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MPTP 诱导的帕金森病小鼠模型黑质脑组织 DNA 甲基化研究 *

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摘要 为研究 DNA 甲基化在帕金森病发病机制中的作用，本研究用环境毒素 1- 甲基 -4- 苯基 -1, 2, 3, 6- 四氢吡啶(MPTP)连续腹腔给药诱导小鼠帕金森病(Parkison's disease，PD)模型，应用 ELISA 检测小鼠黑质脑组织总体甲基化水平，应用实时荧光定量 PCR 方法检测 DNA 甲基转移酶表达水平，探讨 MPTP 诱导的小鼠 PD 模型黑质部位是否存在 DNA 甲基化异常．进一步应用甲基化 DNA 免疫共沉淀结合 DNA 甲基化芯片方法，构建 MPTP 诱导的小鼠 PD 模型黑质脑组织 DNA 甲基化谱，并寻找 DNA 甲基化修饰异常的 PD 相关基因对其进行验证．结果表明，模型组小鼠黑质脑组织 DNA 总体甲基化水平较对照组显著降低，Dnmt1 的表达水平显著增高．利用 DNA 甲基化芯片在全基因组内筛选出甲基化差异修饰位点共 48 个，涉及 44 个基因，这些甲基化差异基因参与信号转导、分子转运、转录调控、发育、细胞分化、凋亡调控、氧化应激、蛋白质降解等生物学过程．在甲基化差异修饰基因中，对 *Uchl1* 基因及 *Arih2* 基因进行了甲基化水平以及表达水平的验证．结果表明，模型组小鼠黑质脑组织 *Uchl1* 启动子区域甲基化水平较对照组增高，mRNA 及蛋白质表达水平降低，*Arih2* 启动子区域甲基化水平较对照组降低，mRNA 及蛋白质表达水平增高．实验结果进一步证实，DNA 甲基化修饰异常在帕金森病发病机制中有重要作用，环境因素(如 MPTP)可以通过改变 DNA 甲基化修饰参与帕金森病的发生发展．

关键词 帕金森病，DNA 甲基化，总体甲基化，DNA 甲基转移酶，泛素羧基末端水解酶 1
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帕金森病(Parkison's disease，PD)是一种常见的神经退行性疾病，在中国 65 岁以上的老年人中患病率约为 1%～2%，且随着年龄增长有增加的趋势[1]．PD 的发病机制十分复杂，目前认为与遗传因素和环境因素的相互作用有关．最新研究表明，表观遗传学机制在神经退行性疾病中有重要作用．表观遗传是指 DNA 序列不发生变化的前提下，基因表达却发生可遗传的改变．DNA 甲基化是发现最早的、最基本的，也是研究最多的表观遗传学机制．DNA 甲基化与神经退行性疾病的关系已逐渐成为研究的新热点之一．

在 PD 发病机制中 DNA 甲基化作用的相关研究不多．有研究者将内源性甲基供体 SAM 注入啮齿类动物脑内可导致 PD 样改变，提示过度甲基化可能为 PD 的诱发因素[2-3]，但有研究发现 PD 患者外周血中 SAM 相对含量下降[4]．PD 相关基因甲基化研究发现，PD 患者黑质密部细胞中的 TNF-α 启动子甲基化水平降低，SNCA 内含子 1 甲基化水平降低[5]，提示低甲基化可能与 PD 发病相关．这些研究结果存在一些矛盾之处，且尚无从基因组水平对 PD 的 DNA 甲基化进行全面探索研究．

1- 甲基 -4- 苯基 -1, 2, 3, 6- 四氢吡啶(1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine hydrochloride,

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MPTP)作为最早被发现、最经典的可导致选择性黑质部位多巴胺能神经元变性损伤的环境因素，其毒性作用依赖 N- 甲基基团的存在[6]. 在 PD 患者和 MPTP 诱导的动物模型的黑质部位存在蛋白质和脂质的 N- 甲基化修饰异常[7-8]，提示 MPTP 模型诱导的 PD 模型黑质部位可能存在 DNA 甲基化修饰异常. 表达谱研究表明，MPTP 诱导的灵长类和啮齿类 PD 模型黑质脑组织基因的表达存在显著的改变，而 DNA 甲基化作为一种基因表达的重要调控机制，是否参与了 PD 动物模型黑质部位神经元变性损害和 PD 症状的发生目前尚不清楚.

本研究利用环境毒素 MPTP 建立 PD 动物模型，从总体甲基化、全基因组甲基化以及 PD 相关特异基因甲基化三个角度对此动物模型黑质部位的 DNA 甲基化改变进行研究，探讨 DNA 甲基化异常在环境因素诱导的 PD 动物模型中的作用.

1 实验方法

1.1 实验材料

1.1.1 实验动物

雄性 C57/BL 小鼠，鼠龄 8 周，体重 25～28 g, 符合国家卫生部颁发的实验动物 SPF 清洁级标准，由中南大学医学遗传学国家重点实验室提供.

1.1.2 主要试剂

1- 甲基 -4- 苯基 -1, 2, 3, 6- 四氢吡啶(MPTP)(Sigma 公司)，DNA 提取试剂盒(Qiagen 公司)，逆转录试剂盒(Fermentas 公司)，荧光定量 PCR 试剂盒(大连宝生物公司)，总体甲基化试剂盒(Epigentek 公司)，Trizol(Sigma 公司)，重亚硫酸盐转化试剂盒(Qiagen 公司)，凝胶回收纯化试剂盒(Promega 公司)，pGEM-T Easy Vector System Ⅰ(Promega 公司)，DH5α 感受态细胞(天根生化公司)，anti-Uchl1 (Abcam 公司)，anti-Arih2 (Genetex 公司)，anti-GAPDH(Santa 公司).

