阿尔茨海默病DNA甲基化研究进展

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摘要 阿尔茨海默病(Alzheimer's disease, AD)又称老年痴呆症, 是老年人中发病率最高的神经退行性疾病之一, 以记忆和认知功能损伤为主要特征。AD与表观遗传学如DNA甲基化联系紧密。通常, 基因启动子区域DNA高甲基化会抑制相关基因的表达。目前研究表明, 诸多因素通过影响DNA甲基化修饰显著增加AD的患病风险, 如环境、年龄及AD相关疾病。AD相关基因的DNA甲基化修饰研究已取得较大的进展, 测试外周血中基因DNA甲基化修饰差异可为AD的预测、诊断及治疗开辟新的途径。该文就最近相关的DNA甲基化研究进展进行了系统阐述。

关键词 阿尔茨海默病; 表观遗传学; DNA甲基化

Research Progress of DNA Methylation in Alzheimer's Disease

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Abstract Alzheimer's disease (AD) is one of the most common types of neurodegenerative diseases in the elderly. AD is characterized by progressive memory and cognitive dysfunction. The pathogenesis of AD is complex and contributed by both genetic and environmental factors. AD is closely linked with epigenetic modifications which comprise DNA methylation. Generally, gene expression will be down-regulated by promoter hypermethylation. Recent studies have found that multiple factors might exert their influences on the risk of AD by the changes of DNA methylation, such as environment, age and AD related diseases. Recently, AD related DNA methylation research has made great progress. Testing for aberrant DNA methylation in peripheral blood may be potentially applied for the prediction, diagnosis and treatment of AD. This review provides a systemic landscape of recent AD related DNA methylation studies.

Key words Alzheimer's disease; epigenetics; DNA methylation

收稿日期: 2014-05-15 接受日期: 2014-06-12
国家自然科学基金(批准号: 81271209、81070873、31100919)、科技部973项目(批准号: 2013CB835100)、浙江省自然科学基金杰出青年项目(批准号: LRl3H020003)、宁波市科技局/农业与社会发展攻关项目(批准号: 2012C50032)、宁波市自然科学基金项目(批准号: 2007A610077、200701A6304004)和宁波大学重点科学项目(批准号: XKLlD2117)资助的课题
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Received: May 15, 2014 Accepted: June 12, 2014
This work was supported by the National Natural Science Foundation of China (Grant No.81271209, 81070873, 31100919), National Program on Key Basic Research Project of China (973 Program) (Grant No.2013CB835100), Natural Science Foundation of Zhejiang for Distinguished Young Scholars (Grant No.LRl3H020003), Ningbo Science and Technology Bureau/Program for Agriculture and Society Development (Grant No.2012C50032), Ningbo Natural Science Foundation (Grant No.2007A610077, 200701A6304004) and Project of Key Disciplines in Ningbo University (Grant No.XKLlD2117)
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网络出版时间: 2014-10-24 09:20 URL: http://www.cnki.net/kcms/doi/10.11844/cjcb.2014.11.0172.html

