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污染物与生物大分子互作及效应专题 评 述
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环境污染物的毒性作用与线粒体DNA的变化

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摘要 线粒体作为细胞有氧呼吸的主要场所, 是为细胞供能的细胞器, 具有一套独立于核DNA的遗传物质, 即线粒体DNA(mitochondrial DNA, mtDNA). mtDNA编码了37个基因, 包括13个呼吸链相关的多肽、22种tRNA以及2种rRNA基因. 线粒体异常可直接导致细胞三磷酸腺苷(adenosine triphosphate, ATP)合成减少、细胞能量不足. mtDNA拷贝数及表观遗传的调控对于线粒体行使基本功能至关重要. 环境污染物进入生物体内后诱导产生大量活性氧(reactive oxygen species, ROS), 造成生物体氧化应激反应, 从而引发代谢异常, 进而诱发各种疾病. 因为靠近氧化磷酸化(oxidative phosphorylation, OXPHOS)发生的场所, 并且缺乏组蛋白的保护和足够的DNA损伤修复能力, 与核DNA相比, mtDNA更容易受到氧化应激反应的影响. 而多种环境污染物暴露可引发过多的活性氧活动, 进而可导致mtDNA拷贝数及mtDNA表观遗传修饰的改变. mtDNA异常也正在作为一种可能的环境污染物暴露后的生物标志物, 受到越来越多的研究关注. 本文简要介绍了mtDNA拷贝数、mtDNA可能的表观遗传修饰以及相关调控机制, 并总结了各种类型环境污染物暴露引起mtDNA拷贝数和甲基化变化的研究结果, 同时对如何深入研究环境污染物影响mtDNA及分子机制进行了展望.

关键词 mtDNA, mtDNA拷贝数, 甲基化, 环境污染物

线粒体是真核生物细胞内的重要细胞器, 处于生物能量物质生产与转换的中心, 在生命活动中发挥极其重要的作用, 同时也影响细胞的衰老、死亡, 甚至导致肿瘤或其他疾病的发生. 线粒体基因组的完整性对于生物体的生存有重要意义. 人们对线粒体的结构和功能已经有一定的认识, 但仍然存在太多的未解之谜. 本综述主要对线粒体DNA拷贝数及其调控、线粒体DNA表观遗传修饰进行了简要总结, 并介绍了多种环境污染物暴露导致疾病与mtDNA拷贝数以及mtDNA甲基化之间的关联.

1 线粒体及mtDNA

线粒体通过氧化反应为细胞供能, 是真核生物的能量代谢中心, 也是调控细胞凋亡的关键细胞器[1]. 线粒体作为真核细胞的一种半自主细胞器, 由双层膜组成囊状结构, 其内膜向腔内突起形成许多嵴, 成熟线粒体DNA(mitochondrial DNA, mtDNA)呈超螺旋结构[2], 没有组蛋白缠绕, 几乎裸露于线粒体基质中. 一个线粒体中一般含多个mtDNA分子, 而根据细胞种类的不同, 单个细胞可以包含数百或数千个mtDNA拷贝[3]. 例如, 外周血单核细胞(peripheral blood mononuclear cell,

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PBMC)mtDNA数约为400[4], 而人骨骼肌细胞中mtDNA拷贝数约为1800[5].

哺乳动物mtDNA是双链闭合环状分子, 两条链根据G+C含量不同分为重链(H链)和轻链(L链), 外环为重链, 内环为轻链, 人类mtDNA长度为16569 bp. mtDNA编码37个基因, 包括13个呼吸链相关的多肽、22种tRNA以及2种rRNA基因[6], 无内含子(图1). Lee等人[7]的研究显示, 除13个电子传递链相关多肽外, 线粒体基因组还编码一类有生物活性的内源性肽. 线粒体基因组中的非编码区包括D-环(displacement-loop, D-loop)区和L链复制起始区, D-环区包括重链和轻链的2个转录启动子(HSP和LSP)与H链复制起始区. 因此, D-环区在mtDNA的复制和转录中起重要作用, 该区域的任何变化, 包括点突变、序列缺失、表观遗传修饰改变等, 都可能引起mtDNA复制和转录水平的改变, 从而可能造成线粒体功能的损伤.

