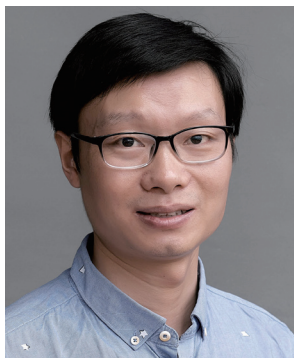


实验室介绍



周小龙博士, 2019年5月起, 任中国科学院分子细胞科学卓越创新中心/生物化学与细胞生物研究所研究员、研究组长、博士生导师。该实验室主要运用生物化学、分子生物学、细胞生物学、遗传学等技术方法, 研究包括: (1) 探究tRNA、氨基酰-tRNA合成酶与tRNA修饰酶介导的人细胞质与线粒体蛋白质合成的分子机制; (2) 阐明临床鉴定的蛋白质合成缺陷相关线粒体疾病的致病机制; (3) 探索线粒体疾病靶向诊断与干预策略。

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人线粒体tRNA修饰与疾病

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摘要 人线粒体作为细胞中重要的能量工厂, 拥有自己的基因组。线粒体基因组编码2种rRNA和22种tRNA, 用于翻译产生13种氧化磷酸化复合物亚基。线粒体tRNA(mt-tRNA)作为接头分子携带特定氨基酸参与线粒体蛋白质合成。线粒体tRNA上含有18种转录后修饰。这些修饰对于稳定tRNA结构, 介导密码子与反密码子的相互作用具有重要作用。因此, 线粒体tRNA修饰水平和修饰酶的异常变化与线粒体功能紊乱及疾病的发生发展密切相关。该文综述部分线粒体tRNA修饰的研究进展并介绍它们与线粒体脑肌病、神经系统疾病和癌症的关系。

关键词 线粒体; tRNA修饰; 线粒体脑肌病; 神经系统疾病; 癌症

Human Mitochondrial tRNA Modification and Diseases

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Abstract As the pivotal energy hub of cells, human mitochondria have their own genomes and translation system to synthesize thirteen mitochondrial DNA-encoded subunits for OXPHOS (oxidative phosphorylation) complexes. Mt-tRNA (mitochondrial transfer ribonucleic acid) serves as an adaptor in mitochondrial protein synthesis by carrying the specific amino acid to the ribosome. Mt-tRNAs harbor 18 types of posttranscriptional modifications, which play important roles in stabilizing tRNA tertiary structure and/or mediating precise codon-anticodon interactions. Thus, abnormal mt-tRNA modification and modification enzymes are closely related to mitochondrial dysfunctions and human diseases. This review summarizes the recent advances in the study of mt-tRNA modifications and discusses their relevance to various mitochondrial encephalomyopathies, neurological disorders and cancers.

Keywords mitochondria; tRNA modification; mitochondrial encephalomyopathy; neurological disorder; cancer

线粒体在细胞的能量转化过程中发挥着关键作用,对于生命活动至关重要^[1-3]。一般认为线粒体来源于被真核细胞的前体细胞吞噬的 α -变形菌,结构组分上与 α -变形菌祖先类似。线粒体由线粒体内膜、外膜、内外膜间隙及线粒体基质所组成,基质内有其环状双链DNA基因组^[4]。在漫长的进化过程中,线粒体基因组编码的基因逐渐转移到核基因中,或者发生基因丢失,由此导致自身编码的基因越来越少。人线粒体基因组共编码37个基因,分别是13种参与氧化磷酸化复合物组装的蛋白质基因、22个线粒体转移核糖核酸(tRNA)基因以及2个线粒体核糖体RNA(rRNA)基因^[5-8]。

tRNA作为接头分子,被线粒体氨基酰-tRNA合成酶接载上对应的氨基酸,并被延伸因子转运至核糖体上^[9-12],它的反密码子序列和信使RNA(mRNA)的密码子序列互补配对,按照基因序列参与蛋白质的合成,在遗传信息的翻译过程中发挥着关键的作用^[13]。1965年,HOLLEY等^[14]首次解析了酵母tRNA^{Ala}的化学结构,发现了tRNA有着三叶草型的二级结构,包括D环/茎(二氢尿嘧啶环/茎)、反密码子环/茎、可变环、T Ψ C茎环、氨基酸接受茎和3'CCA尾,其中D环和T Ψ C环进一步相互作用并形成了倒L型的三级结构。他们还预言了酵母tRNA^{Ala}的34位反密码子可能是一个被修饰的腺苷酸-肌苷(I),促使Francis CRICK^[15]提出了密码子“摆动”假说。此后,大量的tRNA的修饰核苷从不同的组织和物种中被鉴定出来,揭示了成熟tRNA含有多种转录后修饰^[16-17]。

目前,在三界生物中已经鉴定到约170多种RNA修饰,其中约80%的修饰发生在tRNA上^[17-18]。对于人线粒体编码的22种线粒体tRNA(mitochondrial trans-

fer ribonucleic acid, mt-tRNA),在它们的137个位置上发现了18种修饰核苷^[19](图1)。这些发生在mt-tRNA上的修饰对线粒体蛋白质合成速率、翻译的精确性以及线粒体tRNA结构稳定性起重要作用^[20]。已有研究表明,mt-tRNA上修饰的缺失可能会使线粒体基因组编码蛋白质的翻译出现问题,引起线粒体功能异常^[21-22]。作为细胞的能量工厂,线粒体功能的失调会对各组织器官尤其是高耗能的组织器官如脑、肌肉、心脏等产生破坏性的影响,导致多种人类重大疾病,统称为线粒体病,但是多数致病机制尚不清楚^[22-23]。

