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线粒体tRNA突变与神经系统疾病

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摘要：线粒体是真核细胞能量代谢的核心细胞器。人线粒体基因组编码2个rRNA基因、22个tRNA基因和13个氧化磷酸化复合物亚基基因。除了tRNA^{Leu}和tRNA^{Ser}具有2种等受体外，其他18种氨基酸分别对应唯一一种tRNA。线粒体tRNA基因是线粒体DNA的突变热点区域，任何一种tRNA结构和功能发生改变，都可能对线粒体蛋白质合成系统造成明显的影响。线粒体功能障碍通常累及多个器官系统，如神经系统、肌肉、心脏等。本文将着重介绍6种神经系统线粒体疾病及其相关线粒体tRNA基因突变，总结分子致病机制，讨论相关的基因治疗手段。

关键词：线粒体；tRNA；神经系统疾病；致病机制；基因治疗

Mitochondrial tRNA mutations and neurological diseases

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Abstract: Mitochondria are the key organelles in eukaryotic cells, responsible for energy production. Human mitochondrial DNA encodes two ribosomal RNAs, 22 species of mitochondrial tRNAs, and 13 essential subunits of the inner membrane complex responsible for oxidative phosphorylation. Two tRNA^{Leu} and tRNA^{Ser} isoacceptors are found in human mitochondria, while only one tRNA is for the other 18 amino acids respectively. Mitochondrial tRNA genes are hot spots for pathogenic mitochondrial DNA point mutations. Any change in the structure and function of tRNA may introduce a significant impact on the mitochondrial protein translation system. Mitochondrial dysfunctions affect more than one type of cell, tissue or organ, for example, nervous system, muscle, heart and so on. Herein, we summarized the latest research progress of 6 neurological diseases related with mitochondrial tRNA gene mutations, as well as the molecular pathogenic mechanisms and gene therapy of mitochondrial diseases in the future.

Key Words: mitochondria; tRNA; neurological disease; pathogenic mechanism; gene therapy

线粒体疾病是指由线粒体基因或核基因突变导致的、以氧化磷酸化缺陷引起的线粒体功能障碍为特点的一组复杂疾病，通常累及多个器官和系统。其中神经系统的能量需求高，对能量代谢

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障碍非常敏感，因此神经系统是较为常见的发病部位，且中枢神经系统和外周神经系统都会受到影响[1]。本文将重点介绍与神经系统线粒体疾病相关的线粒体tRNA突变。

1 线粒体tRNA

线粒体是真核细胞的能量代谢中心，通过氧化磷酸化(oxidative phosphorylation，OXPHOS)提供细胞各种生理活动所需约90%的ATP。同时，线粒体也是物质代谢和细胞凋亡的核心细胞器，参与血红素、类固醇合成，并与内质网、细胞外基质等结构协同作用，控制细胞中钙离子浓度的动态平衡[2]。

人线粒体DNA(mitochondrial DNA，mtDNA)全长16 569 bp，编码37个基因，包括2个rRNA基因、22个tRNA基因和13个参与线粒体氧化磷酸化的复合物亚基基因[3]。核基因组则编码其余90%以上的与线粒体结构和功能相关的蛋白质[4]。与核基因组不同，mtDNA没有核小体保护，缺少有效的突变修复机制，突变频率是核基因的10~20倍[5]。mtDNA作为细胞内的核外遗传物质，表现为典型的母系遗传，即母亲将mtDNA传递给她的子女，但只有女儿能将其mtDNA传递给下一代[6]。若细胞或组织中的所有mtDNA都相同，称之为同质性；若同一细胞或组织中同时存在突变型和野生型mtDNA，则称之为异质性。

tRNA是指携带并转运氨基酸参与蛋白质生物合成的一类非编码RNA，通常由60~90个核苷酸组成，二级结构呈现三叶草模式——包括二氢尿嘧啶茎/环、反密码子茎/环、可变环、TΨC茎/环和接受氨基酸的接受茎(图1)[3]。人核基因组编码超过400种细胞质tRNA，而22种线粒体tRNA则全部由mtDNA编码。除了亮氨酸和丝氨酸分别对应2种tRNA等受体外，其他18种氨基酸各对应唯一一种tRNA，因此任何一种tRNA的结构或功能发生改变，都可能对线粒体蛋白质合成造成明显的影响；线粒体tRNA二级结构中的A•U配对和不稳定的非Watson-Crick配对含量明显偏高，并且往往具有缩短的茎区和环区，tRNA$^{Ser(AGY)}$甚至缺失了整个D-茎环结构[3]。除此之外，人线粒体tRNA上存在大量的转录后修饰，18种修饰位于137个不同位