线粒体在调节细胞增殖、维持胞内离子稳态和凋亡等重要细胞过程中发挥作用, 线粒体功能损伤会导致细胞功能受损[1]. mtDNA编码提供细胞大部分能量的氧化磷酸化通路中所需的蛋白, 其质量直接决定线粒体功能是否正确, 因此mtDNA拷贝数可作为表征线粒体功能的生物学指标[8]. 同时, 越来越多的研究表明, mtDNA甲基化与多种神经退行性疾病[9]、癌症[10]、糖尿病[11]、心血管疾病[12]等的发生发展以及早期胚胎发育不良[13]有关.

环境污染物进入细胞后诱发过量活性氧(reactive oxygen species, ROS)的生成, 造成细胞和组织的氧化损伤. 线粒体是细胞氧化应激的来源和靶点. 由于其位于活性氧(ROS)产生的场所(线粒体), mtDNA更容易受到环境因素诱发的活性氧影响而发生拷贝数、甲基化水平改变, 甚至是DNA片段缺失等变化. mtDNA稳态被破坏可能是环境污染物暴露导致人类健康受损的早期事件.

2 mtDNA拷贝数

2.1 mtDNA拷贝数

单个细胞基因组中的mtDNA分子个数即为mtDNA拷贝数. mtDNA拷贝数受到组织类型和发育阶段的影响.

mtDNA拷贝数具有组织类型特异性. 肌细胞[5]、神经元细胞[14]、肝细胞[15]等对三磷酸腺苷(adenosine triphosphate, ATP)需求高的细胞, mtDNA拷贝数较高, 而主要通过糖酵解途径产生ATP的组织细胞则含有较少mtDNA拷贝数, 如脾细胞[16]. 因此, mtDNA拷贝数可被认为是细胞通过氧化磷酸化途径生产ATP能力的有

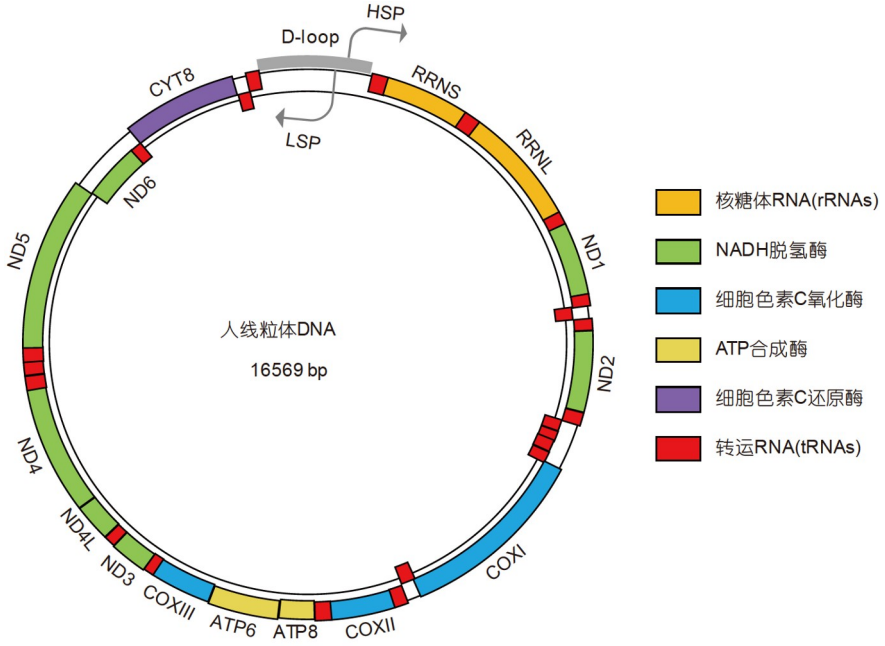

图 1 人类线粒体基因组[6]
Figure 1 Human mitochondrial genome[6]

效指标.

mtDNA拷贝数还具有发育阶段特异性. mtDNA的复制在发育的关键阶段受到严格调控. mtDNA拷贝数在原始生殖细胞分化成为卵子的过程中逐渐增加, 在受精过程中达到峰值, 而对于受精过程, 卵子mtDNA拷贝数必须超过一个临界值, 未达到临界值可能会导致受精失败或胚胎停止发育[17]. 早发性卵巢功能不全(premature ovarian insufficiency, POI)的重要表型之一即为患者卵母细胞中mtDNA数量明显减少[18]. 卵母细胞在受精后分裂成2细胞胚胎之前的合子阶段, mtDNA会有一次复制过程, 而在此之后, mtDNA不再复制, mtDNA拷贝数下降, 直到胚胎到达着床发育前的囊胚阶段, mtDNA重新开始复制[17]. 因而在早期生殖发育过程中, 控制细胞mtDNA拷贝数对生殖细胞功能至关重要.