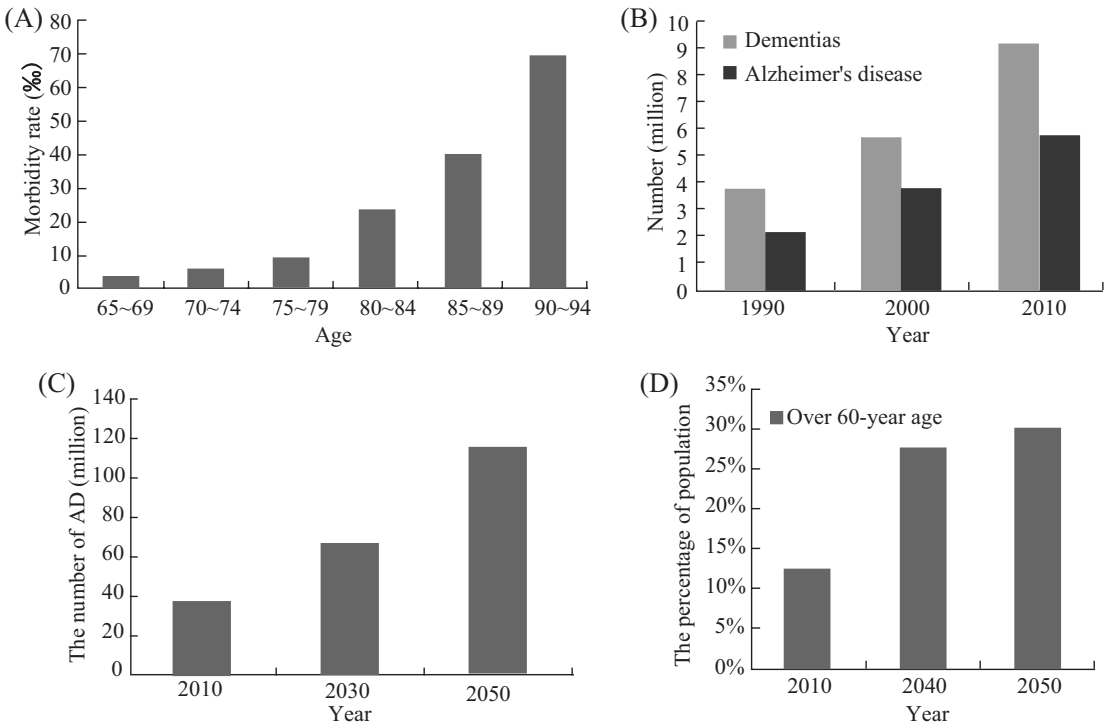
阿尔茨海默病(Alzheimer's disease, AD)是一种以进行性认知障碍和记忆力损害为主的中枢神经系统退行性疾病[1]。AD多潜隐起病于老年期, 临床表现以智力损害为主, 病程缓慢且不可逆。随着社会老龄化趋势加剧, AD对个人、家庭及社会的影响日益突出。最近研究表明, 环境对AD的发生和发展都具有较大影响, 而表观遗传学正是连接环境和疾病研究之间的重要桥梁[2]。DNA甲基化是一种重要的表观遗传修饰模式, 与AD的发病及发展密切相关。本文将系统阐述AD相关的DNA甲基化研究进展。

1 阿尔茨海默病危害显著

阿尔茨海默病(AD)是一种神经系统退行性疾病[1], 在临床上以认知和记忆功能的不断恶化为主要特征, 同时伴随着社会生活能力和专业领域能力的进行性减退、各种神经精神症状和行为障碍等表现[3]。AD的主要神经病理学特征有以下两种: 细胞外β样淀粉蛋白(β amyloid, Aβ)沉积所形成的老年斑(senile plaque, SP)增多及细胞内的神经元纤维缠结(neurofibrillary tangles, NFT)[4]。AD在病理改变方面还包括皮质弥漫性萎缩、沟回增宽、脑室扩大以及神经元大量丢失[5]。由于发病机制复杂, AD有多种分型, 从遗传角度可分为散发型AD(sporadic AD, SAD)和家族型AD(familial AD, FAD), 其中以SAD居多; 从发病年龄角度, AD可分为早发型AD(early-onset AD, EOAD)和晚发型AD(late-onset AD, LOAD), 其中以LOAD居多[6]。

1.1 AD面临的社会形势十分严峻

AD发病年龄常在65岁以上, 发病率随年龄递增(图1A)[7]。近年来AD患者人数几乎成倍上升(图1B), 在中国每7秒就增加一位AD患者, 患者平均生存期仅为5.9年, 严重威胁老年人的健康[8]。当前全球人口呈老龄化趋势, 随着未来社会老龄化的进一步加剧, AD患者人数倍增(图1C), 在2050年全球将有1/85的人受到AD的威胁[9]。AD给社会经济带来沉重负担, 全球痴呆症社会总成本约为每年6 040亿美元, 占全世界各国国内生产总值的1.0%[10]。在中国, 伴随着改革开放的成功和计划生育政策的实施, 人均寿命得到极大延长, 与此同时, 我国人口结构老龄化趋势显著, 60岁以上人口所占比例迅增(图1D), AD发病



A: AD发病率在年长者中增长快速; B: 中国AD患者近年来增势迅猛; C: 社会老龄化将导致AD患者倍增; D: 中国60岁以上人口所占比例增加。
A: the incidence of AD showed a fast increasing trend in the elderly; B: AD patients increased dramatically recent years in China; C: the number of AD patients will increase with aging society; D: the proportion of over 60-age in China increased.

