·文献综述·

DNA 甲基化在眼科疾病中的研究进展

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摘要

DNA 甲基化作为表观遗传修饰的重要形式,通过调控基因表达,在疾病发生发展中发挥重要作用。近年来,随着 DNA 甲基化研究的迅速开展、检测技术的不断提升,DNA 甲基化修饰已成为探究疾病发病机制及探寻新的治疗方案的重要方法;眼科不同亚专业疾病在 DNA 甲基化的基础研究方面也取得了很多突破,包括角膜上皮的修复、结膜上皮的细胞黏附与异常的基质重塑、眼组织纤维化与青光眼、氧化应激和炎症反应与细胞损伤、不同 DNA 甲基化水平与眼部肿瘤的关系等。本文旨在通过对不同眼科疾病 DNA 甲基化调控机制的相关研究进行概述,为眼病发病机制的研究、筛查、诊断及预防提供新思路。
关键词:DNA 甲基化;眼科疾病;发病机制
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Research progress on DNA methylation in ocular diseases

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Abstract

• DNA methylation, as an important form of epigenetic modification, plays vital roles in the occurrence and development of diseases by regulating gene expression. In recent years, with the rapid development of DNA methylation research and the continuous updates of the detection method, it is reported that DNA methylation has become an important way to explore the pathogenesis of various diseases and new treatment methods. Many breakthroughs have been made in the basic research of DNA methylation in different ophthalmological diseases, including corneal epithelium repair, cell adhesion and abnormal matrix remodeling of conjunctival epithelium, ocular fibrosis and glaucoma, oxidative stress, inflammatory response and cell damage, the relationship between different DNA methylation levels and ocular tumors, *etc.* This review aims to provide a new idea for the pathogenesis, examination, diagnosis and prevention of different ocular diseases by summarizing the relevant studies on the regulatory mechanisms of DNA methylation in ophthalmological diseases.
• KEYWORDS: DNA methylation; ophthalmological diseases; pathogenesis

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0 引言

DNA 甲基化是指 DNA 序列上特定的碱基在 DNA 甲基转移酶(DNA methyltransferase,DNMT)的催化作用下,以 s-腺苷甲硫氨酸作为甲基供体,以共价键结合方式获得一个甲基基团的化学修饰过程[1-2]。异常病理条件下,DNA 甲基化状态发生改变,引起机体内环境紊乱,产生病变。DNA 甲基化作为遗传表观学的重要研究内容之一,已被认为在形成复杂的表型中发挥重要作用,包括疾病的病因学等。这些修饰已被证明具有高度的可塑性,能够响应环境变化,并且以遗传的方式改变基因表达。近年来,在角、结膜等眼表疾病、青光眼、白内障以及视网膜疾病、眼部肿物等各领域,有关 DNA 甲基化的研究层出不穷,为不同眼部疾病发病机制的研究,提供了新的探索方向。

1 角膜疾病

异常 DNA 甲基化参与角膜疾病发生发展[3],了解 DNA 甲基化在角膜生理病理过程中的作用至关重要。正常生理条件下角膜表达 DNMT1、DNMT2、DNMT3[4],角膜上皮伤口愈合期间,DNMT1 表达上调,整体 DNA 甲基化水平升高,而下调 DNMT1 可抑制角膜上皮细胞增殖及迁移,延缓伤口愈合[5]。

Fuchs 角膜内皮营养不良(Fuchs endothelial corneal dystrophy,FECD)患者存在大量差异甲基化位点[6],其中离子通道相关基因低甲基化对角膜上皮功能至关重要,可能调控角膜上皮功能使 FECD 角膜透明度丧失。细胞外

基质异常沉积是 FECD 的特异性改变,Snail 和 ZEB1 是细胞外基质诱导基因,在 FECD 患者中高表达[7],miRNA 基因的启动子作为 FECD 异常甲基化位点,miR-199B 高甲基化下调 miR-199B-5p,调控 Snail 和 ZEB1 基因的表达[8],提示调控 miRNA 甲基化可引起细胞外基质沉积从而导致疾病发生发展。