本文将介绍mt-tRNA的修饰与疾病之间的关系,重点总结mt-tRNA修饰及其修饰酶突变在线粒体脑肌病、神经系统疾病以及癌症发生中的研究进展,为mt-tRNA修饰缺陷导致疾病的潜在机制的研究提供思路 and 参考。

1 线粒体脑肌病

1.1 牛磺酸修饰

2-氨基乙磺酸又称牛磺酸(Taurine),是哺乳动物血浆及组织中丰度最高的氨基酸之一,具有调节钙离子流、维持感光细胞、调节神经兴奋性、调节渗透压和调节细胞增殖等功能。2002年,Takeo SUZUKI等^[24]第一次发现牛磺酸可以作为底物修饰到mt-tRNA上形成5-牛磺酸甲基尿嘧啶($\tau\text{m}^5\text{U}$)以及5-牛磺酸甲基-2-硫尿嘧啶($\tau\text{m}^5\text{s}^2\text{U}$)(图2)。目前已经鉴定到有5种人mt-tRNA的34位上含有牛磺酸修饰,其中人mt-tRNA^{Leu}(UUR)和mt-tRNA^{Trp}的34位为 $\tau\text{m}^5\text{U}$ 修饰,而人mt-tRNA^{Lys}、mt-tRNA^{Glu}和mt-tRNA^{Gln}的34位为 $\tau\text{m}^5\text{s}^2\text{U}$ 修饰^[25]。人GTP结合蛋白质3(GTP binding protein 3, GTPBP3)和线粒体翻译优化蛋白质1(mitochondrial translation optimization 1, MTO1)

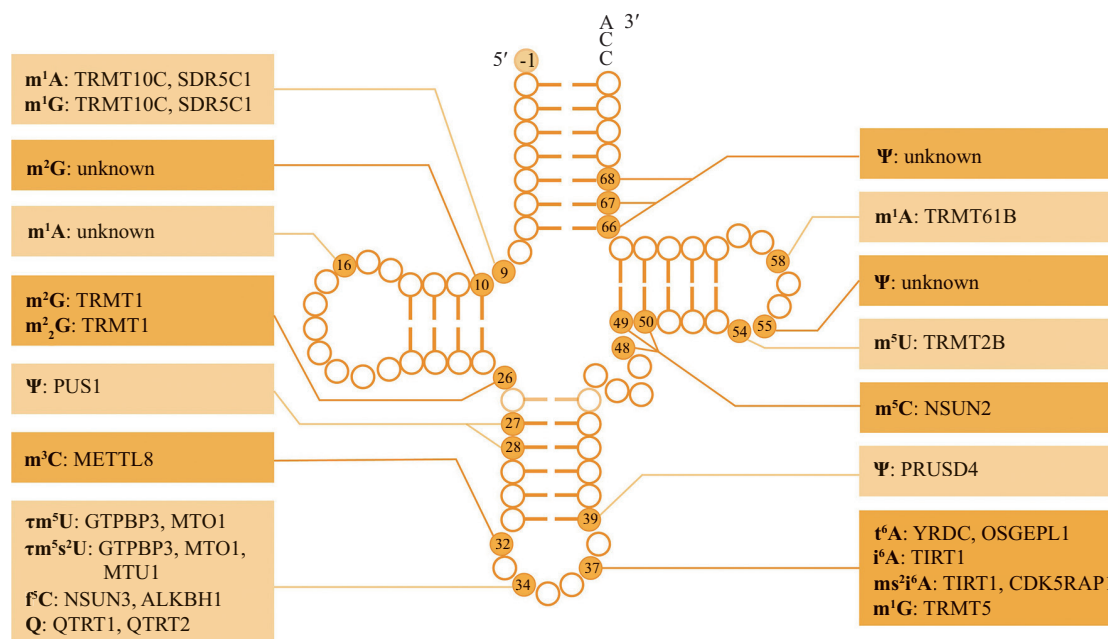


图1 人mt-tRNA修饰及修饰酶

Fig.1 Human mitochondrial tRNA modification and modification enzymes

以牛磺酸、5,10-亚甲基四氢叶酸、黄素腺嘌呤二核苷酸、烟酰胺腺嘌呤二核苷酸、鸟苷三磷酸等为底物共同催化形成 $\tau\text{m}^5\text{U}$ 修饰^[25-28]。线粒体tRNA特异性2-硫脲酰化酶1(mitochondrial tRNA specific 2-thiouridylase 1, MTU1)则独立催化 s^2U 修饰,使tRNA 34位尿苷的第二位氧原子被硫原子所取代,这一修饰与 $\tau\text{m}^5\text{U}$ 修饰的催化过程没有前后之分,两者共同形成 $\tau\text{m}^5\text{s}^2\text{U}$ 修饰^[29-30](图3)。