图1 AD流行病学相关数据及其未来趋势

Fig.1 Epidemiological data and trends of AD

率正不断接近西方国家[11]。

1.2 疾病发病机制研究进展

大量文献已对AD的发病机制进行阐述, 然而AD发病过程涉及环节众多, 其发病机制始终不明。目前公认的AD发病机制有胆碱性假说、类淀粉蛋白质假说及微管相关蛋白质假说等。胆碱性假说认为, AD是由于神经系统内的胆碱能神经元受损减少, 导致乙酰胆碱(acetyl choline, ACH)合成和释放减少, 进而引起以记忆和识别为主的功能性障碍[12]。类淀粉蛋白质假说则认为, 淀粉样前体蛋白基因(amyloid precursor protein, *APP*)、早老素基因1(presenilin 1, *PS1*)、淀粉样前体蛋白β位点裂解酶1(β-site APP-cleaving enzyme l, *BACE1*)等基因的突变导致Aβ分泌异常, 在细胞外沉积形成SP, 对周围的突触和神经元产生毒性作用, 破坏突触膜, 引起神经细胞坏死[6]。微管相关蛋白质假说则认为, Tau蛋白异常磷酸化而聚集, 降低其与微管蛋白结合的能力, 导致微管的形成和稳定性下降, 其病理性沉积导致神经元细胞内NFT(neurofibrillary tangles)的形成, 而NFT与痴呆程度相关[13]。另外, 炎症和免疫功能异常[14]、自由基和氧化应激作用[15]、胰岛素相关糖代谢异常[16]、钙稳态失调[17]、脂质代谢异常[18]、高半胱氨酸血症[15]等都对AD的病理过程产生一定的影响(图2)。

2 AD相关基因DNA甲基化研究进展

表观遗传学研究在基因核苷酸序列不发生改变的情况下, 基因组功能发生改变的现象[19], 主要有DNA甲基化(DNA methylation)、组蛋白共价修饰(histone covalent modification)[20]、染色质重塑(chromatin remodeling)[21]、RNA编辑(RNA editing)[22]等。DNA甲基化是指在DNA甲基转移酶(DNA methyltransferases, DNMTs)催化下, 将S-腺苷甲硫氨酸中的甲基由四氢叶酸转移到胞嘧啶的第5位上形成5-甲基胞嘧啶(5-mC)[20]。DNA甲基化的研究有助于阐述疾病、环境和遗传三者之间的关系。AD作为一种复杂的精神疾病, 是环境和遗传因素共同作用的