2 结膜疾病

翼状胬肉是结膜疾病中的常见病,为睑裂部球结膜与角膜上一种赘生组织。Arish 等[9]发现翼状胬肉患者胬肉组织中鼠双微基因 2(murine double minute 2,MDM2)启动子低甲基化,蛋白表达上调, p53 蛋白表达下调。MDM2 是肿瘤抑制因子 p53 的负调控基因[10],DNA 甲基化修饰增加与 p53 蛋白的结合,抑制 p53 转录调节功能[11]。此外,研究发现,翼状胬肉组织中转谷氨酰胺酶 2(transglutaminase 2, TGM-2)的转录起始位点及 E-cadherin 基因启动子 CpG 岛高甲基化,基质金属蛋白酶 2(matrix metalloproteinases 2,MMP2)的转录起始位点下游及 CD24 的转录起始位点上游 CpG 岛低甲基化,提示基质(MMP2)重塑和细胞(TGM-2、CD24、E-cadherin)黏附相关基因的异常 DNA 甲基化与翼状胬肉高度相关[12]。

3 青光眼

青光眼是一组以视乳头萎缩及凹陷、视野缺损及视力下降为共同特征的疾病。研究表明青光眼患者 Schlemm 管内皮细胞及小梁网细胞存在全基因组甲基化水平升高,小梁网细胞促纤维化因子转化生长因子(transforming growth factor, TGF)β1 表达增加,抗纤维化基因 RASAL1 表达降低,DNA 甲基化抑制剂 5-氮杂胞苷(5-azacytidine, 5-AC)可抑制纤维化[13-15],提示 DNA 甲基化改变导致的眼组织异常纤维化可能是青光眼发病机制之一。此外,跨筛板压差是青光眼视神经受损的主要原因,青光眼患者巩膜筛板变薄,巩膜筛板细胞 TGFβ1 启动子甲基化水平降低[16],纤维化水平增加,提示青光眼跨筛板压形成可能与筛板纤维化有关。

4 白内障

白内障是由各种原因引起晶状体代谢紊乱,从而导致晶状体蛋白质变性而发生混浊的疾病。研究发现,晶状体上皮细胞中 DNA 甲基化相关基因表达上调[17],外周血差异甲基化基因与晶状体上皮细胞凋亡相关通路相关[18]。晶状体上皮细胞机能减退,无法清除自由基,导致氧化应激反应发生,细胞凋亡,造成晶状体氧化损伤,白内障发生。

机体抗氧化基因[19-20]如沃纳综合征基因、谷胱甘肽转移酶 P1 等,其启动子在白内障患者晶状体中高甲基化,氧化与抗氧化作用失衡,氧化应激反应发生。Nrf2/Keap1 是机体抗氧化机制的重要通路,核因子 E2 相关因子(nuclear factor erythroid 2-related factor 2,Nrf2)是转录调节因子,可激活多种抗氧化酶;Keap1 即 Kelch 样环氧氯丙烷相关蛋白 1,是 Nrf2 的负调控蛋白。研究发现,白内障患者晶状体的 Keap1 基因启动子 DNA 甲基化水平降低,Nrf2 活性减弱,导致氧化系统失衡,晶状体上皮细胞氧化凋亡[21]。

5 视网膜疾病

5.1 年龄相关性黄斑变性 年龄相关性黄斑变性(age-related macular degeneration,ARMD)是一种神经退行性疾病。氧化应激和炎症反应会降低视网膜色素上皮细胞(retinal pigment epithelium, RPE) DNMT 的表达及活性,下调甲基化水平[22],然而研究发现 ARMD 患者外周血 DNMT 表达及活性上升[23],表明不同样本之间 DNA 甲基化存在差异,可能由于模拟的氧化应激及炎症反应不能完全替代 ARMD 的病理环境,或外周血在经过复杂的生物过程后甲基化修饰发生改变而表现出差异,白藜芦醇可以抑制氧化应激及炎症反应对细胞甲基化水平的影响。谷胱甘肽-S-转移酶作为氧化应激反应的关键蛋白,其启动子在 ARMD 患者 RPE/脉络膜和视网膜神经感觉层中甲基化水平上调[24];同型半胱氨酸作为氧化应激反应的重要因子,异常堆积可显著提高 DNMT 的活性,增加视网膜 DNA 甲基化水平[25]。ELOVL2 基因与年龄相关性疾病有关,其功能受损会干扰脂质合成,增加内质网应激和线粒体功能障碍。研究发现,ELOVL2 在 ARMD 患者中高甲基化,玻璃体腔注射甲基化抑制剂可逆转其高甲基化状态,恢复部分视网膜功能[26]。