研究表明,线粒体脑肌病伴乳酸中毒和中风样发作综合征(mitochondrial encephalomyopathy-lactic acidosis and stroke-like episodes, MELAS)和致肌阵挛性癫痫伴红纤维病(myoclonus epilepsy, ragged-red fibers, MERRF)与人体内牛磺酸修饰的丰度息息相关^[31-32]。约80%的MELAS患者的线粒体基因组的3 243位出现了腺苷酸变为鸟苷酸的突变(A3243G),另有10%的MELAS患者的线粒体基因组的3 271位出现了胸腺嘧啶到胞嘧啶的点突变(T3271C),这两个点突变都发生在负责编码mt-tRNA^{Leu}(UUR)的线粒体基因组片段上,并导致人mt-tRNA^{Leu}(UUR) 34位上的 $\tau\text{m}^5\text{U}$ 修饰水平显著下降,使mt-tRNA^{Leu}(UUR)只能识别UUA密码子而不能识别UUG密码子进而阻滞线粒体编码MT-ND6的翻译使其稳态水平降低,最终影响线粒体功能^[33]。MELAS是一种具有广泛临床表型的多器官疾病,包括卒中

样发作、痴呆、癫痫、乳酸血症、肌肉病变、反复头痛、听力障碍、糖尿病和身材矮小等。儿童时期是其典型的发病阶段,约65%~76%的受影响人群在20岁之前发病;只有1%~5%的人在2岁前表现出症状,1%~6%的人在40岁之后表现出症状^[32,34]。MELAS病人一般会出现两种以上的临床表型,其中最常见的为卒中样发作、心肌肥厚、肌无力以及乳酸血症^[35]。MERRF则是另一种与牛磺酸修饰有关的线粒体脑肌病,约90%的MERRF病人的发病原因与mt-tRNA^{Lys}上的突变有关,其中80%的病人线粒体基因组的8 344位的腺苷酸变为鸟苷酸(A8344G),另有三种发生在mt-tRNA^{Lys}上的突变T8356C、G8363A和G8361A与10%的病例有关^[36-37],这些突变导致线粒体tRNA^{Lys}第34位上的 $\tau\text{m}^5\text{s}^2\text{U}$ 修饰缺失,使mt-tRNA^{Lys}不能识别AAA和AAG密码子从而导致整个线粒体基因组编码的蛋白质出现缺陷^[38]。MERRF病的典型表型有肌阵挛、全身性癫痫、共济失调和纤维斑驳肌病,这些临床表型多发生在婴幼儿时期或者青春期^[33-37]。

此外, $\tau\text{m}^5\text{U}$ 和 $\tau\text{m}^5\text{s}^2\text{U}$ 的修饰酶的突变也会导致线粒体脑肌病。根据已有的临床样本和对病人及家属的全外显子基因检测,发现15个病例共19种GTPBP3的致病点突变会导致联合氧化磷酸化缺陷23型(combined oxidative phosphorylation deficiency