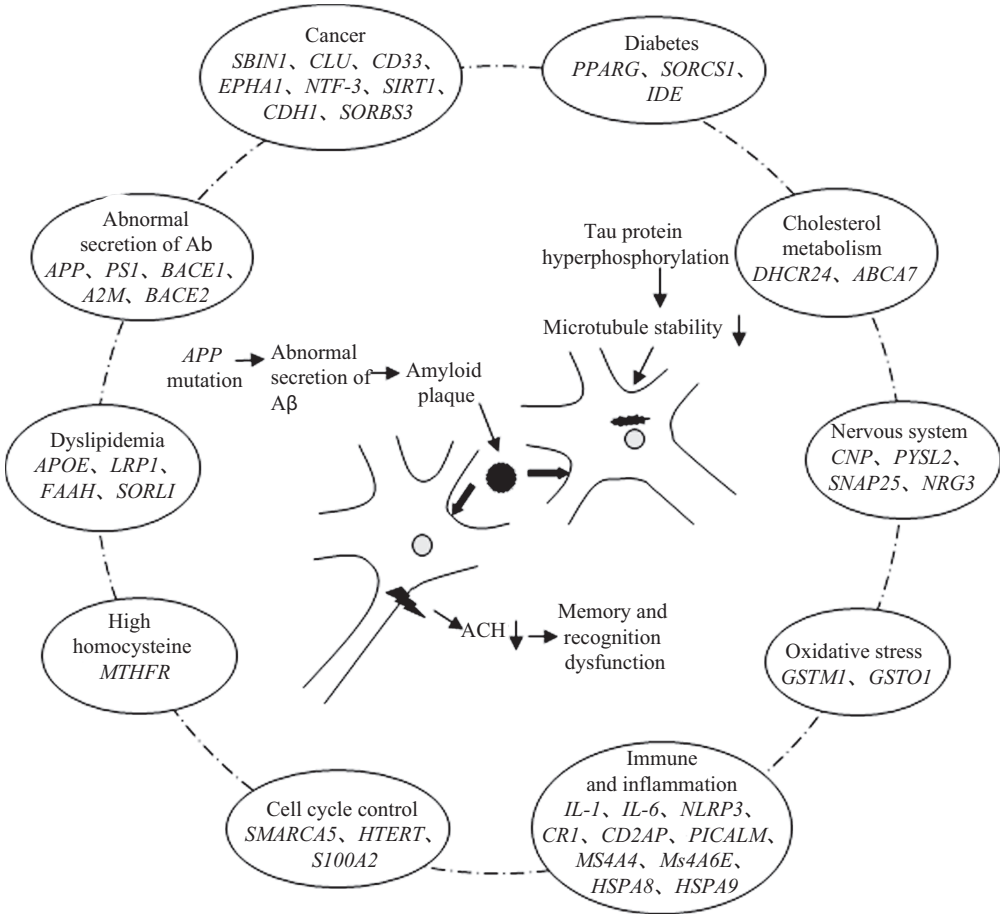


图2 AD发病机制及相关基因

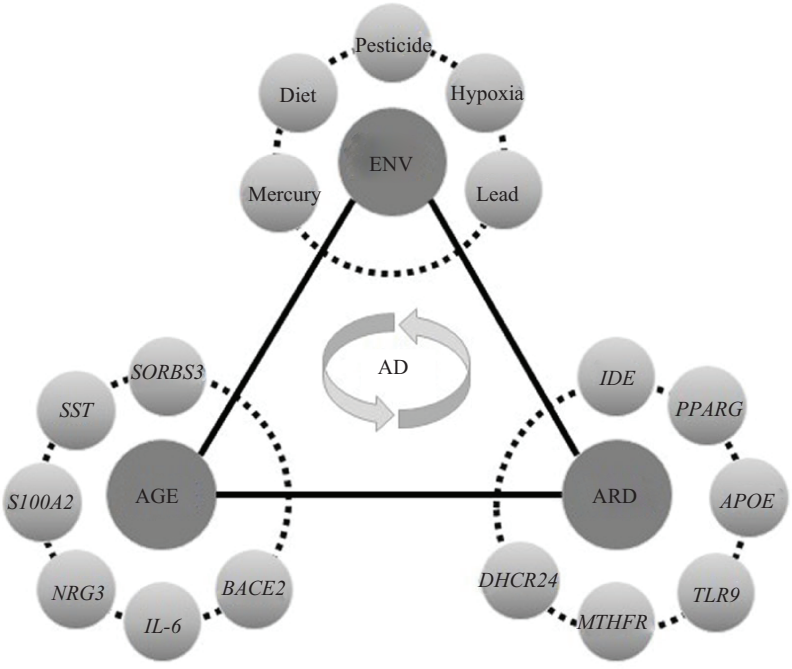
Fig.2 Pathogenesis and related genes to AD