5.2 糖尿病视网膜病变 糖尿病视网膜病变(diabetic retinopathy,DR)是长期高血糖使毛细血管自身调节失常,活性氧自由基堆积引起氧化应激反应,导致线粒体 DNA(mitochondrial DNA,mtDNA)结构受损,内皮细胞屏障功能受损,最终引起的视网膜损伤[27-29]。视网膜线粒体肿胀可导致线粒体融合蛋白(mitofusin 2,Mfn2)甲基化,加剧线粒体损害及氧自由基堆积,DNA 甲基化抑制剂可抑制 Mfn2 甲基化,改善视网膜功能[30-31]。

高血糖水平使视网膜基质金属蛋白酶 9(matrix metalloproteinases 9,MMP9)基因启动子甲基化-羟甲基化过程失衡,视网膜受损[32]。同型半胱氨酸可提高视网膜 DNA 甲基化酶活性,激活 MMP9,引起血视网膜屏障功能障碍,调节同型半胱氨酸可预防或减缓 DR 病变的发展[33]。SOD2 基因是负责清除线粒体超氧物的基因,编码 MnSOD 酶,其启动子在 DR 患者中高甲基化[34]。上调 SOD2 表达可抑制糖尿病引起的 mtDNA 甲基化[35]。MnSOD 酶模拟物可减少 MMP9 启动子区与 DNMT1 结合,防止线粒体损伤[36]。高血糖状态下,Ras 相关的 C3 肉毒素 1(Ras-related C3 botulinum toxin substrate 1,Rac1)启动子区甲基化改变,Rac1 激活,胞浆活性氧水平增加,引起氧化应激[37]。DNMT 抑制剂、抗氧化剂均可调节视网膜抗氧化酶 DNA 甲基化,维持氧化还原平衡,阻止 DR 进展[38-39]。

DR 患者外周血全基因组 DNA 甲基化水平明显上调[40-41],较无视网膜病变的糖尿病患者,DR 患者外周血甲基化程度更高[42],故外周血特异性 DNA 甲基化改变可能是 DR 诊疗的生物标志物。此外,保持良好生活方式和健康体格有助于调节糖尿病患者 DNA 甲基化水平,预防和延缓视网膜病变[43]。

6 眼部肿瘤

6.1 葡萄膜黑色素瘤 葡萄膜黑色素瘤起源于葡萄膜内黑色素细胞,恶性程度较高,易经血行转移,预后较差,了解致病机制有助于改善预后、延长存活时间。

RAS 相关区域家族 1A(Ras association domain family 1 A,RASSF1A)、BRCA1 相关蛋白 1(BRCA1 associated protein1,BAP1)及 $p16^{INK4a}$ 是常见抑癌基因,葡萄膜黑色素瘤中 RASSF1A、BAP1 及 $p16^{INK4a}$ 启动子高甲基化,蛋白表达下降[44],且研究发现启动子的高甲基化与肿瘤转移有关[45]。DNA 甲基化转移酶抑制剂处理可逆转抑癌基因启

动子甲基化状态,减少肿瘤转移[46]。

癌基因经 DNA 甲基化修饰表达上调,促进肿瘤发生发展。黑色素瘤特异性抗原是黑色素瘤患者中筛查得出的表面抗原,在葡萄膜黑色素瘤患者中该基因启动子异常低甲基化,且与转移风险增加有关,是转移性葡萄膜黑色素瘤免疫治疗的潜在靶点[47]。生长分化因子 11(growth differentiation factor 11,GDF11)调节细胞增殖、凋亡等生物过程,Yu 等[48]发现在肿瘤组织中 GDF11 启动子 CpG 位点低甲基化,蛋白表达明显高于邻近正常组织。

6.2 视网膜母细胞瘤 视网膜母细胞瘤基因(RB1)的缺失或失活是视网膜母细胞瘤(retinoblastoma, RB)发生的重要机制,RB 患者中 RB1 基因启动子高甲基化,蛋白表达下调,细胞分裂能力受损,细胞分化方向异常,导致疾病的发生发展[49]。其余抑癌基因如 RASSF1A、MEG3、APC-2等,均受 DNA 甲基化调控,经去甲基化剂处理可逆转其结果[50-51]。

7 小结

表观遗传学可以作为基因-环境的中介,为研究疾病发生发展机制提供新思路。目前,眼科疾病中 DNA 甲基化的研究还处于初步阶段,随着 DNA 甲基化研究技术的发展,有望明确 DNA 甲基化在不同眼科疾病发生发展中的机制,为眼科疾病诊疗提供新思路。

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