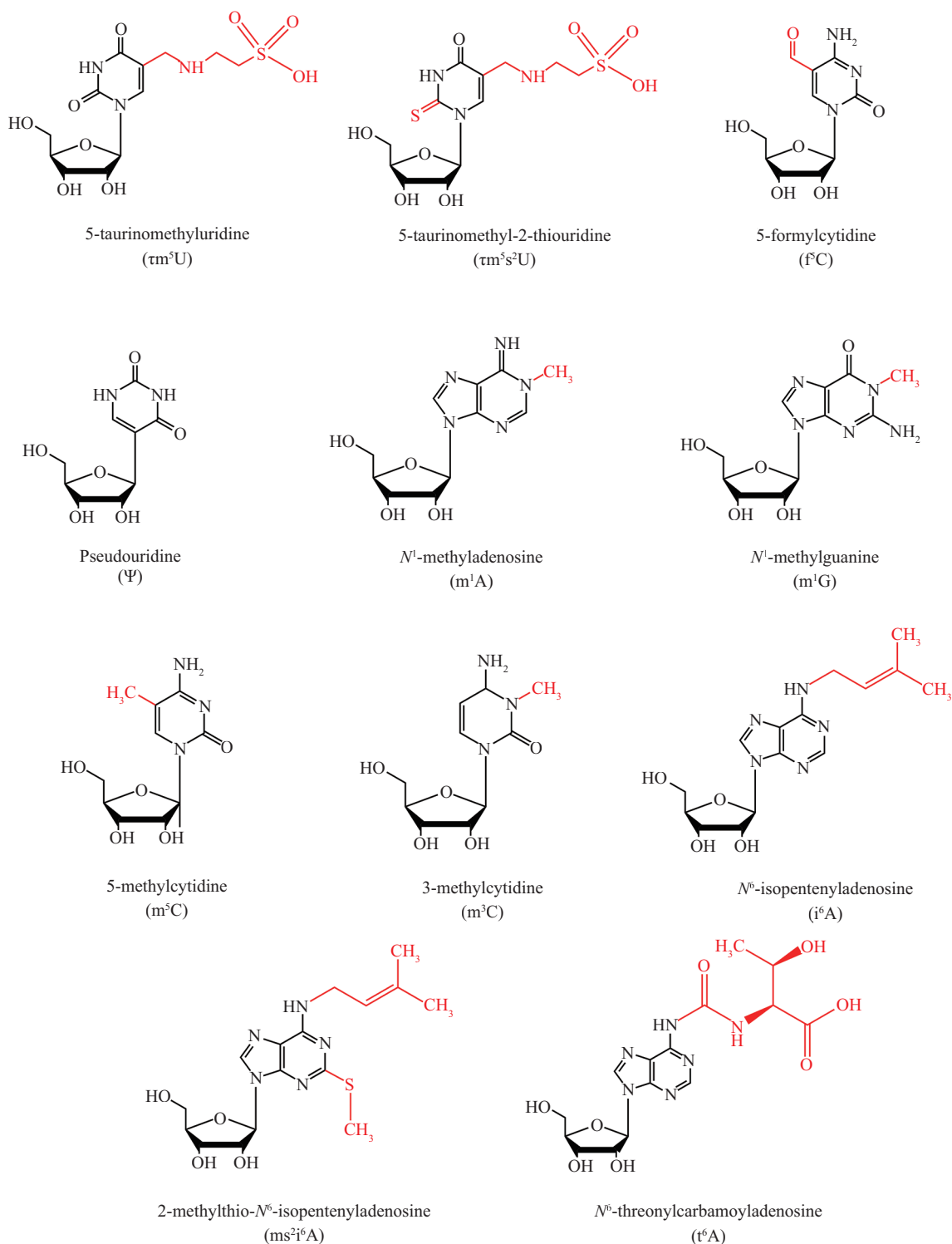


图2 mt-tRNA修饰的化学结构

Fig.2 Chemical structures of mitochondrial tRNA modifications

23, COXPD23), 这种常染色体隐性遗传病以儿童时期的乳酸中毒、肥厚性心肌病和脑病为特征^[39-40]; MTO1上的21种致病点突变会导致线粒体氧化磷酸化复合物I和IV的缺失引起乳酸中毒和肥厚性心肌病^[41-43]; 而MTU1上的致病点突变则会使tm⁵s²U的2-

硫尿嘧啶修饰缺失造成氧化磷酸化功能的损伤, 临床上引起可逆性婴儿肝衰竭(reversible infantile liver failure, RILF)^[44-45]。专一敲除肝脏Mtu1的模型小鼠的肝脏出现损伤及乳酸中毒, 这一结果与病人表型相符, 说明MTU1的突变是导致RILF的直接原因^[46]。

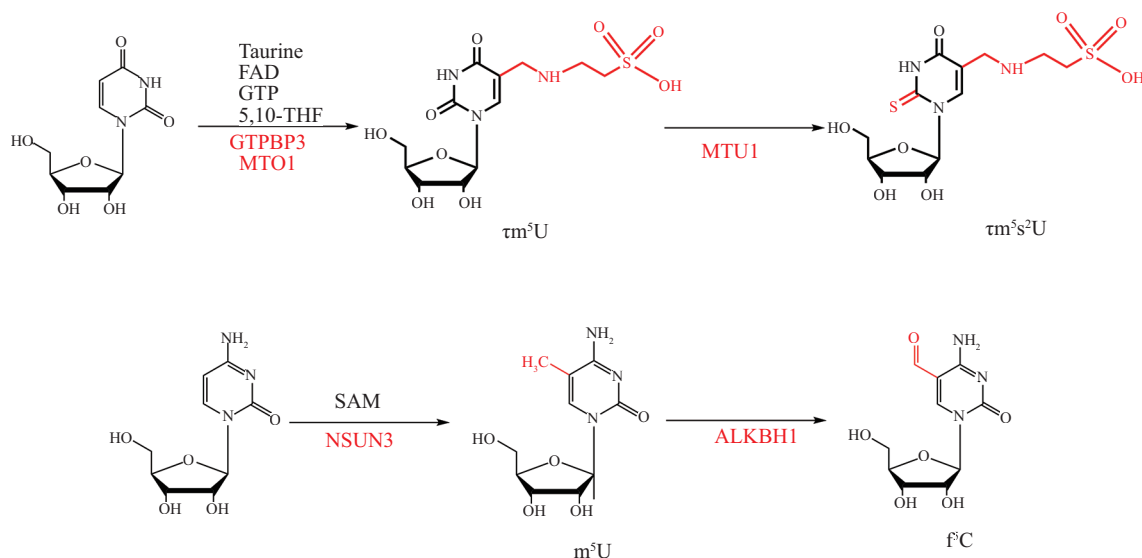


图3 部分mt-tRNA修饰的生物合成过程

Fig.3 The biosynthesis of some mt-tRNA modifications

然而这些修饰酶致病的分子机制仍不清楚。

目前没有特效药根治mt-tRNA牛磺酸修饰及其修饰酶突变造成的疾病,只是针对特定症状进行传统治疗,如MELAS病造成的听力受损可以通过植入人工耳蜗来改善,癫痫可以通过传统的抗癫痫药物进行治疗。此外,基于有限的临床试验,包括抗氧化剂和辅因子在内的补充试剂已被用于治疗MELAS病,例如精氨酸、辅酶Q和肌酸均对MELAS病有一定的治疗效果^[32]。而临床上对于MERRF的治疗也与MELAS病的治疗类似^[47]。有报道表明日本的团队通过让MELAS病人摄入牛磺酸来进行治疗,这一将牛磺酸作为药物的治疗方法已经进入三期临床试验阶段,显示口服摄入牛磺酸可以显著减少卒中样发作并提高mt-tRNA上34位的牛磺酸修饰水平^[48]。