结果[23]。

AD与表观遗传学联系紧密。大量研究指出, DNA甲基化修饰与AD相关[24]。全基因组DNA甲基化分析发现, LOAD患者和正常人两组之间的平均甲基化程度不同, 基因跨膜蛋白59(transmembrane protein 59, *TMEM59*)在患者前额叶皮层中出现7.3%的低甲基化现象[25]; 在经过早期铅暴露的老年大鼠和正常老年大鼠的对比中发现, 早期铅暴露导致约150个基因的表达被抑制, 这些基因主要涉及免疫应答、金属结合、新陈代谢和转录四个方面, 且影响机体修复神经退行性损伤的能力, 从而可能导致AD的发生[26]; Aβ[27]、APP、微管相关蛋白tau(microtubule-associated protein tau, MAPT)[28]等多种蛋白也被证明具有影响基因组甲基化的能力; 在AD病人大脑的额中回和颞中回中发现了甲基化和羟甲基化程度有升高的现象[29], 而在海马体中它们却一致下降[30]。众多研究都表明, DNA甲基化机制对AD的起因及发展都具有影响, 主要集中在环境、年龄和相关疾病的研究上(图3)。在AD患者的神经元细胞、星形胶质细胞和小胶质细胞中存在异常的DNA甲基化修饰现象[30]。早在1990年, 就有AD相关基因的DNA甲基化研究报道[31], 迄今为止已经针对多个基因开展了DNA甲基化修饰的研究, 包括分拣蛋白相关受体L1基因(sortilin-related receptor L1, *SORL1*)[32]、沉默信息调节因子2同源蛋白1基因(sirtuin 1, *SIRT1*)[33]、C型利钠肽基因(c-type natriuretic peptide, *CNP*)[34]、二氢嘧啶酶2基因(dihydropyrimidinase-like 2, *DPYSL2*)[34]、APP[35]、SWI/SNF相关, 基质关联, 肌动蛋白依赖染色质调控因子, 亚家族a, 成员5(SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5, *SMARCA5*)[33]、人端粒酶逆转录酶基因(human telomerase reverse transcriptase, *HTERT*)[33]、钙黏蛋白1基因(cadherin 1, *CDH1*)[33]、热休克蛋白8基因(heat shock protein 8, *HSPA8*)和热休克蛋白9基因(heat shock protein 9, *HSPA9*)[36]、突触相关蛋白基因(synaptosomal-associated protein, *SNAP25*)[37]、核糖体编码基因[38]及线粒体基因[39]等。

2.1 环境因素

有研究认为, 环境与AD的发病机制密切相关。汞、铅和杀虫剂都能够影响正常的表观遗传修饰过程, 从而导致发育不良、精神发育迟缓、女性化或其他复杂疾病的发生[6], 由此可以推断, 环境很可能是AD发病的一个触发因素。脑啡肽酶(neprilysin,



ENV: 环境; ARD: AD相关疾病。
ENV: environment; ARD: AD-related diseases.

图3 AD发病机制相关的异常甲基化修饰基因

Fig.3 Aberrantly methylated genes in the AD pathogenesis

NEP)与神经元突触有关, 主要分布于黑质纹状体通路及海马等易于形成老年斑的区域。NEP分泌剂量与脑内Aβ水平相关, 该物质水平下降时能够引起Aβ水平的升高[40]。低氧条件下的AD患者, 能够通过增加组蛋白H3K9me2的脱甲基化作用和减少组蛋白H3的乙酰化修饰, 从而下调NEP水平[41]。早期铅暴露将增加*APP*的表达甚至诱导AD发生, 在大鼠嗜铬细胞瘤(PC12)细胞中, 铅暴露可导致*APP*基因启动子区域低甲基化, 并导致全基因组DNA甲基化水平下调, 并且*DNMT1*基因表达水平也出现下调的现象[42]。另外, 有相关报道称, 铅暴露会抑制*APP*启动子区域的甲基化修饰过程, 从而上调该基因的表达, 使细胞内Aβ沉淀现象加剧, 并加深DNA氧化损伤程度[2]。此外, 压力相关基因对AD发病机制也具有一定影响[43]。大多数AD患者都属于LOAD, 其形成以环境因素为主、若干敏感基因变异作用为辅[44]。EOAD患者相对较少, 其病因主要是基因突变, 在AD患者中占极少的比例(5%左右)[45]。由此可见, 研究DNA甲基化在AD致病环境因子中的作用机制十分关键。