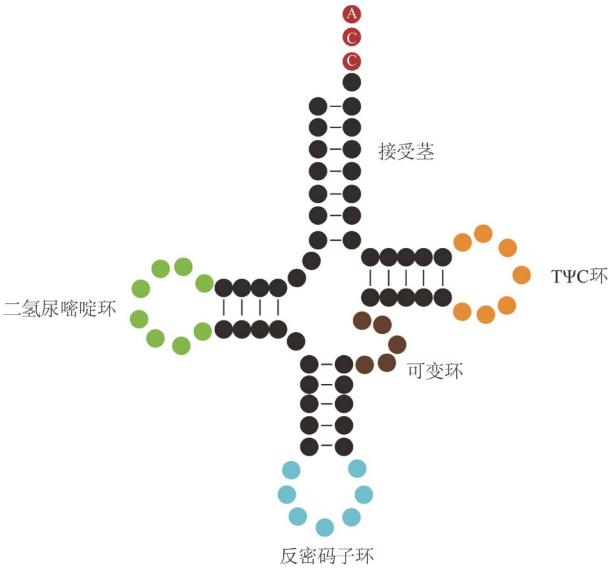


图1 tRNA分子二级结构示意图

点，占核苷酸总数目的8.7%[7]。线粒体tRNA是多顺反子rRNA和mRNA之间重要的衔接子，分别通过5′核酸酶RNase P和3′核酸酶RNase Z的剪切，释放mRNA、rRNA和tRNA[8,9]。tRNA经多种核苷酸修饰酶修饰并折叠成倒L形的成熟tRNA。氨基酰-tRNA合成酶(aminoacyl-tRNA synthetase，aaRS)催化成熟tRNA与其对应的氨基酸之间的酯化反应生成氨基酰-tRNA，人线粒体热不稳定延伸因子(human mitochondrial elongation factor thermo unstable，hmEF-Tu)则介导氨基酰-tRNA进入核糖体空出的A位。该过程需消耗hmEF-Tu水解其复合的GTP产生的能量来完成。EF-Tu通过一种热力学的补偿机制保证翻译的精确性，只有正确的氨基酰化产物才会被EF-Tu识别并保护氨基酰-tRNA的酯键不被自发水解[10]。

线粒体tRNA基因是mtDNA的突变热点区域。人线粒体基因组数据库(https://www.mitomap.org/MITOMAP)中与疾病相关的mtDNA突变超过980个，尽管线粒体tRNA基因片段仅占mtDNA总长度的9.1%，但其突变数目超过360个，占总突变数目的37%。线粒体tRNA基因突变的致病机理不尽相同，分子机制的研究尚不完善。

2 线粒体tRNA基因突变与神经系统疾病

2.1 线粒体脑肌病伴高乳酸血症及卒中样发作

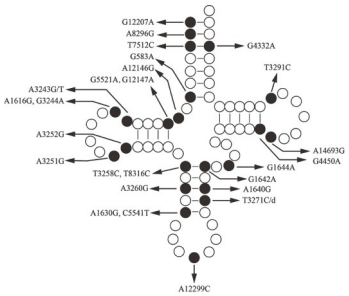
线粒体脑肌病伴高乳酸血症及卒中样发作

(mitochondrial encephalomyopathy with lactate acidosis and stroke-like episodes，MELAS)是最常见和研究最广泛的母系遗传性线粒体疾病之一，发病年龄为2~40岁[11]。常见的临床表现包括：乳酸酸中毒、癫痫发作和卒中样发作的三联征以及痴呆、肌肉无力、运动不耐受、头痛、呕吐、听力下降、学习障碍和身材矮小等[12]。MELAS大多是由mtDNA突变引起的，包括12个tRNA基因：*MT-TF*、*MT-TV*、*MT-TL1*、*MT-TQ*、*MT-TM*、*MT-TW*、*MT-TS1*、*MT-TK*、*MT-TH*、*MT-TS2*、*MT-TL2*和*MT-TE*(表1)。其中超过80%的MELAS患者携带A3243G突变[13]。Koga等[14]发现，A3243G突变还可导致其他不同的临床表型，包括Leigh综合征(Leigh syndrome，LS)、进行性外眼肌麻痹(progressive external ophthalmoplegia，PEO)和线粒体糖尿病等。

2.2 肌阵挛性癫痫伴破碎红纤维

肌阵挛性癫痫伴破碎红纤维(myoclonic epilepsy with ragged red fibers，MERRF)是一种可从儿童期持续至成年期的多系统病，主要临床表现为阵发

表1　MELAS相关线粒体tRNA突变



基因	突变	保守性	编号	位置	同质性	异质性	参考文献
$tRNA^{Phe}$	G583A	95.56%	7	ACC stem	—	+	[15]
$tRNA^{Val}$	A1616G	86.67%	15	D-loop	+	—	[16]
	A1630G	15.56%	31	AC stem	—	+	[17,18]
	A1640G	80.00%	41	AC stem	+	—	[19]
	G1642A	26.67%	43	AC stem	—	+	[20]
	G1644A	91.11%	45	Variable loop	—	+	[21]
$tRNA^{Leu(UUR)}$	A3243G	97.78%	14	D-loop	—	+	[13,22]
	A3243T	97.78%	14	D-loop	—	+	[23,24]
	G3244A	95.56%	15	D-loop	—	+	[25]
	A3251G	93.33%	22	D-loop	—	+	[26]
	A3252G	100.00%	23	D-loop	—	+	[27]
	T3258C	88.89%	27	AC stem	—	+	[28]
	A3260G	100.00%	29	AC stem	—	+	[29]
	T3271C	82.22%	40	AC stem	—	+	[30,31]
	T3271d	97.78%	40	AC stem	—	+	[32]
	T3291C	91.11%	60	T-loop	—	+	[33,34]
$tRNA^{Gln}$	G4332A	97.78%	70	ACC stem	—	+	[35]
$tRNA^{Met}$	G4450A	97.78%	53	T-stem	—	+	[36]
$tRNA^{Trp}$	G5521A	93.33%	10	D-stem	—	+	[37]
	C5541T	100.00%	31	AC stem	—	+	[38,39]
$tRNA^{Ser(UCN)}$	T7512C	31.11%	3	ACC stem	—	+	[40]
$tRNA^{Lys}$	A8296G	93.33%	2	ACC stem	—	+	[41]
	T8316C	75.56%	27	AC stem	—	+	[42]
$tRNA^{His}$	A12146G	100.00%	9	A-D Junction	—	+	[43]
	G12147A	100.00%	10	D-stem	—	+	[44]
$tRNA^{Ser(AGY)}$	G12207A	77.78%	1	ACC stem	—	+	[45]
$tRNA^{Leu(CUN)}$	A12299C	100.00%	35	AC loop	—	+	[46]
$tRNA^{Glu}$	A14693G	91.11%	54	T-loop	+	—	[47]

