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评 述 中国科学院学部 科学与技术前沿论坛 表观遗传学与人类疾病专题

RNA修饰与人类疾病研究进展

高纯纯[1†], 管敏鑫[2*], 何晓[2†], 金昊[3†], 金勇丰[5*], 刘君[6*], 罗慧[4†], 曲慧儒[5†], 谢珊珊[3†], 杨运桂[1*], 张锐[4*], 张薪泞[6†], 张艺凡[6†], 周天华[3*]

1. 中国科学院北京基因组研究所(国家生物信息中心), 北京 100101;
2. 浙江大学医学院第四附属医院线粒体生物医学研究中心, 浙江大学医学院遗传学研究所, 浙江省遗传与发育障碍重点实验室, 杭州 310058;
3. 浙江大学医学院, 杭州 310058;
4. 中山大学生命科学学院, 广州 510275;
5. 浙江大学生命科学学院, 先进药物递释系统全国重点实验室, 杭州 310058;
6. 北京大学生命科学学院, 北大-清华生命联合中心, 蛋白质与植物基因研究国家重点实验室, 北京 100871
† 同等贡献
* 联系人, E-mail: gminxin88@zju.edu.cn; jinyf@zju.edu.cn; junliu1223@pku.edu.cn; ygyang@big.ac.cn; zhangrui3@mail.sysu.edu.cn; tzhou@zju.edu.cn, 作者同等贡献，按姓氏拼音顺序排序

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摘要 转录后修饰广泛存在于各种RNA分子中. 这种化学修饰可通过调控RNA剪接、翻译、运输、定位、稳定性等多种方式发挥生物学功能. 近年来, 高通量测序技术及化学生物学检测等技术的快速发展使多种RNA修饰(如m^6A, m^5C, m^1A)的准确鉴定和定位问题取得突破. RNA修饰与编辑作为一类新的表观转录生物标志物, 在肿瘤、神经系统疾病、免疫性疾病等多种疾病的诊断和治疗中受到广泛关注. 本文主要综述了RNA修饰与编辑的生物学功能以及参与蛋白调控的分子机制, 并重点回顾了其在疾病诊疗中的应用进展.

关键词 RNA修饰, RNA编辑, 表观遗传学, 疾病, 生物标记物

转录后修饰广泛存在于各种RNA分子中, 这类化学修饰是调控分子功能的有效方式. 当前, 对RNA的认识不再局限于蛋白质合成的中间物或效应分子, 而是认识到RNA修饰在不改变基因序列排列方式的前提下极大地提高了碱基编码遗传信息的能力, 扩展了人们对基因调控的理解, 已成为转录后基因调控的新机制. RNA生物学的广泛研究揭示了转录后修饰的多样性, 可通过高通量测序及化学生物学检测等技术[1]对越来越多的RNA修饰进行准确识别和转录组范围的映射及功能表征.

目前, 用于存储和发布RNA修饰的主要数据库MODOMICS[2]列出了百余种这样的修饰, 四种RNA碱基以及核糖都可以成为修饰的靶标, 所有已知类型的RNA都可以被修饰. 目前被人们所熟知的修饰包括N^6-甲基腺嘌呤(N^6-methyladenosine, m^6A)、N^1-甲基腺嘌呤(N^1-methyladenosine, m^1A)、5-甲基胞嘧啶(5-methylcytodcine, m^5C)、假尿嘧啶(pseudouridine, ψ)和核糖的2′-*O*-甲基化(2′-*O*-methylation, Nm)[3]. 当前鉴定

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出的每一种修饰，都可以改变其修饰核苷酸的化学性质，从而影响碱基配对、蛋白质构象以及与RNA结合蛋白相互作用. 同时，RNA修饰可通过调控RNA代谢[4]、剪接[5]、翻译[6]、运输、定位[7]、稳定性[8]等多种方式促进遗传信息的多样化. 与表观遗传标记类似[9], 已经发现了一组相关的效应蛋白, 它们能特异地催化特定修饰的生成(写入器)、去除(擦除器), 并识别和结合修饰的核苷酸(阅读器)[10]，进而参与多种生命过程的调控, 如细胞分化、组织发育和肿瘤发生[11,12].