2.2 mtDNA拷贝数调控

mtDNA拷贝数具有组织类型特异性和发育阶段特异性, mtDNA拷贝数的调控机制至今尚不明确. Montier等人[19]认为, 细胞的mtDNA拷贝数调控存在一个阈值, 高于或低于这个阈值都将触发调节机制, 使mtDNA拷贝数稳定在某个值附近. St John课题组[17,20]提出了多能细胞中mtDNA设定点(set point)的概念, 即多能细胞在发育的某个阶段具有较低的mtDNA拷贝数, 避免因过早或过晚的分化而导致分化失败, 一旦细胞确定了分化方向, 它们将以细胞特异性的方式复制mtDNA, 这使完全分化的细胞具有适当数量的mtDNA拷贝数, 以便它们能够履行其专门功能.

mtDNA拷贝数的调节依赖于mtDNA复制, 而mtDNA复制需要RNA引物, 与转录紧密相关. 因此, mtDNA的复制不仅依赖于包括DNA聚合酶Polγ、解旋酶TWINKLE和线粒体单链结合蛋白(mtSSB)等蛋白在内的复合物, 也受到线粒体转录因子TFAM的协调. Noack等人[21]研究发现, 小鼠成纤维细胞C2C12在暴露氧化应激压力(H_2O_2)后, mtDNA拷贝数减少, 过表达TFAM加快mtDNA拷贝数的恢复. 当减少线粒体中的DNA连接酶LIG3表达时, 尽管细胞能够维持正常的mtDNA拷贝数和呼吸, 但氧化损伤会导致mtDNA降解增加, mtDNA拷贝恢复缓慢[22]. St John课题组[16]的研究发现, Polγ第二外显子上CpG岛的高甲基化与细胞低mtDNA拷贝数有关, 当使用去甲基化试剂5-氮杂胞嘧啶(5-azaC)或维生素C(可促进双加氧酶TET1催化活性, 将5-甲基胞嘧啶转化为5-羟甲基胞嘧啶[23])可增加mtDNA拷贝数, 并且Polγ第二外显子上CpG岛去甲基化水平越高, mtDNA拷贝数的恢复程度越高[10]. Jiang等人[24]发现, 敲低mtSSB能够显著降低mtDNA水平, 通过小鼠基因敲除模型的深度测序和蛋白质的生物化学重组实验, 证明mtSSB除了调控复制起始点外, 还可以限制非特异性RNA的转录以优化RNA引物. 这表明敲低mtSSB导致的mtDNA拷贝数大幅度下降是由于mtSSB的缺失导致复制启动失败.

2.3 环境污染物暴露与mtDNA拷贝数改变

由于环境污染物主要通过引起细胞氧化应激以损伤细胞, 因此线粒体在环境暴露毒性方面也起到关键作用. mtDNA拷贝数的增加可能是人类细胞对内源性或外源性氧化应激反应的早期分子事件[25]. 氧化应激、线粒体功能受损诱发的mtDNA拷贝数增加可以弥补线粒体呼吸功能的下降. 例如, 白细胞mtDNA拷贝数与血浆中氧化应激指标硫代巴比妥酸反应物含量成正比[26].