保守性分析参考MITOMAP中45种物种线粒体tRNA序列比对；核苷酸编号参考文献[48]命名；ACC stem：接受茎；D-loop：D环/二氢尿嘧啶环；AC stem：反密码子茎；variable loop：可变环；T-loop：T环/TΨC环；T-stem：T茎；D-stem：D茎；A-D Junction：接受茎/D茎连接核苷酸；AC loop：反密码子环。+指有同质性或异质性的病例(下同)，—指没有同质性或异质性的病例(下同)

性癫痫，伴有进行性神经系统障碍(智力倒退、共济失调、意向性震颤)，肌纤维紊乱、粗糙，线粒体形态异常并在骨骼肌细胞中积累，肌肉活检时能观察到参差不齐的红色纤维(ragged red fibers，RRF)[49]。临床上，MERRF还表现为眼、耳、内分泌器官、心脏、胃肠道和皮肤的异常，偶尔伴发脂肪瘤[50]。MERRF最常见的原因是*MT-TK*上的突变，少数是由其他tRNA基因突变引起，包括*MT-TF*、*MT-TL1*、*MT-TC*、*MT-TH*、*MT-TE*、*MT-TT*和*MT-TP*(表2)。其中，A8344G突变占MERRF病例的80%~90%，引起呼吸链酶复合物的多种缺陷[51]。MERRF相关tRNA突变大多是异质性的，异质性比例和患者年龄会影响患者OXPHOS缺陷及临床表型的严重程度[52]。

2.3 Leigh综合征

Leigh综合征是一种严重的进行性代谢性神经退行性疾病，通常始于婴儿期或儿童期，亦有成人发病病例。临床症状主要表现为精神和运动发育迟缓、共济失调、肌张力障碍、视神经萎缩、癫痫发作和乳酸酸中毒等[68]。影像学诊断表现为脑干、基底节和丘脑的对称损伤。LS是最常见的线粒体疾病之一，常和其他线粒体病重合出现。迄今为止，已在受影响的患者中鉴定出超过75个基因的致病突变，其中包括7种线粒体tRNA的基因：*MT-TV*、*MT-TL1*、*MT-TM*、*MT-TI*、*MT-TW*、*MT-TK*和*MT-TL2*[69](表3)。

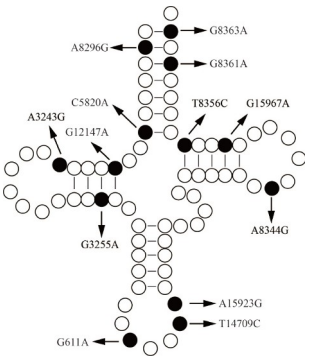
2.4 Kearns-Sayre综合征

Kearns-Sayre综合征(Kearns-Sayre's syndrome，KSS)是一种非常罕见的线粒体脑肌病，由以下三联征定义：20岁前发病、慢性进行性外眼肌麻痹(chronic progressive external ophthalmoplegia，CPEO)和色素视网膜病变。KSS患者随时可能出现心脏传导障碍，容易因心率失常或心脏栓塞而中风或猝死。此外，患者还可能出现小脑性共济失调、身材矮小、耳聋、痴呆和内分泌异常等症状。90%的KSS是由线粒体DNA的一个或多个碱基缺失引起的，少数KSS由线粒体tRNA基因突变造成[85]。目前，已报道3个线粒体tRNA基因与KSS相关：*MT-TL1*、*MT-TK*和*MT-TL2*(表4)。

2.5 Leber遗传性视神经病

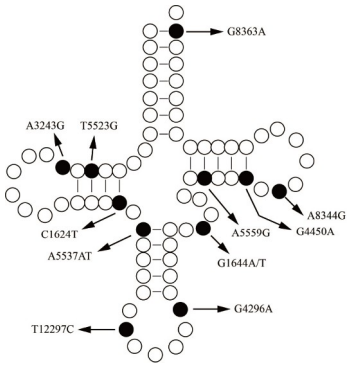
Leber遗传性视神经病(Leber hereditary optic neuropathy，LHON)是一种罕见的线粒体视神经病。LHON的眼底三联征特征是视乳头周围毛细血管扩张、视盘周围神经纤维层肿胀、荧光素眼底血管造影术视盘无渗漏。临床表现为单侧、无痛、亚急性、中央视力丧失，在接下来的6个月内对侧眼开始受累。LHON由mtDNA的致病突变造成，男性好发，发病高峰年龄在15~35岁[90]。95%以上的LHON病例是由位于*MT-ND4*基因(G11778A)、*MT-ND1*基因(G3460A)和*MT-ND6*基因