1.2 实验方法

1.2.1 动物的分组及造模

C57/BL 小鼠共 92 只，饲养于中南大学动物实验中心，随机分为对照组和模型组，每组 46 只. 模型组小鼠按体重给予 MPTP 25 mg•kg^{-1}•d^{-1} 腹腔注射，对照组小鼠给予等容积的生理盐水腹腔注射，每天于同一时间给药，连续给药 10 天. 分别在给药 10 天并稳定 3 天后取材.

1.2.2 黑质组织分离

10%水合氯醛麻醉小鼠后，断头法迅速处死. 参照 ALLEN BRAIN ATLAS 在线图谱(http://mouse.brain-map.org/)和 Karunakaran 等[9]的方法分离黑质部位脑组织. 组织分离后迅速于电子天平称重，立即置于液氮中，–80℃ 保存.

1.2.3 DNA 总体甲基化水平的测定

采用 DNA 提取试剂盒提取小鼠脑组织基因组 DNA. 用总体 DNA 甲基化试剂盒进行 DNA 总体甲基化水平检测，具体操作参照试剂盒说明书. 所有样品(标准品，待测样本及阴性对照样品)均于培养板设置复孔；每板均设置标准曲线，避免板间差异. 根据已知浓度的标准品吸光度(A)值绘制标准曲线，根据待测样本的 A 值由标准曲线求得待测样本对应的 DNA 甲基化水平.

1.2.4 实时荧光定量 PCR

采用 Trizol 试剂提取总 RNA. 用逆转录试剂盒进行 mRNA 逆转录成 cDNA 反应. 用荧光定量 PCR 试剂盒检测目的基因的表达水平. PCR 反应在 ABI Prism 7900 荧光定量 PCR 仪上进行，用两阶段法 PCR，反应条件：预变性(95℃，30 s)；变性(94℃，5 s)，退火(退火温度 T_m 见表 1，30 s)，进行 40 个循环；熔解(95℃，15 s；60℃，15 s；95℃，15 s). 引物在参照文献[10−11]的基础上，由 PRIMER 5.0 软件设计，深圳华大基因有限公司合成. 采用 β-actin 基因作为内参，各引物序列及扩增片段大小及退火温度见附件表 S1.

1.2.5 甲基化 DNA 免疫共沉淀结合甲基化芯片(methylated DNA immunoprecipitation microarray, MeDIP-Chip)

该实验由博奥生物有限公司完成. 基因组 DNA 用 *Mse* Ⅰ酶切消化，并对酶切产物进行纯化，电泳质检，一部分酶切后的 DNA 样品预留为对照 Input 样品，另一部分进行免疫共沉淀(称为 IP 样品)；将抗 5- 甲基化胞嘧啶抗体与 DNA 样品进行孵育，磁珠富集抗体 -DNA 复合物；蛋白酶 K 消化 DNA- 抗体 - 磁珠复合物，分离 DNA 片段，并进行纯化；取等量 IP 和 Input 样品进行 WGA 扩增，并对扩增产物进行纯化；用 Klenow 酶进行标记(IP 标记 cy5+Input、标记 cy3)，将标记好的 IP 样品和 Input 样品杂交到同一个点阵上进行芯片杂交，扫描、图像采集并进行数据分析，利用 IP/Input 计算并确定 peak，有显著意义的 peak 即为 DNA 甲基化区域；比较两组样品之间的 peak，定位差异甲基化 peak 的位置并进行基因注释.

1.2.6 重亚硫酸盐测序(bisulfate sequencing PCR,

BSP)

采用 Methyl Primer Express v1.0 及 MethPrimer 针对 CpG 岛设计 BSP 的引物，引物由深圳华大基因有限公司合成．*Uchl1* 引物序列：上游引物为 5′ GGGGGTTTGGTTTGTATTATTTT 3′；下游引物为 5′ CCACCTCCATTACACAAAAC 3′．BSP 扩增区域为包括转录起始位点(transcriptional start site, TSS)在内的长度为 453 bp 的片段(TSS –185 bp～+268 bp)，覆盖启动子的主要活性部分以及 CpG 岛，包含 26 个 CpG 位点(图 1)．*Arih2* 引物序列：上游引物为 5′ GTTAGAAGTTTGGGAGTTTTT-AGGG 3′；下游引物为 5′ ACCAATCAAAAAT-AAACAAAACCTC 3′．BSP 扩增区域为包括转录起始位点(TSS)在内的长度为 239 bp 的片段(TSS –61 bp～+178 bp)，包含 34 个 CpG 位点(图 2)．采用重亚硫酸盐转化试剂盒将未甲基化胞嘧啶(C)转化为尿嘧啶(U)，而甲基化的 C 不变．以修饰后的 DNA 作为模板进行 PCR 扩增：总反应体系为 10 μl，其中上、下游引物各为 0.2 μl，甲基化修饰后的 DNA 为 1.0 μl．反应条件为：预变性(95℃，5 min)；变性(94℃，30 s)，退火(53℃，45 s)，延伸(72℃，45 s)，进行 35 个循环；延伸(72℃，10 min)．产物采用 TA 克隆，将其连接到 pGEM-T 质粒载体，转化至 DH-5α 感受态细胞后，挑选 10 个阳性克隆测序，测序结果采用 DNA STAR 软件进行分析．