来源于自然和人为因素的空气污染物, 主要包括气态物质、挥发性物质、半挥发性物质和颗粒物质(particulate matter, PM). 一些研究探讨了空气污染和mtDNA拷贝数之间的关系, 但现有数据所得出的论点并不一致[27~29]. 黑碳是含碳物质在不完全燃烧时的产物, 是车辆交通污染相关空气颗粒物的常见成分, 与呼吸系统疾病和不良心血管事件有关[30,31]. 与$PM_{2.5}$颗粒物相比, 黑碳的暴露将导致更严重的血压升高[32]. 在对675名老年男性进行每日环境黑碳含量和血压监测, 并测定了血液中mtDNA丰度后, 发现短期及中期暴露的黑碳水平与血压和血液线粒体丰度的增加相关, 并表明血液线粒体丰度的增加是一种代偿反应, 减弱了黑碳暴露对心脏的影响[29]. 职业暴露于更高水平的颗粒物PM(PM_1、$PM_{1\sim10}$和PM_{10})也被发现与在意大利铸造厂工作的健康男性(n=63)全血mtDNA拷贝数增加有关[28]. 在一项对中国60名卡车司机的研究中, 以60名办公室工作人员为对照. 研究显示, 短期(2~8 d)元素碳和PM_{10}暴露与全血mtDNA拷贝数减少有关[27]. 一项针对148名来自中国农村的健康非吸烟女性的横向研究也发现, 短期接触$PM_{2.5}$与白细胞mtDNA拷贝数之间存在负相关关系[33]. Wang等人[34]针对2758名女性的研究结果也表明, 长期暴露于环境$PM_{2.5}$与健康女性血液中mtDNA拷贝数下降有关. 独立研究结果显示, 暴露于相同的空气污染物可导致mtDNA拷贝数不同的变化,

尽管具体的原因尚不明确，但这可能与暴露人群、污染物暴露浓度以及暴露时间的不同有关，同时这也提示，同种环境污染物也可能通过不同的机制和通路影响细胞内mtDNA拷贝数目.

尼古丁是室内空气污染物香烟烟雾的主要成分之一. 长期接触尼古丁或吸烟会造成外周血DNA拷贝数下降和线粒体功能损伤. 大鼠暴露研究模型显示, 尼古丁引起神经细胞和海马体中mtDNA拷贝数的减少是由线粒体自噬介导的[35]. 二氧化硫主要来源含硫燃料的燃烧、含硫矿石的冶炼等，在2017年被世界卫生组织国际癌症研究机构列入了3类致癌物清单中. 大鼠在吸入大气污染物二氧化硫后，其肺中mtDNA含量显著降低, 这可能与二氧化硫抑制核编码的线粒体转录因子A(TFAM)表达有关[36].

Shen等人[37]的研究显示，职业暴露苯导致白细胞减少和mtDNA拷贝数增加；而Carugno等人[38]的研究显示，即使暴露低于职业安全与健康标准(OSHA标准)100倍的低剂量苯也会导致外周血细胞mtDNA拷贝数增加. 多环芳烃(polycyclic aromatic hydrocarbons, PAHs)是一种已知的具有诱发肺癌风险的环境污染物. Pavanello等人[39]的研究显示，职业暴露于PAHs的焦炉工人外周血淋巴细胞中具有更高的mtDNA拷贝数，提示淋巴细胞mtDNA拷贝数异常可能是一个潜在的癌症风险的生物标记物.

二噁英是一种持久性有机环境污染物，是钢铁冶炼、纸浆的氯漂白、某些杀虫剂的生产和焚烧等工业过程的副产物. 二噁英是具有相似结构和理化特性的一组多氯取代的平面芳烃类化合物，包括多氯二苯并对二噁英、二苯并呋喃、联苯和其他相关化合物. 在经除草剂四氯二苯并-*p*-二噁英(tetrachlorodibenzo-*p*-dioxin, TCDD)暴露后, 大鼠肝和肺中mtDNA拷贝数明显增加，在暴露类二噁英多氯联苯(polychorinatedbiphenyls, PCBs)后，大鼠肺部mtDNA拷贝数呈剂量依赖增加[40]. VanEtten等人[40]的研究结果表明, mtDNA拷贝数或许可以作为类二噁英物质毒性发展的一个早期氧化应激生物标志物.

环境重金属污染物，包括砷、铅、铍、铬、钴、镉、镍和钒, 可通过产生氧化应激而损害细胞[41~43], 并可能影响mtDNA的拷贝数. 小鼠卵母细胞经相对较低浓度的三氧化二砷处理后线粒体活性氧水平升高, ATP含量降低，导致mtDNA拷贝数降低以及mtDNA片段严重缺失，这些线粒体损伤可能会影响卵母细胞的发育[44]. 低剂量甲基汞暴露则会导致人类神经祖细胞中产生剂量依赖的ROS，导致mtDNA拷贝数的增加以及D-loop区、*ND1*基因、*Cyt1*基因及*ATP6*基因区域突变位点的增加, 使线粒体代谢功能降低, 细胞活力受抑制, 从而诱导人神经祖细胞凋亡[45]. 镉暴露导致骨髓间充质干细胞线粒体数量减少、形态异常以及线粒体膜电位下降, 并且在镉暴露24 h后, mtDNA拷贝数显著减少(约50%). 这些结果表明，镉暴露明显损伤骨髓间充质干细胞的线粒体结构和功能. 另外, 镉诱导的骨髓间充质干细胞的损伤也预示镉暴露与其早衰密切相关[46].