表2 MERRF相关线粒体tRNA突变



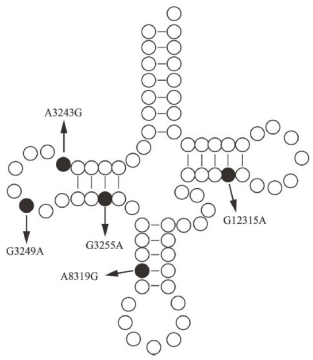
基因	突变	保守性	编号	位置	同质性	异质性	参考文献
$tRNA^{Phe}$	G611A	100.00%	34	AC loop	—	+	[53]
$tRNA^{Leu(UUR)}$	A3243G	97.78%	14	D-loop	—	+	[54]
	G3255A	100.00%	24	D-stem	—	+	[55]
$tRNA^{Cys}$	C5820A	91.11%	7	ACC stem	+	—	[56]
$tRNA^{Lys}$	A8296G	93.33%	2	ACC stem	—	+	[57]
	A8344G	37.78%	55	T-loop	—	+	[51,52,58]
	T8356C	26.67%	65	T-stem	—	+	[59-61]
	G8361A	26.67%	70	ACC stem	—	+	[62]
	G8363A	95.56%	72	ACC stem	—	+	[61]
$tRNA^{His}$	G12147A	100.00%	10	D-stem	—	+	[44]
$tRNA^{Glu}$	T14709C	95.56%	37	AC loop	—	+	[63.64]
$tRNA^{Thr}$	A15923G	100.00%	38	AC loop	—	+	[65,66]
$tRNA^{Pro}$	G15967A	35.56%	62	T-stem	—	+	[67]

表3 LS相关线粒体tRNA突变



基因	突变	保守性	编号	位置	同质性	异质性	参考文献
$tRNA^{Val}$	C1624T	97.78%	25	D-stem	+	—	[70]
	G1644A	91.11%	45	Variable loop	+	—	[71]
	G1644T	91.11%	45	Variable loop	—	+	[72]
$tRNA^{Leu(UUR)}$	A3243G	97.78%	14	D-loop	—	+	[14,73]
$tRNA^{Ile}$	G4296A	95.56%	38	AC loop	—	+	[74,75]
$tRNA^{Met}$	G4450A	97.78%	53	T-stem	—	+	[69]
$tRNA^{Trp}$	T5523G	20.00%	12	D-stem	—	+	[76]
	A5537AT	97.78%	27	AC stem	—	+	[77,78]
	A5559G	33.33%	50	T-stem	+	+	[76,79]
$tRNA^{Lys}$	A8344G	37.78%	55	T-loop	—	+	[80-82]
	G8363A	95.56%	72	ACC stem	—	+	[83]
$tRNA^{Leu(CUN)}$	T12297C	100.00%	33	AC loop	—	+	[84]

表4 KSS相关线粒体tRNA突变



基因	突变	保守性	编号	位置	同质性	异质性	参考文献
$tRNA^{Leu(UUR)}$	A3243G	97.78%	14	D-loop	—	+	[86]
	G3249A	97.78%	20	D-loop	—	+	[87]
	G3255A	100.00%	24	D-stem	—	+	[55]
$tRNA^{Lys}$	A8319G	82.22%	30	AC stem	—	+	[88]
$tRNA^{Leu(CUN)}$	G12315A	97.78%	52	T-stem	—	+	[89]

(T14484C)的三种主要突变引起，也有少数病例是由线粒体tRNA基因突变造成的。大多数与LHON相关的tRNA突变是继发突变，突变本身不足以引起LHON症状，但对原发突变的LHON表型表达有修饰作用。目前已报道8个线粒体tRNA基因与LHON相关：*MT-TF*、*MT-TL1*、*MT-TQ*、*MT-TM*、*MT-TA*、*MT-TE*、*MT-TT*、*MT-TP*(表5)。

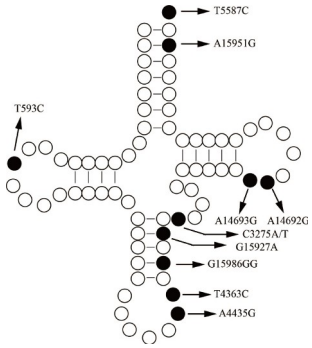
2.6 慢性进行性外眼肌麻痹

慢性进行性外眼肌麻痹(chronic progressive external ophthalmoplegia，CPEO)与线粒体功能障碍的其他症状同时发生时，临床特征表现为外眼肌麻痹、上睑下垂、眼球运动障碍、近端肌无力、高频感音神经性听力损失和进行性吞咽困难，并可伴有视网膜、心脏和大脑异常[103]。肌肉活检是诊断CPEO的重要方法，可以观察到COX阴性纤维和超过2%总纤维的参差不齐的红色纤维(RRF)。大多数CPEO病例是散发性的，由单个异质性的mtDNA大片段缺失引起，长度范围为1.3~9.1 kb。少数CPEO病例是遗传性的，包括母系遗传或常染色体遗传[104]。 CPEO病例可能由*MT-TF*、*MT-TL1*、*MT-TI*、*MT-TA*、*MT-TN*、*MT-TY*、*MT-TS1*、*MT-TK*、*MT-TL2*、*MT-TE*和*MT-TP*等11种线粒体tRNA基因突变引起(表6)。其中高达15%的CPEO患者携带A3243G突变[105]。