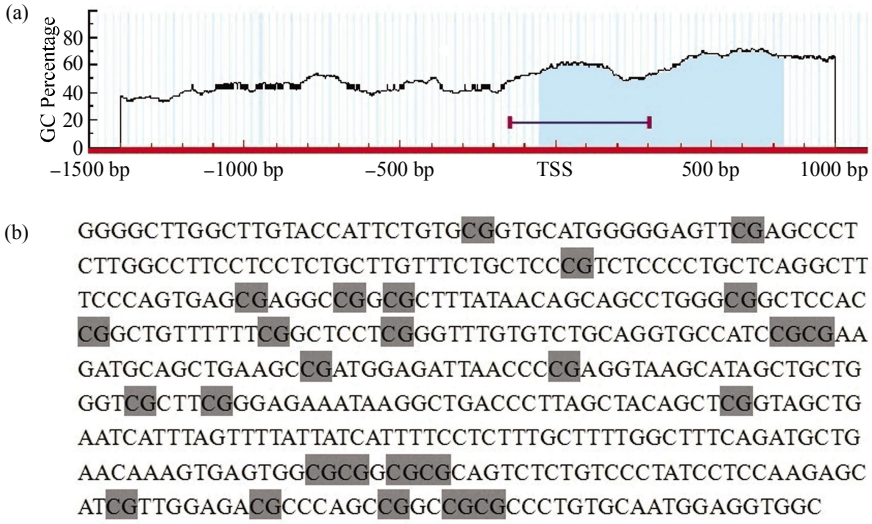

Fig. 1　Schematic drawing of the amplified region of *Uchl1* by BSP

(a) The transcriptional start site is marked by TSS. The blue region represents CpG island, the amplified fragment located between bisulfate PCR primers( ), including part of the CpG island and entire activated promoter region. (b) Sequence of amplified fragment of *Uchl1* (TSS –185 bp～+268 bp) and 26 CpG sites (highlighted in grey) in this region.

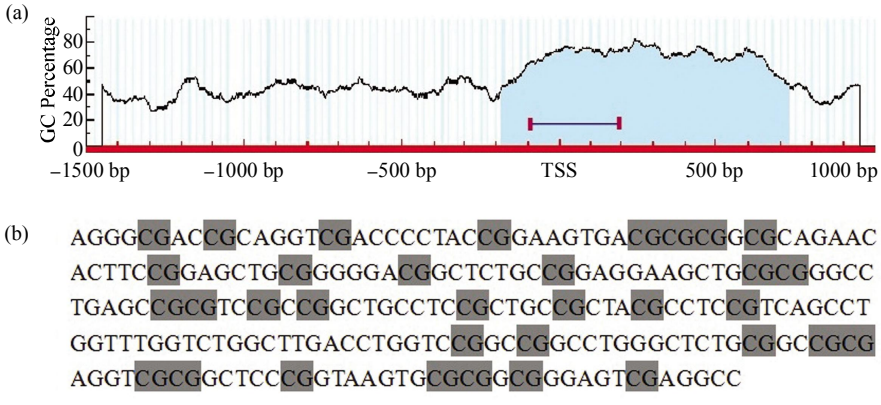

Fig. 2　Schematic drawing of the amplified region of *Arih2* by BSP

(a) The transcriptional start site is marked by TSS. The blue region represents CpG island, the amplified fragment located between bisulfate PCR primers( ). (b) Sequence of amplified fragment of *Arih2* (TSS –61 bp～+178 bp) and 34 CpG sites (highlighted in grey) in this region.

1.2.7　Western blot 检测蛋白质表达水平

将切除的脑组织迅速置于预冷的生理盐水中，漂洗数次至清洁干净．加入裂解液冰上超声裂解组织细胞，离心(4℃，13 000 r/min，5 min)后取上清，采用 BCA 法定量蛋白质浓度．将组织细胞裂解液加入等体积 2×SDS 样本缓冲液，煮沸变性 5 min．取 15 g 样品在 10%十二烷基硫酸钠聚丙烯酰胺凝胶上电泳后，半干电转至硝酸纤维素膜，以标准蛋白 (marker)为参照，取相应条带分别加入 anti-Uchl1 抗体(1∶4 000)、anti-Arih2 抗体(1∶4 000)以及 anti-GAPDH 抗体(1∶2 000)，4℃过夜，TBST 洗 4 次，每次 5 min；然后分别加入二抗 4℃过夜，TBST 洗 4 次，每次 10 min．加入辣根过氧化物酶室温下孵育 10 min 后显色．将特异性蛋白条带扫描后用 Gel pro4.0 凝胶吸光度分析软件测定吸光度并进行分析．

1.2.8　统计分析

数据结果以均数±标准误($\bar{x} \pm s$)表示，用 SPSS13.0 统计软件进行相关数据处理及统计分析，$P < 0.05$ 为差异具有统计学意义．

2　实验结果

2.1　DNA 总体甲基化

如图 3a 所示，模型组小鼠中脑黑质 DNA 总体甲基化水平较对照组显著降低[对照组(8.66±0.29)% *vs*. 模型组(7.60±0.33)%，P=0.028].

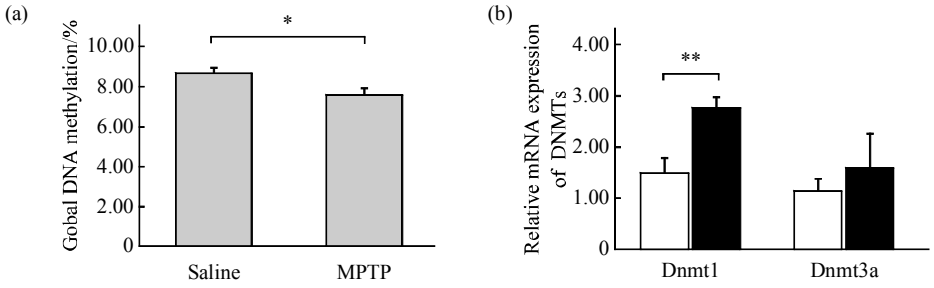

Fig. 3　Global methylation and Dnmts expression in SN region of MPTP-treated mice and saline controls

(a) Global DNA methylation in SN region was decreased in MPTP-treated mice compared to saline controls (P=0.028). (b) Relative mRNA expression of Dnmt1 and Dnmt3a in SN region of MPTP-treated model and saline controls. Dnmt1 expression was significantly increased in MPTP-treated mice compared to sanline controls ($P < 0.001$). *$P < 0.05$, **$P < 0.01$. □: Saline; ■: MPTP.