3 mtDNA表观遗传修饰

3.1 mtDNA 5-甲基胞嘧啶(5mC)修饰

DNA甲基化是表观遗传修饰的重要方式之一，S-腺苷甲硫氨酸作为甲基供体在DNA甲基转移酶的作用下, 将甲基转移到特定的碱基, 直接或间接参与调控细胞内的生理过程. 尽管核DNA表观遗传学领域发展迅速，mtDNA表观遗传学领域由于其研究困难而未受到应有的关注[47]. 5mC是哺乳动物核DNA中最重要的表观修饰，但是否存在于mtDNA中仍然存在一些争议(图2)[48,49].

2011年, Taylor课题组[50]在哺乳细胞中发现线粒体5mC，并且发现了具有线粒体定位序列(MTSs)的DNA甲基转移酶1(mtDNMT1)亚型与mtDNA之间存在CpG依赖的相互作用，证实了线粒体基质中存在DNA甲基转移酶，而线粒体重链和轻链的转录水平不对称地受到mtDNMT1表达改变的影响. 此外，Martin课题组[51]从小鼠运动神经元细胞系分离出的线粒体中鉴定出了DNA甲基化转移酶3a(DNA methyltransferase 3a, DNMT3a). 但在之后的一项研究中，Hong等人[52]通过亚硫酸氢盐测序证明，人结肠癌细胞系以及人胚肾上皮细胞系线粒体基因组上不存在具有生物学意义的CpG甲基化. 最新的研究使用纳米孔测序, 避免了亚硫酸氢盐和聚合酶链式反应(polymerase chain reaction, PCR)扩增引入的偏差，在终末分化肝细胞中检测到低水平的mtDNA CpG甲基化[53]. 这些研究结果可能说明mtDNA甲基化是一种潜在的细胞和组织特异性修饰.

3.2 mtDNA 5-羟甲基胞嘧啶(5hmC)修饰

5hmC由Tet酶氧化5mC生成, 是5mC去甲基化过程

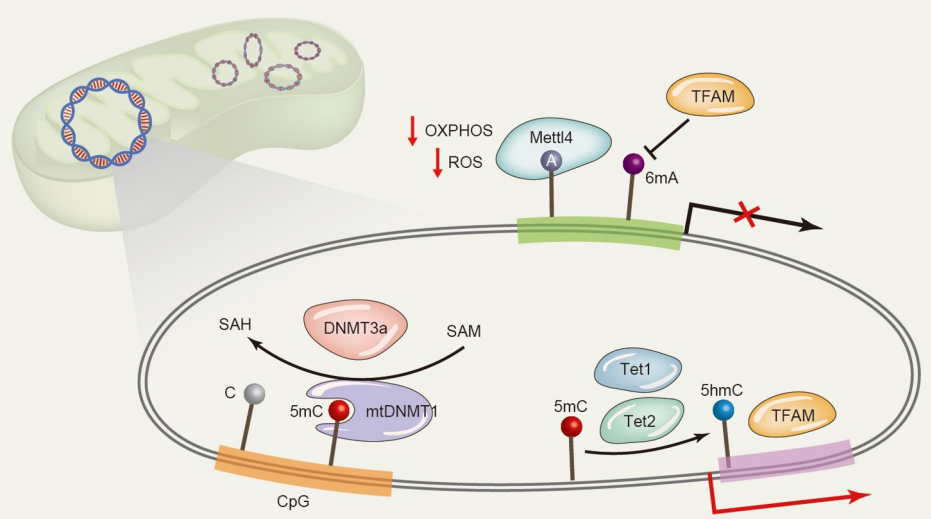

图 2 mtDNA可能存在的表观遗传修饰(5mC、5hmC和6mA)及其调控
Figure 2 Possible epigenetic modifications (5mC, 5hmC, and 6mA) of mtDNA and the regulation