3 线粒体tRNA突变导致的神经系统疾病的分子机制

线粒体tRNA基因突变导致的神经系统疾病临床表现多样，基因型和表型之间的相互作用复杂，携带相同突变的患者表现出截然不同的表

表5 LHON相关线粒体tRNA突变



基因	突变	保守性	编号	位置	同质性	异质性	参考文献
$tRNA^{Phe}$	T593C	33.33%	17	D-loop	+	—	[91,92]
$tRNA^{Leu(UUR)}$	C3275A	37.78%	44	Variable loop	+	—	[93]
	C3275T	37.78%	44	Variable loop	+	—	[94]
$tRNA^{Gln}$	T4363C	64.44%	38	AC loop	+	—	[94]
$tRNA^{Met}$	A4435G	100.00%	37	AC loop	+	—	[95]
$tRNA^{Ala}$	T5587C	91.11%	73	ACC stem	+	—	[96]
$tRNA^{Glu}$	A14692G	88.89%	55	T-loop	+	—	[97]
	A14693G	91.11%	54	T-loop	+	—	[98]
$tRNA^{Thr}$	G15927A	35.56%	42	AC stem	+	—	[99]
	A15951G	75.56%	71	ACC stem	+	—	[100,101]
$tRNA^{Pro}$	G15986GG	46.67%	40	AC stem	+	—	[102]

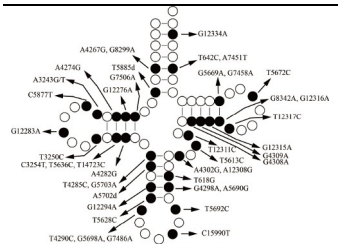
型，而携带不同异质性突变的患者表现出临床上相同的症状[146]。迄今为止，线粒体tRNA功能障碍与神经系统疾病发生之间仍没有找到统一的联系。与神经系统疾病相关的线粒体tRNA突变可能通过影响tRNA的高级结构、tRNA的加工修饰、密码子识别这三个方面，导致tRNA稳定性丧失、线粒体mRNA翻译缺陷，进而影响线粒体蛋白质合成，导致呼吸缺陷等。以下将从突变所在位置区域进行举例分析。

D环：A3243G突变位于$tRNA^{Leu(UUR)}$ D环第14位，进化上高度保守，被认为是线粒体DNA重链转录本H1的转录终止因子的结合区域，因此突变很可能阻碍了线粒体DNA的正常转录。A3243G突变不仅破坏了$tRNA^{Leu(UUR)}$结构稳定性，使之几乎丧失了氨基酰化活力[33]，同时密码子摆动位点34位牛磺酸甲基化修饰(5-taurinemethyluridine，τm^5U)的缺失影响了氨基酰-tRNA在核糖体解码过程中密码子的识别，使本应只识别亮氨酸密码子UUR的tRNA错误地识别了苯丙氨酸密码子UUY，进而引发蛋白质合成错误[147]。另有研究表明，A3243G突变还抑制了细胞对谷氨酸的摄取以及线粒体ATP合成酶的活性[148,149]。

TΨC环：A8344G突变位于$tRNA^{Lys}$ TΨC环第55位[51]。质谱检测表明A8344G突变导致$tRNA^{Lys}$第34位5-牛磺酸甲基2-硫代尿苷(5-taurinomethyl-2-thiouridine，τm^5S^2U)修饰缺失，密码子-反密码子不能稳定匹配，使$tRNA^{Lys}$解码其对应的AAA密码子的能力丧失了95%，且几乎完全不能解码另一对应密码子AAG，线粒体蛋白质合成体系的功能失调致使热休克蛋白质27等多种蛋白质合成量下降，并引发细胞凋亡反应[150,151]。A14693G突变位于线粒体$tRNA^{Glu}$ TΨC环的第54位。细菌和酵母中U54和A58形成的Hoogsteen配对是Ψ55修饰酶Trub和Pus4识别tRNA的关键，因此该位点突变破坏了U54-A58配对，阻碍了Ψ55修饰，导致tRNA代谢异常[152]。

反密码子环：A12299C突变位于$tRNA^{Leu(CUN)}$的反密码子的第35位，直接导致反密码子识别由亮氨酸转变为精氨酸，即在线粒体蛋白质合成过程中由$tRNA^{Leu(CUN)}$负责的4种亮氨酸密码子被误掺入精氨酸，导致线粒体蛋白质合成异常[46]。G7486A、T14709C和A15923G分别位于$tRNA^{Ser(UCN)}$，$tRNA^{Glu}$和$tRNA^{Thr}$反密码子环的第32、37和38位，虽然没有导致识别密码子的直接改变，但都可能引起反密码子环构象的改变[63-66]。反密码子环第32位在进化中高度保守，并携带3-甲基胞苷(3-methylcytidine，m^3C)修饰。同时该位点突变也破坏了对反密码子环的U型转弯构象至关重要的C32:A38配对，影响反密码子环的构造，干扰与核糖体的相互作用，减少密码子识别接触时间。当线粒体tRNA的37位碱基由A突变为G时，会引入第37位1-甲基鸟苷(1-methylguanosine，m^1G)修饰，引起线粒体tRNA前体5′剪切效率的下降，导致tRNA稳态水平和氨基酰化水平的明显下降和线粒体内蛋