2.2 年龄因素

年龄一直被认为是AD最重要的影响因素, DNA甲基化的改变不仅与衰老过程有关[46], 而且与AD发生也有相关性[47]。DNA甲基化修饰早在子宫内就已发生, 从胎儿时期开始, 基因组DNA甲基化修饰程度便不断发生变化[48]。在冰岛人群的外周血细胞中进行了DNA甲基化与年龄的关联研究, 在11年内29%的冰岛人群显示出大于10%的甲基化程度改变, 这表明DNA甲基化程度在人体内是动态变化的[49]。在对孕期17周到104岁的125个样本进行DNA甲基化的研究中发现, AD患者的颞叶皮层样本中出现钙结合蛋白A2(S100 calcium binding protein A2, *S100A2*)基因的低甲基化修饰以及山梨糖和SH3结构域包含蛋白3(sorbin and SH3 domain containing 3, *SORBS3*)基因的高甲基化修饰现象[50]。在AD患者大脑皮质中生长抑素受体(somatostatin, *SST*)基因表达显著减少[51], 并且SST甲基化程度随着年龄增长呈现上升趋势[52]。此外, 在中枢神经系统中, 炎症相关因子白细胞介素-6(interleukin-6, *IL-6*)基因的表达水平与年龄相关[53]。还有研究发现, 神经调节蛋白3(neuregulin 3, *NRG3*)基因的SNPs位点和AD的发病年龄有关[54], 以及在唐氏综合症患者中*BACE2*基因多态性和AD发病时间有关系[55]。DNA羟甲基化修饰通过DNA羟化酶TET(ten eleven translocation)蛋白介导参与DNA去甲基化过程[56], 且和基因表达水平的上调有关[29]。近年来, DNA羟甲基化修饰被认为与AD的发病机制具有关联[29]。小鼠实验表明, 5-羟甲基胞嘧啶(5-hydroxymethylcytosine, 5-hmC)的富集程度随着年龄的增长而增加[57]。显然, 年龄可以作为AD发病过程中的重要风险因素, 并通过DNA甲基化修饰水平的改变影响疾病的发生和发展。

2.3 AD相关疾病

最近研究发现, AD与多种疾病之间存在相同的候选基因[58]。其中以胰岛素抵抗和进行性β细胞衰竭为特点的2型糖尿病(type 2 diabetes mellitus, T2D)就与AD的发生有一定的关系。研究已经发现, T2D病人中高发AD, T2D病人患AD的危险性是非T2D病人的2倍[59], 主要是由于胰岛素抵抗与神经元的葡萄糖摄取量下降、Aβ的生成和分泌、老年斑的形成及tau蛋白磷酸化都有紧密联系[60]。此外, 过氧化酶增殖因子活化受体γ(peroxisome proliferator-activated receptor gamma, *PPARγ*)基因和胰岛素降解酶(insulin-degrading enzyme, *IDE*)基因对AD和T2D的发生均有影响[61]。对糖尿病小鼠模型的研究发现, *PPARγ*基因的表达会被DNA甲基化所抑制[62], 而*IDE*基因被认为是T2D和AD之间的连接点[63], 可能作为同时治疗这两种疾病的潜在靶点。临床研究表明, AD和脑血管疾病(cerebrovascular disease, CVD)有关[64], 且两种疾病与脂质代谢异常密切相关[65], 载脂蛋白E(apolipoprotein E, *APOE*)基因是胆固醇代谢机制中的重要基因[66], 同时还参与免疫调节及神经组织的再生[67], 此外, 该基因的DNA甲基化修饰程度表现出显著的个体差异[68]。在记忆功能障碍人群中, 血管风险因子对伴有AD的CVD患者影响更显著[69]。因为AD的发病机制与免疫炎症有关, 其中免疫相关基因Toll样受体9(Toll-like receptor 9, *TLR9*)基因低甲基化修饰会引起脑内炎症, 甚至产生胰岛素抵抗[70]。研究发现, 高半胱氨酸血症会导致认知功能障碍, 甚至有发展为AD的风险[71], 而叶酸还原酶基因(methylene tetrahydrofolate reductase, *MTHFR*)的异常表达将影响同型半胱氨酸水平, 从而增加患LOAD的风险, 有研究发现, *MTHFR*在AD患者大脑组织中甲基化水平有明显的升高[68]。高胆固醇血症与AD相关, 而3β-脱氢胆固醇-△24还原酶基因(24-

dehydrocholesterol reductase, *DHCR24*)与胆固醇合成相关[72]。有研究表明, *DHCR24*基因在AD患者大脑中表达量下降[73], 而该基因启动子活性受DNA甲基化的调节[74], 在神经瘤细胞研究中发现其过度表达能赋予细胞抗Aβ毒性[75]。综上, 进一步研究AD及其相关疾病的表观遗传学进展是特别有意义的, 有助于我们更好地理解AD的分子病理机制。