的中间产物，发挥重要的生理作用[54]. 5hmC不仅存在于核DNA, 也可能存在于mtDNA[50](图2). 如同5mC, 线粒体5hmC是否存在至今尚未有定论. mtDNA 5hmC含量改变被认为与衰老[55]和疾病[56]相关. 衰老造成小鼠额叶皮层中线粒体5hmC含量减少，且小鼠神经元细胞中Tet1、Tet2蛋白与线粒体共定位, 这表明Tet家族蛋白可能参与了mtDNA 5hmC的形成[55]. 另一项研究也发现，小鼠脑缺血再灌注损伤后的脑区线粒体5hmC丰度和Tet2表达均发生上调，在抑制Tet2表达后，mtDNA 5hmC水平也降低[56]. 高含量的mtDNA 5hmC可能是缺血再灌注损伤后部分线粒体基因mRNA水平升高的原因[56].

3.3 mtDNA N6-甲基腺嘌呤(6mA)修饰

DNA 6mA修饰在原核生物中是一种常见的表观遗传学修饰. 2015年, DNA 6mA甲基化首次被证明存在于衣藻[57]、线虫[58]和果蝇[59]基因组中. 近两年来也相继有报道显示DNA 6mA甲基化形式存在于小鼠胚胎干细胞[60], 甚至是在人类[61]基因组中. 然而, 该修饰的生物学功能和调控机制仍不清楚. 我们最近的研究表明, 在哺乳动物中存在由DNA聚合酶复制引入错误掺入形成的DNA 6mA[62,63]. Koh等人[64]的研究显示, 人胚肾上皮细胞中mtDNA 6mA水平高于整体基因组6mA水平. 相似地, 何川课题组[65]使用超高效液相色谱-质谱分别检测了不同组织和细胞基因组及线粒体6mA含量, 结果显示6mA在mtDNA中更加富集, 并且缺氧可以进一步提高线粒体6mA水平. 另外, 6mA有可能通过抑制线粒体转录因子TFAM与mtDNA的结合，从而抑制DNA转录. METTL4蛋白可能是mtDNA 6mA甲基化转移酶(图2).

3.4 环境污染物暴露与mtDNA甲基化水平

环境污染物暴露可能改变mtDNA甲基化水平. 一项针对不同空气污染物对职业暴露者外周血mtDNA的3个不同位点(*MT-TF*、*MT-RNR1*以及D-loop区)甲基化影响的研究发现，空气中苯和交通来源碳暴露对mtDNA甲基化没有影响，而高暴露于富含金属PM1的钢铁工人表现出较高水平的线粒体*MT-TF*和*MT-RNR1*基因甲基化，同时还发现线粒体*MT-TF*和*MT-RNR1*基因甲基化与mtDNA拷贝数之间呈正相关关系[66]. Janssen等人[67]对381对母亲和新生儿进行了调研，发现妊娠期间$PM_{2.5}$浓度的增加与胎盘组织中mtDNA *MT-RNR1*和D-loop区甲基化水平呈正相关，与mtDNA含量呈负相关. 与上述研究结果相反的是, 职业铬暴露工人的*MT-TF*和*MT-RNR1*基因的甲基化水平显著低于对照组, 并且血铬离子浓度越高, 甲基化水平越低, mtDNA拷贝数则不受影响[68]. 相似的结果还出现在职业焊接烟尘[69]和地下水砷污染[70]暴露中，分别导致线粒体D-loop区及*MT-TF*区甲基化的降低和D-loop区及*ND6*区域显著的低甲基化，而这些区域的低甲基化又使得mtDNA拷贝数增加. 对于职业暴露于焊接烟尘的电焊工人而言，这种拷贝数增加在一定程度上降低了焊接

烟尘对血压的影响[69]. 这些结果提示, mtDNA甲基化水平变化有可能作为环境污染物, 尤其是颗粒污染暴露的替代效应生物标志物(图3). 上述研究结果表明, 部分环境污染物暴露引起的D-loop区甲基化水平改变可能会影响mtDNA复制相关蛋白, 如TFAM、PolG等与mtDNA的相互作用, 从而影响mtDNA复制事件的发生, 进而使mtDNA拷贝数发生变化. 更深入了解环境污染物毒性对mtDNA与蛋白质的相互作用及功能的影响, 需要更系统的研究.

多种营养(如维生素C[23])和环境因素(如卤代醌[71]、药物[72,73]、重金属镍[74]、环境雌激素[75])可引起核DNA的5hmC的改变. 类似地, 这些环境因素也可能影响mtDNA 5hmC, 有待将来开展研究.