表6 CPEO相关线粒体tRNA突变

	基因	突变	保守性	编号	位置	同质性	异质性	参考文献
	tRNA^{Phe}	T618G	26.67%	41	AC stem	—	+	[106]
		T642C	91.11%	68	ACC stem	—	+	[107]
	tRNA$^{Leu(UUR)}$	A3243G	97.78%	14	D-loop	—	+	[105,108]
		A3243T	97.78%	14	D-loop	—	+	[109]
		T3250C	68.89%	21	D-loop	—	+	[110]
		C3254T	6.67%	23	D-stem	+	—	[111]
	tRNA^{Ile}	A4267G	93.33%	5	ACC stem	—	+	[112]
		A4274G	95.56%	12	D-stem	—	+	[113]
		A4282G	97.78%	24	D-stem	—	+	[114]
		T4285C	95.56%	27	AC stem	—	+	[115]
		T4290C	95.56%	32	AC loop	+	—	[107]
		G4298A	95.56%	40	AC stem	—	+	[116]
		A4302G	97.78%	44	Variable loop	—	+	[117]
		G4308A	95.56%	50	T-stem	—	+	[118]
		G4309A	24.44%	51	T-stem	—	+	[119]
	tRNA^{Ala}	T5613C	97.78%	46	Variable loop	—	+	[120]
		T5628C	95.56%	31	AC stem	—	+	[121]
		T5636C	100.00%	23	D-stem	—	+	[122]
	tRNA^{Asn}	G5669A	100.00%	61	T-stem	—	+	[123]
		T5672C	64.44%	58	T-loop	—	+	[124]
		A5690G	97.78%	40	AC stem	—	+	[125,126]
		T5692C	100.00%	38	AC loop	—	+	[127]
		G5698A	97.78%	32	AC loop	—	+	[128]
		A5702d	97.78%	28	AC stem	—	+	[123]
		G5703A	8.89%	27	AC stem	—	+	[124]
	tRNA^{Tyr}	C5877T	80.00%	15	D-loop	—	+	[129]
		T5885d	97.78%	7	ACC stem	—	+	[130]
	tRNA$^{Ser(UCN)}$	A7451T	95.56%	68	ACC stem	—	+	[126]
		G7458A	97.78%	61	T-stem	—	+	[131]
		G7486A	97.78%	32	AC loop	—	+	[132]
		G7506A	82.22%	10	D-stem	—	+	[133]
	tRNA^{Lys}	G8299A	33.33%	5	ACC stem	—	+	[134]
		G8342A	62.22%	53	T-stem	—	+	[135]
	tRNA$^{Leu(CUN)}$	G12276A	97.78%	11	D-stem	—	+	[136]
		G12283A	100.00%	18	D-loop	—	+	[137]
		G12294A	95.56%	30	AC stem	—	+	[138]
		A12308G	95.56%	44	Variable loop	—	+	[139]
		T12311C	31.11%	47	Variable loop	—	+	[140]
		G12315A	97.78%	52	T-stem	—	+	[141]
		G12316A	97.78%	53	T-stem	—	+	[142]
		T12317C	100.00%	54	T-loop	—	+	[126]
		G12334A	86.67%	71	ACC stem	—	+	[143]
	tRNA^{Glu}	T14723C	100.00%	23	D-stem	—	+	[144]
	tRNA^{Pro}	C15990T	100.00%	36	AC loop	—	+	[145]

白质稳态失衡[153,154]。

接受茎：G12207A位于tRNA$^{Ser(AGY)}$ 5′末端的第1位，参与氨基酸接受茎的形成，突变可能影响了前体RNA的加工、tRNA的稳定性和氨基酸接受效率以及蛋白质合成的整体效率[45]。G8363A、T5587C分别位于tRNA^{Lys}、tRNA^{Ala}接受茎的第72、73位，造成tRNA前体分子3′末端剪切异常，tRNA稳定性下降，进而导致线粒体蛋白质合成紊乱，诱发线粒体功能障碍[96]。