2.2　DNA 甲基转移酶

Dnmt1 和 Dnmt3a 在小鼠黑质部位组织均有表达(图 3b)，Dnmt3b 在小鼠黑质部位的表达低于检测阈值．模型组小鼠黑质 Dnmt1 表达水平较对照组显著增高[对照组(1.50±0.13)% *vs*．模型组(2.76±0.09)%，$P < 0.001$]，两组 Dnmt3a 表达水平无显著差异[对照组(1.15±0.09)% *vs*. 模型组(1.58±0.27)%，P=0.165].

2.3　甲基化差异修饰基因筛选

全基因组 DNA 甲基化芯片分析发现对照组小鼠黑质部位 DNA 有 723 个位点发生高甲基化，模型组小鼠黑质部位 DNA 有 640 个位点发生高甲基化．和对照组小鼠相比，在全基因组内筛选出甲基化差异修饰位点共 48 个，涉及 44 个基因，其中甲基化程度增高的基因 5 个(附件表 S2)，甲基化程度降低的基因 39 个(附件表 S3)．这些甲基化差异基因参与信号转导、分子转运、转录调控、发育、细胞分化、凋亡调控、氧化应激、蛋白质降解等生物学过程．

2.4　*Uchl1* 启动子区 DNA 甲基化水平及表达水平

BSP 测序得出 26 个 CpG 位点在两组中的甲基化程度如图 4a 所示．CpG1 位点在两组中甲基化率都为 0%，在其他所有 25 个位点中，除 CpG3 和 CpG24 位点外，模型组的甲基化程度均较对照组增高，但仅 CpG7 位点的甲基化程度在两组中的差异具有统计学意义(对照组 0.00% *vs*．模型组 11.58%，P=0.001)．模型组小鼠 *Uchl1* 启动子区平均甲基化水平较对照组增高[对照组(1.20±0.28)% *vs*. 模型组(4.55±0.53)%，$P < 0.001$](图 4b)，mRNA 表达水平较对照组降低[对照组(1.35±0.09)% *vs*. 模型组(1.06±0.03)%，P=0.017](图 4c)，蛋白质表达水平较对照组降低[对照组(0.92±0.05)% *vs*. 模型组(0.22±0.04)%，P=0.004](图 4d, e).

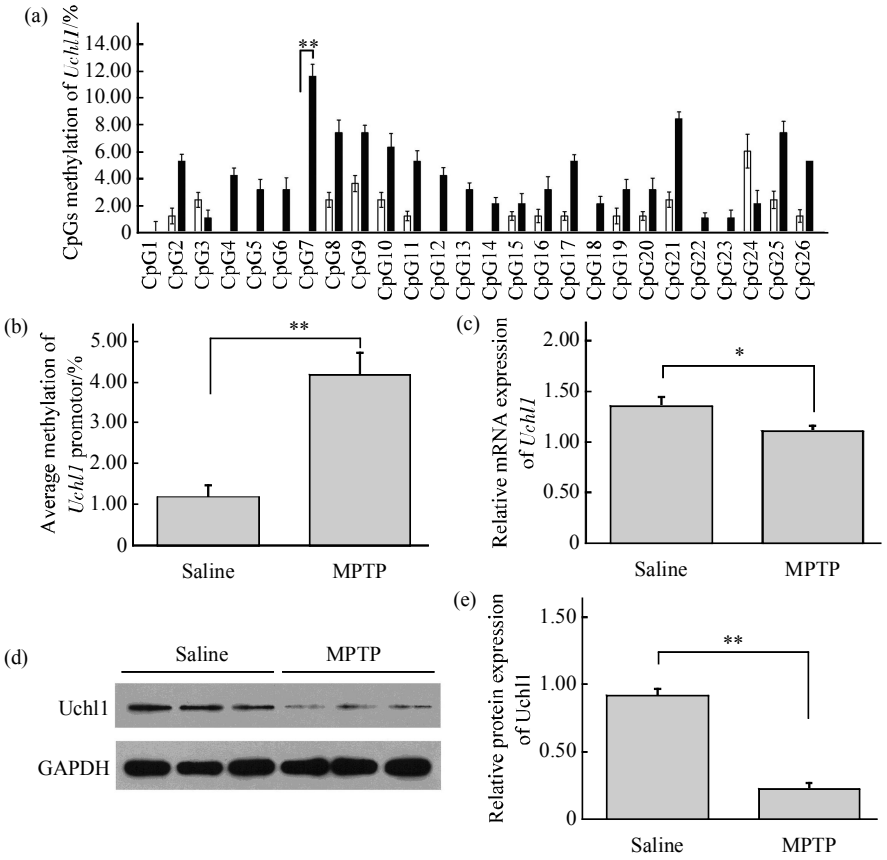

Fig. 4　Methylation and expression of *Uchl1* in SN region of MPTP-treated mice and saline controls

(a) Site specific methylation levels (percentages of methylated CpG at particular CpG site) of 26 CpG sites. CpG7 was hypermethylated in MPTP-treated group compared to controls (P=0.001). □: Saline; ■: MPTP. (b) Average methylation levels of *Uchl1* promoter (average percentages of methylated CpG of all 26 CpG sites). The average methylation level was higher in MPTP-treated group compared to controls ($P < 0.001$). (c) Relative mRNA expression of *Uchl1* in SN was decreased in MPTP-treated group compared to controls (P=0.017). (d) Western blot of Uchl1 protein expression in SN. (e) Relative protein expression of Uchl1 in SN was decreased in MPTP-treated group compared to controls (P=0.004). *$P < 0.05$, **$P < 0.01$.

2.5　*Arih2* 启动子区 DNA 甲基化水平及表达水平

BSP 测序得出 34 个 CpG 位点在两组中的甲基化程度如图 5a 所示，单个 CpG 位点的甲基化程度在两组中均无显著差异．模型组小鼠 *Arih2* 启动子区平均甲基化水平较对照组降低[对照组(1.38±0.22)% *vs*. 模型组(0.71±0.13)%，P=0.011](图 5b)，mRNA 表达水平较对照组增高[对照组(0.99±0.19)% *vs*. 模型组(1.78±0.09)%，P=0.005](图 5c)，蛋白质表达水平较对照组增高[对照组(1.24±0.13)% *vs*. 模型组(2.34±0.28)%，P=0.024](图 5d, e).