4 受损伤线粒体的代谢

环境污染物的暴露及体内代谢可引起线粒体的损害. 受损严重的线粒体如果不能被及时清理, 则会释放大量的死亡因子, 从而影响周围健康线粒体的稳态维持, 并可能诱导细胞凋亡. Wang等人[35]的研究显示, 尼古丁引起神经细胞和海马体中线粒体功能缺陷, 功能失调的线粒体通过线粒体自噬被降解和消除. 俞立课题组[76]通过透射电子显微镜在正常大鼠肾脏细胞中观察到一种新的“细胞器”: 迁移体. 这是一种位于胞外的囊泡结构, 当迁移细胞移动时, 其移动路径上会出现一个蛋白膜管网络, 迁移体就位于膜管的顶端和交叉处. 胞质内容物可以转运到迁移体, 并通过迁移体从细胞释放. 而当细胞遭受细胞饥饿等外界刺激时, 受损的线粒体会进入迁移体中, 再进行线粒体分泌, 这个过程可以控制胞内线粒体质量、维持线粒体稳态[77]. 这种受损伤线粒体的代谢新途径或许可以为研究环境暴露引发线粒体损伤的代谢通路提供新的想法.

5 展望

虽然环境污染暴露引起mtDNA损伤的潜在作用机制仍有待研究, 但可以确定的是, 当暴露于导致ROS增加的多种环境污染物时, mtDNA明显比核DNA积累更多的损伤, 这可能使其成为暴露的敏感生物标志物. 尽管研究表明, mtDNA受到环境污染物进入生物体后引起氧化应激的影响, 然而很多研究结果相互矛盾. 这可能与不同污染物的毒物代谢动力学不同有关, 也可能与现有检测手段不够准确有关. 尽管细胞中mtDNA分子拷贝数较多, 但由于分子量相对基因组DNA依旧过小, 虽然已有商业化的线粒体提取方法, 但是囿于回收效率低, 需要较大量的细胞或组织样品才能满足用于检测的mtDNA量. 为实现mtDNA作为生物标志物检测的临床应用, 发展更准确、更灵敏的少量样品mtDNA提取与分析方法将成为线粒体研究的一个热点. 同时, 由于单个细胞mtDNA拷贝数量多, 且mtDNA突变率较高, 使用传统的重亚硫酸盐的测序方法(bisulfite genomic sequence, BSP)进行甲基化测序时, mtDNA本身的突变导致相邻组织甚至不同细胞出现甲基化水平的“个体差异”, 这种差异又会影响研究者对mtDNA甲基化水平的判断. 因此, 进一步优化mtDNA甲基化测序

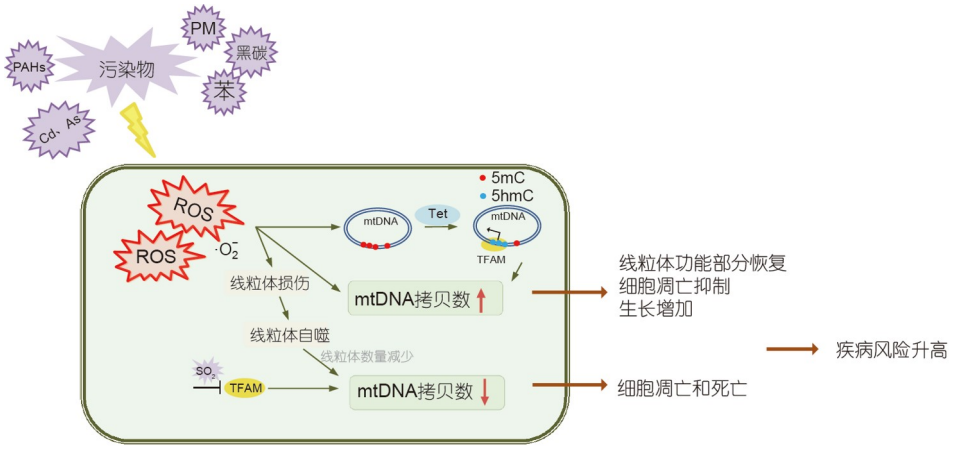

图 3 环境污染物暴露与mtDNA变化
Figure 3 Environmental pollutants exposure and changes of mtDNA

方法，实现少量细胞mtDNA甲基化位点的精确检测也是目前研究的一个难点. 现有的大部分研究只能说明环境污染会造成一定程度的线粒体功能损伤，但随着研究手段的更新与研究的深入，在明确了污染物作用的分子机制以及mtDNA调控机制后，mtDNA或许可以作为预防和诊断环境污染物暴露造成相关疾病的潜在靶点，而针对线粒体的靶向给药也为疾病治疗提供了新的思路.