D茎/TΨC茎/反密码子茎：G3255A位于tRNA$^{Leu(UUR)}$ D茎的第24位，破坏了与C11之间的碱基配对。由于tRNA$^{Leu(UUR)}$ D茎中存在A12•C23和G13•U22两对不稳定的非Watson-Crick配对，所以G3255A很可能破坏了tRNA$^{Leu(UUR)}$ D茎环区域的构象及与TΨC环之间的相互作用，导致tRNA$^{Leu(UUR)}$结构和功能的紊乱[55]。与之类似的如位于tRNA^{Pro} TΨC茎第62位的G15967A突变，导致TΨC茎的非Watson-Crick配对增加为3对，即原有的G49•U65。G50•U64以及突变导致的G52•U62致使tRNA^{Pro}结构的不稳定[67]。G12315A在T茎引入了A52•C62一对错配碱基，破坏了原有的具有较强氢键作用的G52-C62碱基对，导致T茎结构稳定性被削弱，使tRNA前体3′末端加工受到抑制，并且导致了碱基修饰的缺失，进一步抑制了tRNA的氨基酰化活力以及与hmEF-Tu的结合能力[155]。G15927A突变位于反密码子茎第42位，转线粒体细胞实验结果表明突变导致tRNA^{Thr}高级结构发生改变，稳态水平和氨酰化水平降低，而线粒体tRNA^{Thr}功能的改变导致线粒体蛋白质水平降低，氧化呼吸功能障碍，线粒体膜电位降低，以及活性氧的增加。线粒体功能缺陷最终导致细胞凋亡水平增高[156]。

可变环：G1644A突变可能导致tRNA^{Val}的构象变化，或影响线粒体DNA重链转录本H1中12S rRNA和16S rRNAs的剪接，导致线粒体蛋白质合成障碍[21]。T12311C位于可变环第48位，是可变环和TΨC茎的连接碱基。人线粒体tRNA$^{Leu(CUN)}$分子TΨC茎存在独特的滑动机制，调控TΨC茎环结构及氨基酰化活力，突变则可导致tRNA结构柔性的丧失[157]。

综上，神经系统线粒体疾病的发生是多因素影响、多步骤调控的结果。与神经系统疾病相关的线粒体tRNA基因突变不同程度影响了tRNA的成熟(前体分子5′和3′末端剪切、3′末端CCA添加、核苷酸修饰)和功能行使(氨基酰化活力、密码子识别)等步骤，导致线粒体和细胞功能障碍(蛋白质合成、ATP产量下降、ROS生成增加、线粒体自噬和细胞凋亡)，最终引起神经系统疾病(图2)。

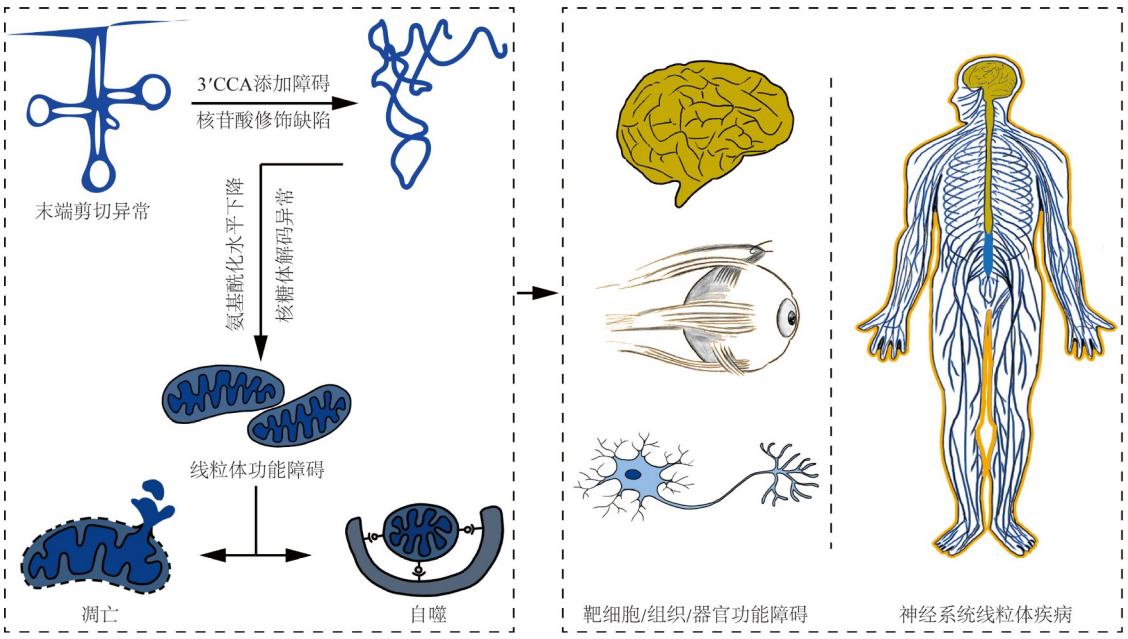


图2 神经系统疾病相关的线粒体tRNA基因突变的分子致病机制

4 线粒体tRNA基因突变导致的神经系统疾病的治疗

线粒体tRNA基因突变导致的神经系统疾病危害严重、致病机制复杂，有效治疗手段匮乏，临床上通常通过补充氧化磷酸化辅助因子、添加呼吸链所需辅酶等常规手段减轻患者症状。基因治疗为有效防治线粒体疾病带来新的希望(图3)。

Kawamura等[158]利用八精氨酸(octaarginine，R8)修饰的脂膜分子(MITO-Porter)携带野生型线粒体前体tRNA^{Phe}(pre-WT-tRNA^{Phe})转染携带G625A异质性突变的病人来源的成纤维细胞，发现tRNA^{Phe}的突变比例降低，线粒体呼吸活性增加。除了线粒体tRNA异位表达外，越来越多的研究表明，线粒体aaRS的过表达可以提高对应突变tRNA的氨基酰化水平，进而恢复线粒体功能，校正细胞的疾病表型，例如分别在携带线粒体tRNA$^{Leu(UUR)}$ A3243G、tRNA^{Ala} A5655G、tRNA^{His} T12201C突变的病人来源的细胞中高表达亮氨酰-tRNA合成酶、丙氨酰-tRNA合成酶、组氨酰-tRNA合成酶，以校正由线粒体tRNA突变导致的线粒体和细胞功能障碍[159-161]。