1.2 5-甲酰基胞嘧啶修饰

人类线粒体的遗传密码由于存在四个非通用密码子而偏离了规范遗传密码,这四个非通用密码子为:AUA编码甲硫氨酸(Met)、UGA编码色氨酸(Trp)、AGA或AGG(AGR)编码“终止密码子”^[49]。为解码编码线粒体甲硫氨酸的AUA密码子,人mt-tRNA^{Met}反密码子环的34位进化出了5-甲酰基胞嘧啶(f⁵C)修饰^[50](图2)。f⁵C34修饰可以让人线粒体tRNA^{Met}同时识别AUA和AUG密码子。一个关于核糖体A位点密码子-反密码子相互作用的结构研究发现在规范的沃森-克里克几何结构中,密码子第三位的A和G均可以和f⁵C配对,表明由甲酰基稳定的胞苷

亚氨基-氧基互变异构体参与了f⁵C-A配对^[51]。f⁵C由NOL1/NOP2/Sun结构域蛋白质3(NOL1/NOP2/Sun domain-containing protein 3, NSUN3)和烷羟化酶同源蛋白质1(alkane hydroxylase homologue 1, ALKBH1)分两步进行催化,NSUN3负责将S-腺苷甲硫氨酸(S-adenosylmethionine, SAM)上的甲基转移到mt-tRNA^{Met} 34位胞苷的第5位上形成5-甲基胞嘧啶(5-methylcytidine, m⁵C),并进一步由ALKBH1负责羟基化和氧化形成f⁵C修饰^[52-53](图3)。

人线粒体基因组上的A4435G和C4437U点突变会使人mt-tRNA^{Met}的f⁵C修饰水平降低,导致疾病发生^[54],这是由于这两个位点的突变会使NSUN3不能有效催化生成m⁵C,进而无法进行由ALKBH1催化的羟基化和氧化^[52]。这些突变临床表现为母系遗传的高血压、利伯氏遗传性视神经病变、张力低下、癫痫、肌肉无力、乳酸性酸中毒和听力丧失^[54-55]。MELAS和MERRF多在青少年时期发病,患者寿命受到影响,而A4435G和C4437U突变多在成年后发病且它们对患者寿命造成的影响相对有限,暗示人mt-tRNA^{Met}的这两个突变虽然影响修饰的生成但是并没有造成破坏性的后果。有研究表明A4435G和C4437U两种突变会使m⁵C的修饰效率下降至野生型的40%左右,但完全不影响ALKBH1的进一步催化,说明仍有部分f⁵C修饰生成,并发挥其生物学功能^[53,56]。然而修饰酶失活的突变则会对病人产生严重影响,如发生在NSUN3基因上的复合杂

合突变(p.Glu42Valfs*11/p.Arg99*)会使两条染色体编码的NSUN3分别产生剪切移码突变以及提前终止,使人体内NSUN3完全失活。这一病例在出生后三个月即开始发病,表现为发育停滞、小头畸形、血浆中乳酸水平提升、肌无力、近端突出、外眼肌麻痹及汇聚性眼球震颤^[56]。研究表明,NSUN3全身敲除的小鼠在10.5~12.5天胚胎致死,特异性敲除心脏中的NSUN3会导致小鼠的心脏收缩力增强以及年龄相关的心脏轻微增大^[57]。这说明Ψ及其修饰酶对生物的线粒体功能及生命活动至关重要。

1.3 假尿嘧啶修饰

假尿嘧啶(pseudouridine, Ψ)是一种广泛存在于核小RNA(small nuclear RNA, snRNA)、tRNA和rRNA的碱基修饰,用于加强碱基配对和稳定碱基堆叠(图2)。在snRNA中Ψ参与pre-mRNA的剪切,在tRNA中Ψ参与抑制“终止密码子”、提高解码效率和翻译特异性。同时在核糖体大亚基上的rRNA上,大量Ψ富集在肽酰基转移酶中心和其他功能区^[58-59]。假尿嘧啶合成酶(pseudouridine synthases)负责Ψ在RNA上的合成,其分为truA家族(包括大肠杆菌的PUS1,酵母的pus1p和pus3p等)、truB家族(包括pus4p和源自大肠杆菌的truB)、rsuA家族(大肠杆菌的rsuA)以及rlu家族(大肠杆菌的rluA和rluC)。这些蛋白质在家族内有明显的同源性但是在家族间除了假定的尿嘧啶结合基序外几乎没有同源性^[60]。所有假尿嘧啶合成酶都发挥相同的功能,即破坏碱基第1位的C-N糖苷键,旋转尿嘧啶环并在第5位形成C-C糖苷键。假尿嘧啶合成酶1(pseudouridine synthase 1, PUS1)属于truA家族,这一家族的所有假尿嘧啶合成酶都在其活性中心有一个保守的天冬氨酸^[61]。PUS1已经被证明可以在体内和体外修饰tRNA的27、28、34、35位核苷,同时在酵母中PUS1已经被证实可以修饰tRNA其他位点^[62]。

线粒体肌病伴随乳酸中毒和铁粒幼细胞性贫血(mitochondrial myopathy with lactic acidosis and sideroblastic anemia, MLASA)是一种常染色体隐性氧化磷酸化疾病,主要影响肌肉和骨髓^[63]。PUS1上的致病点突变已经被证实与这一疾病相关,表现为先天性铁母细胞性贫血、造血功能异常、肌肉量减少及线粒体氧化磷酸化蛋白质减少^[64]。研究发现从MLASA病人身上分离出的淋巴母细胞mt-tRNA^{Lys}上27、28位的Ψ修饰水平下降,表明低Ψ修饰的tRNA