3　讨　　论

一碳单位循环是生成甲基供体 SAM 的重要步骤，叶酸及 B 族维生素的缺乏，同型半胱氨酸(Hcy)及 S- 腺苷同型半胱氨酸(SAH)水平增高可通过影响 SAM 生成或 / 和抑制 DNA 甲基转移酶活性，从而导致 DNA 甲基化障碍[12]．实验表明，给予 SAH 或 Hcy 能够加重 MPP^+ 对中脑原代细胞的毒性作用[13-14]．给予缺乏叶酸的食物或高 Hcy 的食物可加重 MPTP 对小鼠的多巴胺能神经元毒性作用[15-17]，提示 DNA 低甲基化修饰可能加重 MPTP 诱导的 PD 小鼠模型黑质部位 DA 能神经元损害．本研究显示，与对照组相比，模型组小鼠黑质部位 DNA 总体甲基化水平显著降低，支持低甲基化改变与 PD 黑质部位神经元退行变性相关，并与此前帕金森病患者脑组织中的发现一致．导致 PD 患者及 PD 动物模型脑组织 DNA 甲基化水平降低的原因目前尚不清楚．除上述甲基供体 SAM 生成障碍外，Desplats 等[18]证实，α-syneuclein 能与 DNMT1

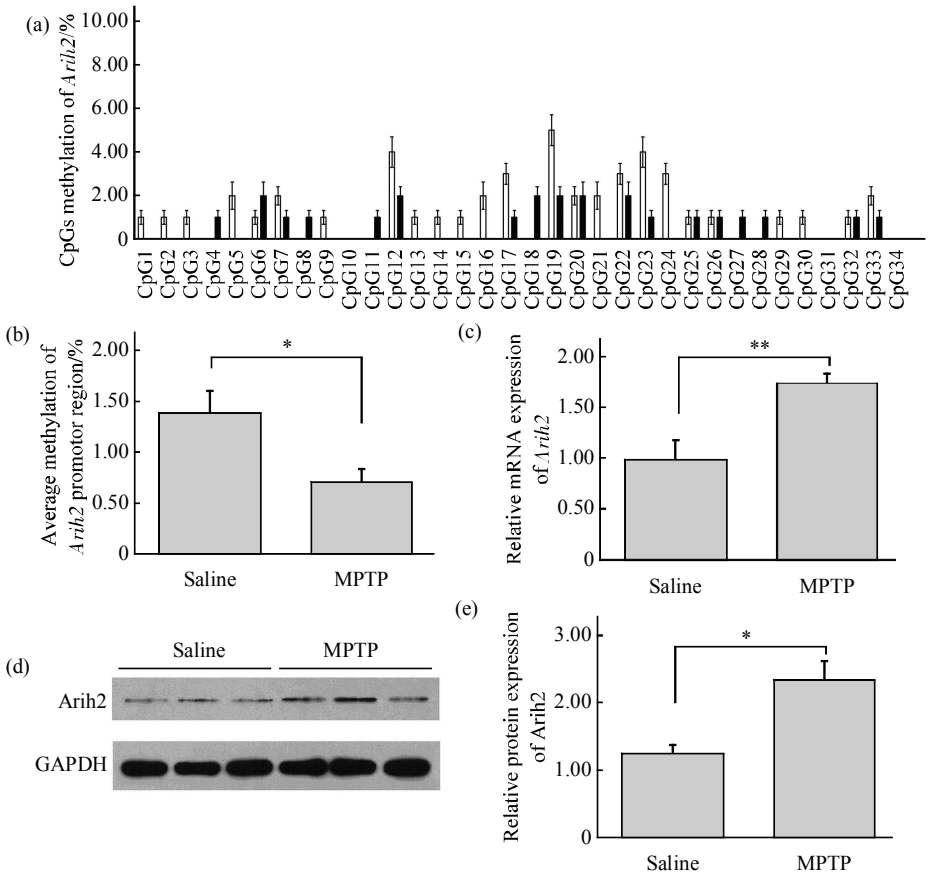

Fig. 5　Methylation and expression of *Arih2* in SN region of MPTP-treated mice and saline controls

(a) Site specific methylation levels (percentages of methylated CpG at particular CpG site) of 34 CpG sites. □ : Saline; ■ : MPTP. (b) Average methylation levels of *Arih2* promoter (average percentages of methylated CpG of all 34 CpG sites). The average methylation level was decreased in MPTP-treated group compared to controls (P=0.011). (c) Relative mRNA expression of *Arih2* in SN was decreased in MPTP-treated group compared to controls (P=0.005). (d) Western blot of Arih2 protein expression in SN. (e) Relative protein expression of Arih2 in SN was decreased in MPTP-treated group compared to controls (P=0.024).*P < 0.05, **P < 0.01.

相互作用，将 DNMT 滞留于胞质中，导致细胞核内 DNMT1 缺乏，而 DNMT1 是维持 DNA 甲基化的主要甲基转移酶，细胞核内 DNMT1 缺乏可能影响 DNA 甲基化过程，导致全基因组低甲基化改变.

研究显示在神经元凋亡过程中，Dnmt1 和 Dnmt3a 表达均上调[19]，提示 DNA 甲基转移酶的表达改变可能在神经元退变凋亡的过程中起到了重要作用．因此本研究对 MPTP 诱导小鼠 PD 模型黑质部 Dnmts 的表达水平进行检测，发现 PD 模型组黑质脑组织总体甲基化降低，但 Dnmt1 的表达水平显著增高，提示 Dnmt1 表达水平的改变不是导致 MPTP 诱导小鼠 PD 模型黑质组织 DNA 甲基化水平降低的原因．Slack 等[20]研究显示，在肿瘤细胞中，全基因组低甲基化可以导致 Dnmt1 基因启动子区 AP-1 依赖调节元件低甲基化改变，从而激活转录，增加 Dnmt1 表达水平．这种负反馈机制提示，Dnmt1 表达水平增高可能是全基因组甲基化水平降低的补偿机制．此外，研究表明，给予 DNA 甲基转移酶抑制剂可以导致全基因组甲基化水平降低，同时 DNA 甲基转移酶表达水平增高[21]，这与本研究的结果相似，提示 MPTP 可能通过抑制 DNA 甲基转移酶的活性起作用.

