly isolated rabbit cornea[J]. Arch Biochem Biophys, 1990, 276(1):70-76.
- 50 Aguayo J B, McLennan I J, Graham Jr C, et al. Dynamic monitoring of corneal carbohydrate metabolism using high-resolution deuterium NMR spectroscopy[J]. Exp Eye Res, 1988, 47(2):337-343.
- 51 Hernández-Quintero M, García-Villegas R, Castro-Muñozledo F. Differentiation-dependent increases in lactate dehydrogenase activity and isoenzyme expression in rabbit corneal epithelial cells[J]. Exp Eye Res, 2002, 74(1):71-82.
- 52 Ottino P, Taheri F, Bazan HE. Growth factor-induced proliferation in corneal epithelial cells is mediated by 12 (S)-HETE[J]. Exp Eye Res, 2003, 76(5):613-622.
- 53 Pappa A, Brown D, Koutalos Y, et al. Human aldehyde dehydrogenase 3A1 inhibits proliferation and promotes survival of human corneal epithelial cells[J]. J Biol Chem, 2005, 280(30):27998-28006.
- 54 Gehlsen U, Oetke A, Szaszak M, et al. Two-photon fluorescence lifetime imaging monitors metabolic changes during wound healing of corneal epithelial cells in vitro[J]. Graefes Arch Clin Exp Ophthalmol, 2012, 250(9):1293-1302.
- 55 Bazan HE. Cellular and molecular events in corneal wound healing: significance of lipid signalling[J]. Exp Eye Res, 2005, 80(4):453-463.
- 56 Bhattacharjee P, Hammond B, Williams R, et al. Arachidonic acid lipoxygenase products in ocular tissues and their effect on leukocyte infiltration in vivo[C]. Proceedings of the Proc Int Soc Eye Res, 1980:32.
- 57 Li DQ, Lokeshwar BL, Solomon A, et al. Regulation of MMP-9 production by human corneal epithelial cells[J]. Exp Eye Res, 2001, 73(4):449-459.
- 58 Jäättelä M. Escaping cell death: survival proteins in cancer [J]. Exp Cell Res, 1999, 248(1):30-43.
- 59 Garrido C, Gurbuxani S, Ravagnan L, et al. Heat shock proteins: endogenous modulators of apoptotic cell death [J]. Biochem Biophys Res Commun, 2001, 286(3):433-442.
- 60 Öztürk F, Kurt E, Inan ÜÜ, et al. The effects of acetylcholine and propolis extract on corneal epithelial wound healing in rats[J]. Cornea, 1999, 18(4):466-471.
- 61 Huo Y, Qiu WY, Pan Q, et al. Reactive oxygen species (ROS) are essential mediators in epidermal growth factor (EGF)-stimulated corneal epithelial cell proliferation, adhesion, migration, and wound healing[J]. Exp Eye Res, 2009, 89(6):876-886.
- 62 Wang Y, Zhang J. Effect of epidermal growth factor on expression of cell cycle-regulatory proteins and proliferation of rabbit corneal epithelial cells[J]. Zhonghua Yan Ke Za Zhi, 2009, 45(2):153.
- 63 Lambiase A, Manni L, Bonini S, et al. Nerve growth factor promotes corneal healing: structural, biochemical, and molecular analyses of rat and human corneas[J]. Invest Ophthalmol Vis Sci, 2000, 41(5):1063-1069.
- 64 Wilson G. The effect of hypoxia on the shedding rate of the corneal epithelium[J]. Curr Eye Res, 1994, 13(6):409-413.
- 65 Carrington LM, Boulton M. Hepatocyte growth factor and keratinocyte growth factor regulation of epithelial and stromal corneal wound healing[J]. J Cataract Refract Surg, 2005, 31(2):412-423.
- 66 Ozaki M, Haga S, Zhang H, et al. Inhibition of hypoxia/reoxygenation-induced oxidative stress in HGF-stimulated antiapoptotic signaling: role of PI3-K and Akt kinase upon rac1[J]. Cell Death Differ, 2003, 10(5):508-515.
- 67 He F, Wu LX, Shu KX, et al. HGF protects cultured cortical neurons against hypoxia/reoxygenation induced cell injury via ERK1/2 and PI-3K/Akt pathways[J]. Colloids Surf B Biointerfaces, 2008, 61(2):290-297.

- 68 Sosne G, Szliter EA, Barrett R, et al. Thymosin beta 4 promotes corneal wound healing and decreases inflammation in vivo following alkali injury[J]. *Exp Eye Res*, 2002, 74(2):293-299.
- 69 Saika S, Shiraishi A, Saika S, et al. Role of lumican in the corneal epithelium during wound healing[J]. *J Biol Chem*, 2000, 275(4):2607-2612.
- 70 Gomes J, Amankwah R, Powell-Richards A, et al. Sodium hyaluronate (hyaluronic acid) promotes migration of human corneal epithelial cells in vitro[J]. *Br J Ophthalmol*, 2004, 88(6):821-825.
- 71 Ceresara G, Migliavacca L, Orzalesi N, et al. In vivo confocal microscopy in Terrien marginal corneal degeneration: a case report[J]. *Cornea*, 2011, 30(7):820-824.
- 72 Tremblay M, Dube I. Meesmann's corneal dystrophy: ultrastructural features. *Can J Ophthalmol* 1982;17(1): 24-28.
- 73 Dogru M, Katakami C, Inoue M. Tear function and ocular surface changes in noninsulin-dependent diabetes mellitus[J]. *Ophthalmology*, 2001, 108(3):586-592.
- 74 Cousen P, Cackett P, Bennett H, et al. Tear production and corneal sensitivity in diabetes[J]. *J Diabetes Complications*, 2007, 21(6):371-373.
- 75 Göbbels M, Spitznas M, Oldendoerp J. Impairment of corneal epithelial barrier function in diabetics[J]. *Graefes Arch Clin Exp Ophthalmol*, 1989, 227(2):142-144.
- 76 Inoue K, Kato S, Ohara C, et al. Ocular and systemic factors relevant to diabetic keratoepitheliopathy[J]. *cornea*, 2001, 20(8):798-801.
- 77 陈剑, 黄建艳, 唐福星, 等. 糖尿病早期角膜组织的超微结构变化 [J]. *中国病理生理杂志*, 2003, 19(7): 942-945.
- 78 Gilbard JP. Human tear film electrolyte concentrations in health and dry-eye disease[J]. *Int Ophthalmol Clin*, 1994, 34(1):27-36.
- 79 Png E, Samivelu G, Yeo S, et al. Hyperosmolarity-mediated mitochondrial dysfunction requires Transglutaminase-2 in human Corneal epithelial cells[J]. *J Cell Physiol*, 2011, 226(3):693-699.
- 80 Carlson KH, Bourne WM, Brubaker R. Effect of long-term contact lens wear on corneal endothelial cell morphology and function[J]. *Invest Ophthalmol Vis Sci*, 1988, 29(2):185-193.
- 81 Liesegang TJ. Physiologic changes of the cornea with contact lens wear[J]. *CLAO J*, 2002, 28(1):12-27.

(收稿日期 :2014-01-10)

(本文编辑 :蔡晓珍)

马佰凯, 何昕, 李炜. 角膜上皮细胞代谢的研究进展[J/CD]. *中华细胞与干细胞杂志:电子版*, 2014, 4(1):72-78.