会降低线粒体的翻译效率。此外,PUS1的底物不仅有mt-tRNA同时还有胞质tRNA,表明PUS1的突变不仅会影响线粒体tRNA的Ψ修饰也会影响胞质tRNA的Ψ修饰^[58,62];另有病例表明线粒体酪氨酸-tRNA合成酶2(mitochondrial tyrosyl-tRNA synthetase 2, YARS2)上的致病突变也会引起MLASA病^[65]。缺失Ψ修饰是造成MLASA的原因之一,其致病机制还需要更深入的研究。

1.4 N⁶-异戊烯基腺苷及其衍生修饰

N⁶-异戊烯基腺苷(N⁶-isopentenyladenosine, i⁶A)存在于原核生物、真核生物和古菌的tRNA第37位上,由异戊烯基转移酶(isopentenyltransferase, IPTase)催化^[66](图2)。一般认为IPTase可以通过识别tRNA上A36-A37-A38序列元件来对tRNA进行修饰,但是这一原则并不绝对^[67]。TRIT1是一个抑癌基因,它的突变与癌症的发生相关,其编码的蛋白是细菌的MiaA、酵母的MOD5的同源蛋白质^[68]。其中MOD5被发现同时负责胞质和线粒体中的i⁶A修饰。研究发现TRIT1的敲低能够降低i⁶A在mt-tRNA^{Ser}(UCN)上的丰度^[69]。目前已经鉴定到包括mt-tRNA^{Ser}(UCN)、mt-tRNA^{Cys}、mt-tRNA^{Phe}、mt-tRNA^{Trp}、mt-tRNA^{Tyr}在内的共5种哺乳动物线粒体tRNA上存在i⁶A修饰^[70]。在此基础上,Cdk5调控亚基相关蛋白质1(Cdk5 regulatory subunit-associated protein 1, Cdk5rap1)负责将2-甲基硫(2-methyl-thio, ms²)修饰到哺乳动物mt-tRNA^{Ser}(UCN)、mt-tRNA^{Phe}、mt-tRNA^{Trp}、mt-tRNA^{Tyr}上生成2-甲基硫-N⁶-异戊烯基腺苷(2-methylthio-N⁶-isopentenyladenosine, ms²i⁶A)修饰^[71](图2)。然而近期有研究表明不同于i⁶A同时修饰在胞质和线粒体tRNA上,ms²i⁶A可能只修饰在mt-tRNA上^[72]。

一般认为tRNA反密码子环34和37位上的修饰与密码子识别的效率和保真性息息相关,如i⁶A37修饰会增强A36-U1之间的相互作用,因此这些修饰的缺失和修饰酶的突变也可能对线粒体的翻译造成影响^[20]。已有的研究发现约12种发生在TRIT1基因上的致病点突变,这些常染色体隐性突变会造成联合氧化磷酸化缺陷35型(combined oxydative phosphorylation deficiency 35),患者在临床上表现为头小畸形、发育障碍和癫痫等典型的线粒体肌病^[73-74]。而深入的机制研究发现,TRIT1的突变和缺失会导致线粒体tRNA上i⁶A37的缺失并影响线粒体编码蛋白质的合成^[75]。此外,研究表明全身敲除Cdk5rap1的小鼠

对压力诱导的线粒体重塑十分敏感, 同时在压力刺激状态下表现出线粒体肌病和心功能失调; 研究人员在携带A3243G突变的MELAS病人细胞中发现线粒体tRNA上 ms^2 修饰的水平下调, 然而发生A3243G的mt-tRNA^{Leu}(UUR)上并没有 ms^2 修饰, 这表明该MELAS病的发生不仅是由于mt-tRNA^{Leu}(UUR)上牛磺酸修饰缺失使翻译发生阻滞, 同时也可能是由于包括mt-tRNA^{Ser}(UCN)、mt-tRNA^{Phe}、mt-tRNA^{Trp}、mt-tRNA^{Tyr}等在内多种含有 ms^2 修饰的tRNA共同解码错误使线粒体翻译紊乱的结果^[76]。

1.5 N^6 -苏氨酰氨基甲酰腺苷

N^6 -苏氨酰氨基甲酰腺苷(N^6 -threonylcarbamoyladenine, t⁶A)被发现存在于三界生物中, 是一种仅修饰在解码ANN密码子tRNA 37位的高度保守的化学修饰。目前已经在5种人线粒体tRNA上鉴定到t⁶A37修饰, 分别是mt-tRNA^{Thr}、mt-tRNA^{Lys}、mt-tRNA^{Ile}、mt-tRNA^{Asn}和mt-tRNA^{Ser}(UCN), 它们均由 N^6 -苏氨酰氨基甲酰转移酶结构域包含蛋白质(N^6 -threonylcarbamoyltransferase domain containing, YRDC)和唾液糖蛋白内多肽酶样蛋白质(*O*-sialoglycoprotein endopeptidase-like protein 1, OSGEPL1)负责合成^[70]。其中L-Thr和CO₂或HCO₃⁻在非酶催化下形成氨基甲酸酯中间体, 之后YRDC以ATP和中间体为底物催化合成苏氨酰氨基甲酰-AMP(threonylcarbamoyl-AMP, TC-AMP), OSGEPL1则将TC-AMP上的苏氨酰氨基甲酰基团转移到mt-tRNA上生成t⁶A修饰^[20,77-78]。