通过芯片筛选出的甲基化差异修饰的位点中，泛素羧基末端水解酶 1 (ubiquitin C-terminal hydrolase L1，*UCHL1*) 是一个已知的 PD 致病基因．*UCHL1* 属于泛素蛋白酶体系统 (ubiquitin proteasome system，UPS)，在错误折叠蛋白的降解

过程中发挥重要作用，其表达下调和活性下降可影响神经元泛素化 / 去泛素化机制的功能，导致错误折叠的蛋白聚集、突触退化和神经元变性．研究表明 *UCHL1* 在 PD 患者脑黑质中表达下降[22-24]，在 MPTP 诱导的 PD 动物模型中表达下降[25-26]．调控 *UCHL1* 表达水平的机制尚不明确，DNA 甲基化作为一种重要的表观遗传调控机制，*UCHL1* 启动子区的高甲基化可能是导致其表达降低的原因．多项肿瘤相关研究已证实 *UCHL1* 的表达水平受其启动子甲基化水平的调控[27-28]．在神经退行性领域，已有研究者开展了 *UCHL1* 启动子区甲基化水平的研究．Barrachina 等[29]对帕金森病患者大脑皮质 *UCHL1* 启动子进行甲基化水平检测，没有发现甲基化水平的改变．但是此研究用的标本并非来自黑质 - 纹状体系统，而有研究表明 *UCHL1* 在 PD 患者大脑皮质中的表达并没有显著改变[24]．我们对 PD 动物模型黑质组织中覆盖 *Uchl1* 启动子的主要活性部分以及 CpG 岛的一段长 453 bp 的区域进行重亚硫酸盐测序，结果显示在正常对照组和 PD 模型组中，*Uchl1* 均呈低甲基化状态，这与其高表达的特征是相符合的．模型组小鼠 *Uchl1* 启动子区的平均甲基化程度增高，并且表达水平降低，提示其表达下降可能与其启动子高甲基化修饰有关．

通过芯片筛选出的甲基化水平降低的基因中，*Arih2*(ariadne homolog 2)同样属于泛素蛋白酶体系统．在小鼠中 *Arih2* 基因的功能研究非常有限，但是其人类的同源蛋白 *HHARI* (human homolog of drosophila ariadne-1)有着与另一个已知的 PD 致病基因 *PARK2*(*PARKIN*)非常相似的结构和功能．HHARI 作为一种泛素连接酶参与 USP 蛋白降解途径，对 *PARKIN* 基因表达减少产生的损害有保护作用[30]．本研究通过 BSP 测序发现 *Arih2* 启动子区甲基化水平极低，MPTP 组小鼠黑质脑组织 *Arih2* 启动子区甲基化水平较对照组更低，其甲基化水平的变化与 mRNA 及蛋白质表达水平改变相符合，提示环境因素可能通过影响 *Arih2* 基因的 DNA 甲基化修饰调控其表达，但是其表达异常对 UPS 功能通路的影响是否参与 PD 发病尚无确切依据．

综上所述，本研究首次对环境毒素诱导的 PD 动物模型黑质脑组织进行全基因组 DNA 甲基化研究，发现在 MPTP 诱导的 PD 小鼠模型中脑黑质部位 DNA 总体甲基化水平降低，而 Dnmt1 表达水平增高，*Uchl1* 启动子区甲基化程度增高，mRNA 及蛋白质表达水平下降，*Arih2* 启动子区甲基化水平降低，mRNA 及蛋白质表达水平增高．本实验结果为环境因素通过改变 DNA 甲基化修饰参与帕金森病的发生提供了证据．但是，本研究中发现的 DNA 甲基化修饰异常究竟是 PD 的发病原因还是疾病进展的结果尚难以明确．同时，虽然我们证实了环境因素 MPTP 能够导致这些 DNA 甲基化修饰改变，但是同样的环境因素如何对不同基因的甲基化修饰产生不同影响还需要进一步研究．此外，DNA 甲基化不是一个独立的表观遗传学过程，与表观遗传的其他机制，例如 DNA 羟甲基化、组蛋白修饰、非编码 RNA 等有着复杂的相互作用，因此探讨其他表观遗传机制的改变及其与 DNA 甲基化的复杂平衡才能更有利于全面理解 PD 的表观遗传学发病机制．

附件 表 S1～S3 见本文网络版附录(http://www.pibb.ac.cn)

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Abnormal DNA Methylation in Substantia Nigra Region of MPTP-induced Mouse Model of Parkinson′s Disease*

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Abstract The importance of DNA methylation in neurodegenerative diseases has been increasingly recognized. We explored role of DNA methylation in the pathogenesis of Parkinson's disease in mouse model induced by MPTP. The global DNA methylation levels of substantia nigra region were measured by ELISA Kit. Expression of Dnmt1 and Dnmt3a were measured by Real-time PCR. Genome-wide profile was performed using methylated DNA immunoprecipitation microarray (MeDIP-Chip). Methylation status of differential methylated gene was validated by bisulfate sequencing and expression of differential methylated gene was determined by Real-time PCR. We have demonstrated that global methylation level was significantly decreased while expression levels of Dnmt1 was significantly increased in substantia nigra region of MPTP-induced mouse model compared to saline controls. Genome-wide DNA methylation analysis detected 48 sites, involving 44 genes, with significantly altered DNA methylation. The abnormal-methylated genes involved in the biological processes concerning signal transduction, molecular transport, transcription modulation, development, cell differentiation, regulation of apoptosis, oxidation reduction and protein catabolism. The methylation levels of promoter region of *Uchl1* in substantia nigra region of MPTP-treated mice were significantly higher than that in the saline controls, with significant decreased expression of mRNA and protein. The methylation levels of promoter region of *Arih2* in substantia nigra region of MPTP-treated mice were significantly decreased than that in the saline controls, with significant increased expression of mRNA and protein. These results suggested that DNA methylation were altered in substantia nigra region of MPTP-induced PD model. DNA methylation may play important role in the pathogenesis of PD induced by environmental factors such as MPTP.