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Summary for “环境污染物的毒性作用与线粒体DNA的变化 ”

Toxicity of environmental pollutants for mitochondrial DNA alteration

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Mitochondria, as the main site of cellular aerobic respiration, are organelles that provide energy for cells. The mitochondrial genomes are independent of the nuclear genome, known as mitochondrial DNA (mtDNA). mtDNA encodes 37 genes, including 13 respiratory chain-related polypeptides, 22 tRNAs and 2 rRNA genes. Mitochondrial abnormalities can directly reduce cellular ATP synthesis and thus generate insufficient cellular energy. The regulation of mtDNA copy number and epigenetics is crucial for the basic functions of mitochondria. After entering the cells of organisms, environmental pollutants elevate reactive oxygen species (ROS), causing oxidative stress in the organism and resulting in abnormal metabolism and various diseases. Compared with nuclear DNA, mtDNA is more susceptible to oxidative stress because of its proximity to the site of oxidative phosphorylation (OXPHOS) and lack of histone protection and sufficient DNA damage repair capacity. Exposure to various environmental pollutants can induce excessive accumulation of ROS, which leads to changes in mtDNA copy number and epigenetic modifications of mtDNA. An increasing number of studies focus on abnormal mtDNA as a possible biomarker for the exposure and toxicity of environmental pollutants.

In this review, we briefly introduce the physiological functions and regulatory mechanisms of mitochondria and mtDNA copy number. Environment pollutant exposures often cause mitochondrial damage, which may alter mtDNA copy number, leading to mitochondrial abnormalities and impairing cell function. Studies have found that mitochondrial dysfunction is related to the occurrence and development of various diseases (e.g., cancer, diabetes, cardiovascular disease, neurodegenerative diseases, etc.). Noteworthily, decreased mtDNA copy number in germ cells blocks embryonic development. Next, along with the associated proteins found in recent studies, possible epigenetic modifications present on mtDNA (e.g., 5-methylcytosine, 5-hydroxymethylcytosine and N^6-methyladenine) are also summarized. A study identified mtDNMT1 in the mitochondrial matrix, which was suggested to be a methyltransferase for mtDNA 5mC. Since 5hmC modification on mtDNA was first reported in 2011, the results of studies on mtDNA 5hmC have been conflicting. However, some research groups found that Tet1 and Tet2 may be involved in the formation of mtDNA 5hmC. A higher level of 6mA modification than nuclear DNA was suggested to be detected in mtDNA, and the METTL4 protein is a potential mtDNA 6mA methyltransferase. Nonetheless, the study on mtDNA methylation is of intensive interest.

We reviewed the effects of multiple common environmental pollutants (e.g., PM, black carbon, nicotine, heavy metal particles, etc.) on mitochondrial DNA from two aspects: (1) Environmental pollutants exposure causes mtDNA copy number changes, which may increase disease risk; (2) environmental pollutant exposure alters methylation levels of certain genes in mtDNA. In the first aspect, exposure to different pollutants, or even the same pollutant, resulted in different changes in mtDNA copy number. It suggests that the same environmental pollutants may also affect mtDNA copy number through different mechanisms and pathways. In the second aspect, changes in the methylation levels of D-loop and gene regions on mtDNA caused by pollutant exposure can affect mitochondrial physiological function by affecting mitochondrial DNA replication and mitochondrial-encoded protein expression.

We prospect and discuss how to perform further study on the effect of environmental pollutants on mtDNA and its molecular mechanism. The following two aspects should be improved in future pollutants-mtDNA studies: (1) Develop more convenient methods for the extraction of mtDNA from less than million cells to a high purity. This will facilitate the detection and sequencing of mtDNA for diverse purposes; (2) to achieve accurate identification of mtDNA methylation sites in small number of cells and eliminate the interference of mtDNA heterogeneity, the mtDNA methylation sequencing method should be further innovated.

mtDNA, mtDNA copy number, methylation, environmental pollutants

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