研究表明, mt-tRNA上t⁶A修饰丰度受到CO₂浓度的影响, 细胞内t⁶A修饰的缺失会导致mt-tRNA^{Thr}和mt-tRNA^{Lys}上m¹A9和m²G10修饰的上调并使两者的氨基酰化水平下降, 同时使临近的U36反密码子不仅识别mRNA上的A1, 也识别其他3种碱基使线粒体基因组编码的蛋白质出现氨基酸的误掺, 进一步使线粒体基因组编码氧化磷酸化蛋白质合成下调进而影响线粒体的形态和功能^[79-80]。然而在全身敲除*Osgepl1*的小鼠上, 研究人员并没有看到显著的线粒体疾病的表型, 说明在正常生理条件下t⁶A修饰的缺失并不会对小鼠的生理功能产生影响, 其作用可能类似于 ms^2 修饰, 相应的表型可能在压力刺激下才会出现^[79]。此外, 研究人员发现在mt-tRNA^{Thr}基因上出现的A15923G突变导致的MERRF样综合征的病人细胞中t⁶A修饰水平显著下降, 说明t⁶A修

饰也是这一线粒体脑肌病疾病的成因之一^[80]。

2 神经系统疾病

甲基化修饰广泛存在于生物的DNA和RNA分子中并发挥重要的生物学功能。在tRNA分子中的各不同位点的假尿嘧啶修饰和甲基化修饰作为核心修饰被认为通过增强或者减弱碱基间相互作用来稳定tRNA分子的三级结构^[59]。这其中, 在人mt-tRNA^{Lys}上第9位腺嘌呤的 N^1 -甲基化修饰(N^1 -methyladenosine, m¹A)对于tRNA的折叠影响很大。未修饰的tRNA^{Lys}会折叠生成无功能的扩展茎环结构, 而m¹A9修饰则阻止了第9位腺嘌呤和第64位鸟嘌呤的碱基互补配对并使结构平衡转移为三叶草构型。在22种人mt-tRNA中, 有19种mt-tRNA的第9位是腺嘌呤(A)或者鸟嘌呤(G), 考虑到目前所有动物mt-tRNA的修饰模式, 可以认为绝大多数mt-tRNA的第9位为m¹A或者m¹G^[81](图2)。

研究表明mt-tRNA第9位的m¹R[(R代表腺嘌呤(A)或鸟嘌呤(G))]由tRNA甲基转移酶10C(tRNA methyltransferase 10 C, TRMT10C)和3-羟酰基-辅酶A脱氢酶2(3-hydroxyacyl-CoA dehydrogenase type-2, 又称SDR5C1)共同催化修饰^[82]。TRMT10C是一个多功能蛋白质, 分别在RNA加工和tRNA甲基化过程中发挥作用, 它以SAM为甲基供体对特定残基进行 N^1 -甲基化修饰。TRMT10C包含一个N末端结构域(N-terminal domain, NTD)和一个双特异性的甲基转移酶结构域, 这一甲基转移酶结构域对线粒体tRNA第9位的m¹R甲基化修饰必不可少^[83]。SDR5C1是一个氨基酸和脂肪酸脱氢酶/还原酶, 它和TRMT10C、PRORP作为人线粒体RNase P复合物的组成部分参与线粒体tRNA 5'延伸段的去除, 此外SDR5C1和TRMT10C参与人线粒体tRNA m¹R9的生物合成^[84]。不同于大多数甲基化修饰只需要一个甲基转移酶进行修饰, TRMT10C负责底物的识别和催化, SDR5C1作为辅助蛋白对线粒体tRNA第9位甲基化修饰至关重要, TRMT10C必须和SDR5C1形成复合物才能行使甲基转移酶功能^[82]。

2-甲基-3-羟基丁酰辅酶A脱氢酶(2-methyl-3-hydroxybutyryl-CoA dehydrogenase, MHB)缺陷又称HSD10病, SDR5C1的错义突变会导致这一线粒体疾病的发生。HSD10主要被报道发生在世界范围内的少数家庭中, 大部分病例为男性, 因为编码SDR5C1

的基因 *HSD17B10* 定位于X性染色体上^[85]。SDR5C1的致病点突变会破坏依赖SDR5C1的脱氢作用、mt-tRNA加工以及甲基化。患者临床表现为进行性的神经退化和心肌病,生物化学诊断表现为异亮氨酸降解的两种中间体2-甲基-3-羟基丁酸酯和甲基巴豆酰甘氨酸水平的升高^[86]。通过敲除 *HSD17B10* 基因的小鼠模型发现这一疾病是由于线粒体氧化呼吸链的缺陷以及线粒体形态的异常引起的,但是其具体的分子机制目前仍不十分清楚^[87]。有研究团队在临床上鉴定到两例TRMT10C病例,分别为c.542G>T(p.Arg181Leu)和c.814A>G(p.Thr272Ala)的复合杂合子及纯合子c.542G>T(p.Arg181Leu),这两例病例临床上表现为出生时乳酸中毒、低肌张力、进食困难及失明,出生后5个月即死于呼吸衰竭。研究表明这两个突变影响了TRMT10C的稳定性及mt-tRNA的加工但是并不影响生成m¹R9修饰,未加工的mt-tRNA不能行使其生物学功能并造成线粒体疾病^[88]。在关于阿尔茨海默病的研究中,研究人员通过蛋白质组学发现阿尔茨海默病患者的TRMT10C和SDR5C1蛋白质表达水平显著低于正常人,在Tau蛋白果蝇模型中敲低编码这两个蛋白质的基因后会引发更严重的阿尔茨海默病表型并且发现mt-tRNA第9位上不同的m¹A甲基化水平和成熟mt-tRNA的表达呈正相关,这些结果有力表明缺失m¹A9修饰的mt-tRNA在阿尔茨海默病的发病过程中发挥作用^[89]。