Key words Parkinson's disease, DNA methylation, global methylation, DNA methyltransferases, ubiquitin C-terminal hydrolase L1

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附 录

Table S1 Primers and conditions for real-time PCR analysis

Gene		Primer sequence(5′-3′)	Size/bp	Annealing temperature/(℃)
β-actin	F	TCTCCATGTCGTCCCAGTTG	121	60
	R	ATGGTGGGAATGGGTCAGAAG		
Dnmt1	F	CCTAGTTCCGTGGCTACGAGGAGAA	137	60
	R	TCTCTCTCCTCTGCAGCCGACTCA		
Dnmt3b	F	TTCAGTGACCAGTCCTCAGACACGAA	145	58
	R	TCAGAAGGCTGGAGACCTCCCTCTT		
Uchl1	F	TAGGGCTGGAGGAGGAGA	306	60
	R	CGAAACACTTGGCTCTATCTT		
Arih2	F	GACTTTGTATTTCCCTTGC	256	60
	R	CGAGCCTCAACAAGCAG		

Table S2 Loci with increased methylation in SN region of MPTP-treated mice compared to control

No.	Location	lg Ratio	Description	Biological process	Molecular function
1	Uchl1	1.25	Ubiquitin carboxy-terminal Hydrolase L1	Cell proliferation; ubiquitin-dependent protein catabolism; response to stress; protein deubiquitination; eating behavior; adult walking behavior; neuromuscular physiological process; axon target recognition; axon transport of mitochondrion	protein binding; peptidase activity; ligase activity; ubiquitin thiolesterase activity; ubiquitin binding; omega peptidase activity; cysteine-type endopeptidase activity
2	St8sia1	0.57	ST8 alpha-N-acetyl-neuraminide alpha-2,8- sialyltransferase 1	Cellular response to heat; lipid biosynthetic process; lipid metabolic process; positive regulation of cell proliferation; protein glycosylation; sphingolipid metabolic process	alpha-N-acetylneuraminate alpha-2, 8-sialyltransferase activity; sialyltransferase activity; transferase activity;
3	V1re6	0.55	Vomeronasal 1 receptor, E6.	Response to pheromone	pheromone binding; pheromone receptor activity
4	Alx4	0.37	Aristaless-like homeobox 4	Regulation of transcription; regulation of apoptosis; muscle development; positive regulation of transcription from RNA polymerase Ⅱ promoter; skeletal development; palate development; limb morphogenesis; pattern specification	protein heterodimerization activity; protein binding; transcription factor activity; sequence-specific DNA binding
5	Tln1	0.21	Talin 1	cell motility; cytoskeletal anchoring; cell-substrate junction assembly; cortical actin cytoskeleton organization and biogenesis; intercellular junction assembly	protein binding; actin binding; structural constituent of cytoskeleton; LIM domain binding; vinculin binding

Table S3 Loci with decreased methylation in SN region of MPTP-treated mice compared to control

No.	Location	lg Ratio	Description	Biological Process	Molecular Function
1	Arih2	1.4833	Ariadne homolog 2 (Drosophila)	ubiquitin-dependent protein catabolism	nucleic acid binding; protein binding; zinc ion binding; metal ion binding
2	Fnbp1	1.0750	Biquit binding protein 1	Endocytosis; nervous system development	lipid binding; identical protein binding
3	Schip1	0.9467	Schwannomin interacting protein 1	estrogen metabolic process; nitrogen compound metabolic process; face morphogenesis; female gonad development; fibroblast migration	molecular_function; identical protein binding; protein homodimerization activity
4	Parvg	0.8900	Parvin, gamma	cell adhesion; tumor supressor	protein binding; actin binding
5	Bmp5	0.7875	Bone morphogenetic protein 5	cell differentiation; ossification; pattern specification; growth; cartilage development	protein binding; cytokine activity; growth factor activity
6	Hlx	0.7600	H2.0-like homeobox	regulation of transcription, DNA-dependent; development; cell differentiation	transcription factor activity; sequence-specific DNA binding
7	Klra10	0.6767	Klra10	ND	ND
8	Prom2	0.6650	Prominin 2	ND	ND
9	Yipf1	0.6233	Yip1 domain family, member 1	vesicle transport; membrane integral	ND
10	Fn3k	0.5525	Fructosamine 3 kinase	fructoselysine metabolism; phosphorylation	transferase activity; ructosamine-3-kinase activity
11	Asna1	0.5386	arsA arsenite transporter, ATP-binding, homolog 1	detoxification of arsenic-containing substance; transport	ATP binding; ATPase activity; hydrolase activity; metal ion binding; nucleotide binding
12	Thg1l	0.5300	tRNA-histidine Guanylyltrans-ferase 1-like	tRNA modification; protein homotetramerization	tRNA guanylyltransferase activity; nucleotide binding ; nucleotidyltransferase activity; transferase activity; metal ion binding; magnesium ion binding; tRNA guanylyltransferase activity
13	Acp2	0.5157	Acid phosphatase 2, lysosomal	lysosome organization and biogenesis; response to organic substance	phosphotyrosine binding; acid phosphatase activity; phosphoprotein phosphatase activity;hydrolase activity
14	Eef1e1	0.5125	Eukaryotic translation elongation factor 1 epsilon 1	protein biosynthesis; positive regulation of apoptosis; DNA repair; positive regulation of DNA damage response, signal transduction by p53 class mediator; negative regulation of cell proliferation;	protein binding
15	Cux1	0.4980	Cut-like homeobox	transport; intra-Golgi vesicle-mediated transport; ranscription, DNA-dependent; regulation of transcription, DNA-dependent	DNA binding; chromatin binding; protein binding, bridging; sequence-specific DNA binding transcription factor activity
16	Olfr63	0.4650	Olfactory receptor 63	G-protein coupled receptor signaling pathway; G-protein coupled receptor signaling pathway; detection of chemical stimulus involved in sensory perception of smell; response to stimulus; sensory perception of smell; signal transduction;	G-protein coupled receptor activity; olfactory receptor activity; receptor activity; signal transducer activity
17	Mcm3ap	0.4300	Minichromosome maintenance deficient 3 associated protein	DNA replication; protein import into nucleus	nucleotide binding; DNA binding
18	V1re12	0.4000	Vomeronasal 1 receptor 185	response to pheromone; G-protein coupled receptor protein signaling pathway	pheromone binding; pheromone receptor activity; receptor activity