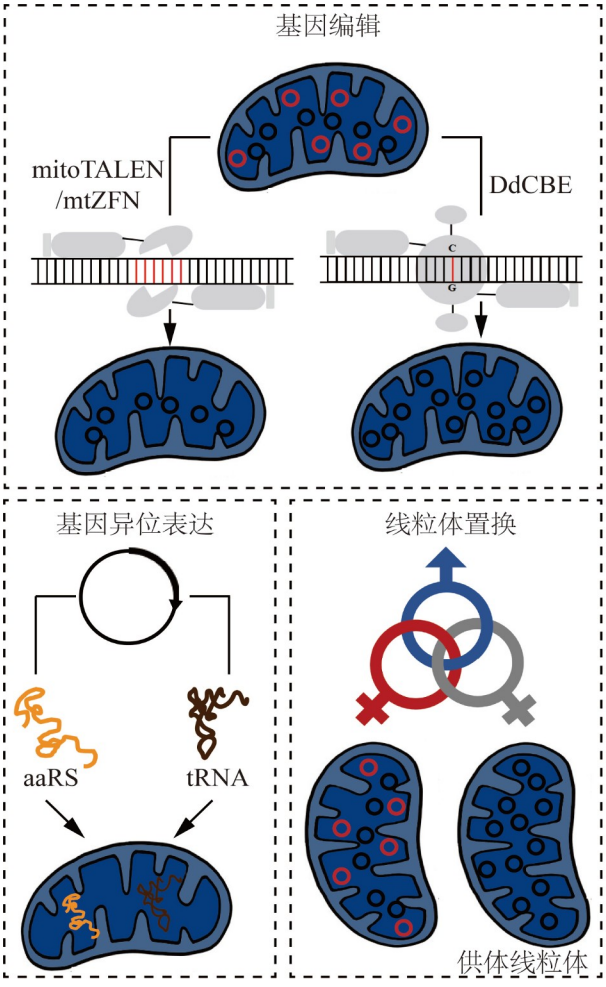


图3 线粒体tRNA基因突变导致的神经系统疾病的治疗

Yang等[162]设计了线粒体靶向转录激活因子样效应核酸酶(mitoTALEN)，成功清除了MELAS患者特异性诱导多能干细胞中的A3243G突变。Gammage等[163]和Bacman等[164]则分别应用线粒体靶向锌指核酸酶(mtZFN)和mitoTALEN，在携带异质性突变C5024T的小鼠体内实现了突变mtDNA的清除，突变mtDNA的异质性水平在心脏和肌肉中分别下降了40%和50%，同时线粒体DNA拷贝数未见明显减少，小鼠的tRNA稳态水平和线粒体功能得以恢复。mtZFN和mtTALEN消除突变mtDNA分子是基于核酸酶产生的mtDNA双链断裂及后续的mtDNA降解，仅适用于异质性突变导致的线粒体功能障碍，并不适用于同质性突变——所有mtDNA将同时被识别并清除。Mok等[165]开发了一种mtDNA单碱基编辑系统——DdCBE，催化mtDNA中的C•G到T•A的转变，实现mtDNA单碱基靶向编辑，编辑效率介于5%和50%之间。然而，DdCBE系统目前只能有效地编辑基因组中紧挨T的C碱基。已有研究通过该技术开发动物疾病模型，用以研究线粒体DNA突变导致的疾病[166-168]。

近年来，核质置换技术的出现为线粒体疾病的防治提供了新思路。核质置换技术，也称为线粒体置换技术，将患者的细胞核移植至正常人健康的去核的细胞质中，重新构建卵母细胞或受精卵，阻断线粒体疾病的母系传播。2016年，Zhang等[169]报道了首例通过纺锤体移植成功诞生的“三亲婴儿”，其携带的mtDNA突变负荷降到6%以下。Wu等[170,171]对核质置换技术进行了优化，建立了人前原核移植、第一/第二极体移植技术，重构胚胎均获得了核型正常的囊胚，在囊胚及干细胞传代后突变mtDNA残留率维持在0.36%低风险水平。但线粒体置换技术中线粒体基因组在传代中的稳定性、线粒体基因与核基因协调性等安全性指标尚未明确，细胞内致病mtDNA残留率、动物生殖效能及表型以及后代的遗传风险等有效的评

估方法也有待明晰，该技术一直存在伦理和安全性的争议。

5 讨论与展望

本文对六种神经系统线粒体疾病——MELAS、MERRF、LS、KSS、LHON和CPEO相关的线粒体tRNA基因突变和潜在的致病机制进行了讨论和总结。目前对线粒体tRNA基因突变导致的神经系统疾病的有效治疗手段匮乏，近年来蓬勃发展的基因治疗新技术为此带来新的希望，但仍有一定的局限性，存在伦理和安全性等多方面争议，距离临床应用仍需要更多相关研究佐证。

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