此外,发生在线粒体基因组上的致病点突变会影响RNase P复合物在mt-tRNA上发挥功能,例如发生在mt-tRNA^{Leu}(UUR)上的所有致病点突变都会影响RNase P复合物的功能,其中U3290C只影响m¹A9甲基化功能,G3242A、G3250C、U3251G、A3288G影响tRNA 5'末端切割和m¹A9甲基化功能,A3243G和G3249A则同时影响RNase P复合物和tRNA的结合、5'末端切割和m¹A9甲基化功能^[90]。

3 癌症

3.1 3-甲基胞嘧啶

3-甲基胞嘧啶(3-methylcytidine, m³C)存在于tRNA和mRNA上,不同种类的RNA上其m³C由不同的甲基转移酶进行催化^[91](图2)。mt-tRNA^{Thr}和mt-tRNA^{Ser}(UCN)的32位m³C由甲基转移酶类似蛋白质8(methyltransferase-like 8, METTL8)催化修饰。研究发现METTL8催化这两种mt-tRNA的机制不同。

mt-tRNA^{Thr}的m³C32修饰由METTL8单独完成修饰反应,不依赖其他修饰酶;而mt-tRNA^{Ser}(UCN)的m³C32修饰则严格依赖37位的i⁶A^[92]。此外,METTL8与线粒体丝氨酰-tRNA合成酶(mitochondrial seryl-tRNA synthetase, SARS2)有相互作用并能稍微提高m³C的修饰速率^[93-94]。

研究发现敲除METTL8会导致mt-tRNA m³C的修饰水平大幅下降,同时影响线粒体氧化磷酸化复合物的活力,表明m³C32修饰对线粒体功能至关重要^[92]。通过分析公开可用的来自癌症病人和组织的基因表达数据,研究人员发现METTL8在弥漫性大B细胞癌(diffuse large B cell carcinoma, DLBC)、胶质母细胞瘤(glioblastoma, GBM)、低级别胶质瘤(low-grade glioma, LGG)、肺鳞状细胞癌(lung squamous cell carcinoma, LUSC)、胰腺癌(pancreatic adeno-carcinoma, PAAD)、胃癌(stomach adenocarcinoma, STAD)和甲状腺癌(thyroid carcinoma, THYM)中上调。由于蛋白质表达失调和发病机制并不一定相关,他们进一步研究了METTL8上调和病人生存率的关系,发现了在高侵袭型的胰腺癌患者中METTL8的高水平表达和患者的生存率存在显著的负相关关系,表明了METTL8可能和胰腺癌的发病有一定关联^[95-96]。进一步的研究发现在胰腺癌细胞系PANC-1中METTL8高表达,并进一步导致mt-tRNA^{Ser}(UCN)的m³C修饰上调,由此使线粒体氧化磷酸化复合物I和II的活力上调,对于癌细胞来说能量供应的提高对其生存和快速发展显然是有利的^[92,97]。

3.2 其他与癌症有关的mt-tRNA修饰

在上文中我们介绍了mt-tRNA第9位m¹R修饰与神经系统疾病的关系。同时,该位点的N¹-甲基化修饰与肿瘤间也存在着紧密的联系。研究表明,mt-tRNA第9位的m¹A和m¹G甲基化在包括浸润性乳腺癌、结肠癌、肝癌、肺癌等在内的多种癌症组织中高表达,同时核苷酸高甲基化的病人其生存率显著降低,说明mt-tRNA第9位m¹R修饰参与了癌症的发生发展^[98-99]。

此外,f⁵C及其前体的m⁵C对癌细胞的迁移和癌症组织的浸润也发挥着重要作用。研究表明,在NSUN3缺失的人口腔癌细胞中糖酵解水平的提高和线粒体功能的变化并不会影响该细胞的存活及原发肿瘤的生长,但是肿瘤组织却无法有效地迁移,说

明转移起始的肿瘤细胞需要mt-tRNA^{Met}的m⁵C34修饰来激活其浸润及扩散功能^[100-101]。

4 总结与展望

mt-tRNA虽然只负责13种氧化磷酸化复合物亚基的翻译,但其功能却至关重要。发生在mt-tRNA上的化学修饰作用不尽相同,如稳定tRNA三级结构、保证mt-tRNA正确识别密码子、稳定tRNA与mRNA间的相互作用等。因此mt-tRNA修饰水平的异常升高或下降都会导致疾病的发生,典型的牛磺酸修饰的缺失会导致严重的线粒体脑肌病,而特定定位点的甲基化修饰的上调则与癌症的发生发展有着千丝万缕的联系。因此,mt-tRNA修饰的研究对于正确认识生命活动有着重要的意义。

然而,我们对于mt-tRNA修饰的研究十分有限。很大一部分的修饰其具体的分子机制甚至催化修饰的酶仍不清楚,而与这些修饰相关的疾病的研究大多数依然停留在表型的描述,缺少具体的致病机理阐释,亟待科研人员不断深入研究。

未来,我们期待能够阐明线粒体tRNA修饰的基本规律,开发专一高效的靶向性干预策略来干预这些由mt-tRNA修饰或修饰酶缺陷导致的人类疾病。

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