Continued

No.	Location	lg Ratio	Description	Biological Process	Molecular Function
19	Sstr1	0.3875	Somatostatin receptor 1	G-protein signaling, coupled to cyclic nucleotide second messenger; glutamate signaling pathway;neuropeptide signaling pathway; cell-cell signaling; response to nutrient digestion; negative regulation of cell proliferation	receptor activity; G-protein coupled receptor activity; somatostatin receptor activity
20	Panx2	0.3750	Pannexin 2	cell-cell signaling; ion transmembrane transport; ion transport; transmembrane transport; transport	protein binding; ion transport; transmembrane transport; cell-cell signaling
21	Sf3b1	0.3700	Splicing factor 3b, subunit 2, 145kDa	nuclear mRNA splicing, via spliceosome; RNA splicing; interspecies interaction between organisms	nucleic acid binding; protein binding
22	Hat1	0.3625	Histone acetyltransferase 1	DNA packaging; chromatin silencing at telomere; internal protein amino acid acetylation; chromatin modification; histone acetylation	histone acetyltransferase activity; protein binding; acyltransferase activity; transferase activity
23	Cyp2c44	0.3478	Cytochrome P450, family 2, subfamily c, polypeptide 44	oxidation-reduction process	arachidonic acid epoxygenase activity; metal ion binding; monooxygenase activity; oxidoreductase activity
24	Csde1	0.3467	Cold shock domain containing E1, RNA-binding	regulation of transcription, DNA-dependent; male gonad development	DNA binding; RNA binding; protein binding
25	Fgfbp1	0.3450	Fibroblast growth factor binding protein 1	signal transduction; cell-cell signaling; negative regulation of cell proliferation	heparin binding; growth factor binding
26	Olfr1444	0.3420	Olfactory receptor 1444	G-protein coupled receptor signaling pathway; detection of chemical stimulus; sensory perception of smell; signal transduction	G-protein coupled receptor activity; olfactory receptor activity; receptor activity; signal transducer activity
27	Ilkap	0.3250	Integrin-linkedkinase- associated serine/threonine phosphatase 2C	protein amino acid dephosphorylation; regulation of S phase of mitotic cell cycle; negative regulation of progression through cell cycle	magnesium ion binding; protein serine/threonine phosphatase activity; hydrolase activity; manganese ion binding
28	Gdf5	0.3238	Growth differentiation factor 5	transforming growth factor beta receptor signaling pathway; cell-cell signaling; limb morphogenesis; positive regulation of chondrocyte differentiation; growth; regulation of body size; regulation of apoptosis	cytokine activity; protein binding; growth factor activity
29	Sfrp5	0.3100	Secreted frizzled-related protein 5	apoptosis; brain development; Wnt receptor signaling pathway; cell differentiation establishment and/or maintenance of cell polarity; signal transduction; development; visual perception; morphogenesis; negative regulation of sequence-specific DNA binding transcription factor activity	protein binding; Wnt-activated receptor activity
30	Tmem211	0.3067	Transmembrane protein 211	NA	NA
31	Gpr101	0.2980	G protein-coupled receptor 101	signal transduction; G-protein coupled receptor protein signaling pathway	receptor activity; G-protein coupled receptor activity
32	Gltpd1	0.2900	Glycolipid transfer protein domain containing 1	glycolipid transport; biological_process	glycolipid binding; glycolipid transporter activity; molecular function
33	Gpsm1	0.2850	G-protein signaling modulator 1 (AGS3-like, C. elegans)	signal transduction; development; nervous system development; cell differentiation	GTPase activator activity; binding

Continued

No.	Location	lg Ratio	Description	Biological Process	Molecular Function
34	Olfr216	0.2667	Olfactory receptor 179	G-protein coupled receptor signaling pathway; detection of chemical stimulus; response to stimulus; sensory perception of smell; signal transduction;	G-protein coupled receptor activity; olfactory receptor activity; receptor activity; signal transducer activity
35	Cnih2	0.2600	Cornichon homolog 2 (Drosophila)	transport; intracellular signaling cascade; regulation of alpha-amino-3-hydrox-y-5-methyl-4-isoxazole propionate selective glutamate receptor activity	channel regulator activity; molecular functions
36	Scfd1	0.2233	Sec1 family domain containing 1	vesicle docking during exocytosis; protein transport; vesicle-mediated transport	NA
37	Jazf1	0.2180	AZF zinc finger	negative regulation of transcription from RNA polymerase II promoter; transcription;regulation of transcription, DNA-dependent	nucleic acid binding; transcription corepressor activity; zinc ion binding; metal ion binding
38	Msrb3	0.1850	Methionine sulfoxide reductase B3	oxidation reduction	protein-methionine-S-oxide reductase activity; zinc ion binding;oxidoreductase activity; metal ion binding
39	V1rc5	0.1700	Omeronasal 1 receptor, C5'	sensory perception of chemical stimulus	receptor activity