3 外周血中DNA甲基化修饰水平检测的应用

由于脑组织在科学研究与临床应用中均不易取得, 构建AD患者外周血中相关生物标记物的表达谱已成为寻找高敏感、高特异性血液生物学标记物的重要前提[36]。理想的生物学标记应该能用于评估AD患者遗传倾向与环境暴露因素相互作用的共同效果。由于中枢神经系统能够影响外周血中淋巴细胞分裂、神经递质、激素等相关基因的表达, 故外周血中相应基因的表达水平与脑组织中的表达具有平行性[76]。研究者们对此进行了探讨, 发现受试者工作特征曲线(receiver operating characteristic, ROC)在AD确诊患者血浆中桥连整合因子1(bridging integrator 1, *BIN1*)基因敏感性和特异性分别是73%和75%, 该研究结果表明可以使用血浆中的*BIN1*作为AD诊断的生物标志物[77]。此外, *SORL1*也可能参与AD的发病机制, 研究表明, 该基因由于启动子甲基化修饰水平的差异使得在AD患者和同龄健康老人的血液及大脑中表达量不同, 是衰老相关基因, 可以作为血液中AD诊断的生物标志物[32]。衰老相关基因*HTERT*也被发现在AD患者外周血中甲基化频率显著高于正常老年对照, 该基因也可以作为诊断AD的标志物[33]。另外, 外周血中组蛋白去乙酰化酶(histone deacetylase, HDAC)[14]、铜离子水平[14]、酰胺水解酶(amidohydrolase)[78]相关基因的异常甲基化修饰均可能导致AD的发生, 也许能够作为诊断AD的血液标志物的候选基因。此外, *IL-8*基因在AD不同病程中甲基化水平不一致, 还可作为监测AD疾病发展状态的生物标志物[14]。

4 结语与展望

AD是一种神经退行性疾病, 多发于65岁以上的老人, 随着年龄增长发病率急剧上升。在中国社会老龄化加剧的大背景下, AD的诊断和治疗就成为亟待解决的重点和难点问题。AD发病机制复杂, 病因至今不明, DNA甲基化是表观遗传学的重要内容, 且已被证明对AD的发生和发展起着重大作用, 我们实验室一直致力于研究DNA甲基化修饰在各种疾病如高血压[79]、冠心病[80-81]、直肠癌[82]、T2D[83-85]和精神分裂症[86]中的潜在作用, 并已发现多个基因启动子区域甲基化修饰程度和疾病密切相关。本文着重从环境、年龄和AD相关疾病三方面来阐述DNA甲基化修饰对AD发病机制的影响, 并详细介绍了AD相关基因甲基化的研究进展。显然, 相关基因DNA甲基化修饰可以通过改变基因表达水平对AD产生直接或间接的影响。AD作为一种脑部神经系统性疾病, 其脑组织的获取方法一直是AD研究项目中的难点, 严重阻滞AD病因研究的进程, 基于此, 从血液中寻找到准确诊断AD的基因标志物就显得尤为重要。通过AD相关基因DNA甲基化程度的检测来做到快速诊断, 并且有望通过目的基因的靶向治疗来延缓AD病程甚至达到治疗目的, 将为AD研究开启新的篇章。

此外, 除DNA甲基化修饰外, 表观遗传学中组蛋白修饰和非编码RNA在AD的发病机制中也存在一定影响。有研究表明, 组蛋白乙酰化程度在AD患者大脑额叶中明显低于年纪相仿的正常人[87], 这暗示组蛋白修饰极有可能和AD相关。值得一提的是, 在哺乳动物中已发现DNA羟甲基化修饰, 而DNA羟甲基化很可能是主动或被动参与到DNA去甲基化的过程中, 但目前DNA羟甲基化的研究和检测方法尚不完善, 仍需要继续研究。综上所述, DNA甲基化修饰在AD发病机制中存在着不可忽视的作用, 但目前相关研究深度依然存在很大的提升空间。

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