RNA修饰已经成为细胞谱系命运决定过程中调节细胞转录组和蛋白质组的基本机制. RNA修饰具有时空特异性和双向可逆性，这种动态化学修饰在细胞状态转变过程中控制转录本的转换和翻译，影响蛋白质的合成效率和保真度，在细胞稳态、组织发育、生理活动和病理变化中通常都发挥着关键作用. RNA表观遗传途径的失调参与了包括免疫系统疾病[13]、肿瘤[12]在内的多种人类疾病. 在基因编辑工具的启发下, 研究人员整合基因靶向编辑工具[14]和相关效应蛋白开发新的工具在体外和细胞内人工靶向RNA进行基因组疾病矫正[15]. 本文综述了RNA修饰与编辑的生物学功能以及其参与蛋白调控的分子机制，并重点回顾了其在疾病诊疗中的应用进展.

1　线粒体tRNA修饰与疾病

1.1　线粒体tRNA修饰概述

线粒体是细胞能量产生与转换、细胞凋亡调控和活性氧产生的重要场所[16]. 作为一种半自主细胞器, 线粒体具备一套独立于核的基因组，因此线粒体功能的调节受到核基因组和线粒体基因组的双重调控. 人类线粒体基因组是由16569个碱基对组成的双链环状分子, 按组成碱基含量的不同分为重链(H链)和轻链(L链)，共可编码22种线粒体tRNA(mitochondrial tRNA, mt-tRNA)、13种氧化磷酸化复合物的蛋白亚基以及2种核糖体RNA. 除线粒体自身编码的13种蛋白外，核基因共编码约1500种蛋白质并转运至线粒体内参与调控[17].

DNA、RNA和蛋白质是生命体内最为重要的三种生物大分子. 在传统的中心法则中，DNA通过转录将遗传信息传向RNA，RNA被解码翻译为蛋白质，因此这三种大分子也是个体遗传信息流的物质基础. 其中, tRNA准确解码mRNA并将所携带氨基酸与肽链结合对于蛋白质翻译过程至关重要. tRNA一级结构为70~90个核糖核苷酸组成的核苷酸单链[18]. 在此基础上，通过碱基互补配对会进一步折叠成经典的三叶草二级结构. 通过核苷酸之间的氢键作用，tRNA可进一步折叠形成倒L形的三级结构[19].

tRNA上存在着大量的化学修饰, 多样化的RNA修饰促使tRNA分子具有更多样的化学性质和更广泛的生物学功能. 随着质谱技术的发展，目前已在tRNA上鉴定出了110多种修饰，且有研究证实这些tRNA上的修饰会根据不同的环境做出快速的变化. 迄今为止, 人们已发现在人类22种线粒体tRNA的137个位置存在着18种修饰, 其中又以线粒体tRNA反密码子环区域修饰丰度最大，修饰种类最多[20](图1). 这些核苷酸修饰对于稳定密码子-反密码子配对，维持翻译可读框，促进tRNA与核糖体A位点的接合并使tRNA从核糖体A位点位移至P位点有着重要作用[21]. 在生物体内，线粒体tRNA最初以前体形式转录, 需经过RNase Z, RNase P剪切后才可释放tRNA转录本[20,22]. 随后tRNA核苷转移酶在序列3′端添加CCA完成tRNA分子的活化, tRNA修饰酶在相应位置添加核苷酸修饰[23], tRNA自身完成高级结构折叠, 最终tRNA才能变为功能性tRNA(图2).

在线粒体编码蛋白进行翻译时，密码子第三位与线粒体tRNA 34位并非严格按照Watson-Crick配对原则进行配对，故该位点也被称为摆动位点[24]. 因此相比mt-tRNA的其他位置，摆动位点成为了tRNA中更容易被修饰的位点之一. 目前人类mt-tRNA 34位点已知存在4种修饰: Q, f^5C, τm^5U以及τm^5s^2U[25~28], 这些转录后修饰在tRNA的结构和功能中具有关键作用, 包括维持小rRNA解码位点的结构稳定，促进tRNA氨基酰化, 提高解码准确性和保证翻译保真度[29]. 其中，由于尿苷具备极高的灵活性，未修饰尿苷可与4种核苷碱基中的任意一种进行配对(四向摆动规则)，而携带的$\tau m^5(s^2)U$修饰会以C3′-内切形式固定尿苷，使其仅能与嘌呤碱基相配对. 这类修饰的添加有效地限制了尿苷的摆动能力，从而加强对嘌呤末端密码子的识别并防止与以嘧啶为末端的近同源密码子的错误识别. 在低等生物, 如大肠杆菌和酵母中, $\tau m^5(s^2)U$修饰以(c)$mnm^5(s^2)U$的形式存在. τm^5U修饰最早发现于线粒体$tRNA^{Leu(UUR)}$和$tRNA^{Trp}$的摆动位点. Suzuki等人[30]通过往细胞培养基内掺入^{18}O标记的牛磺酸，成功在mt-

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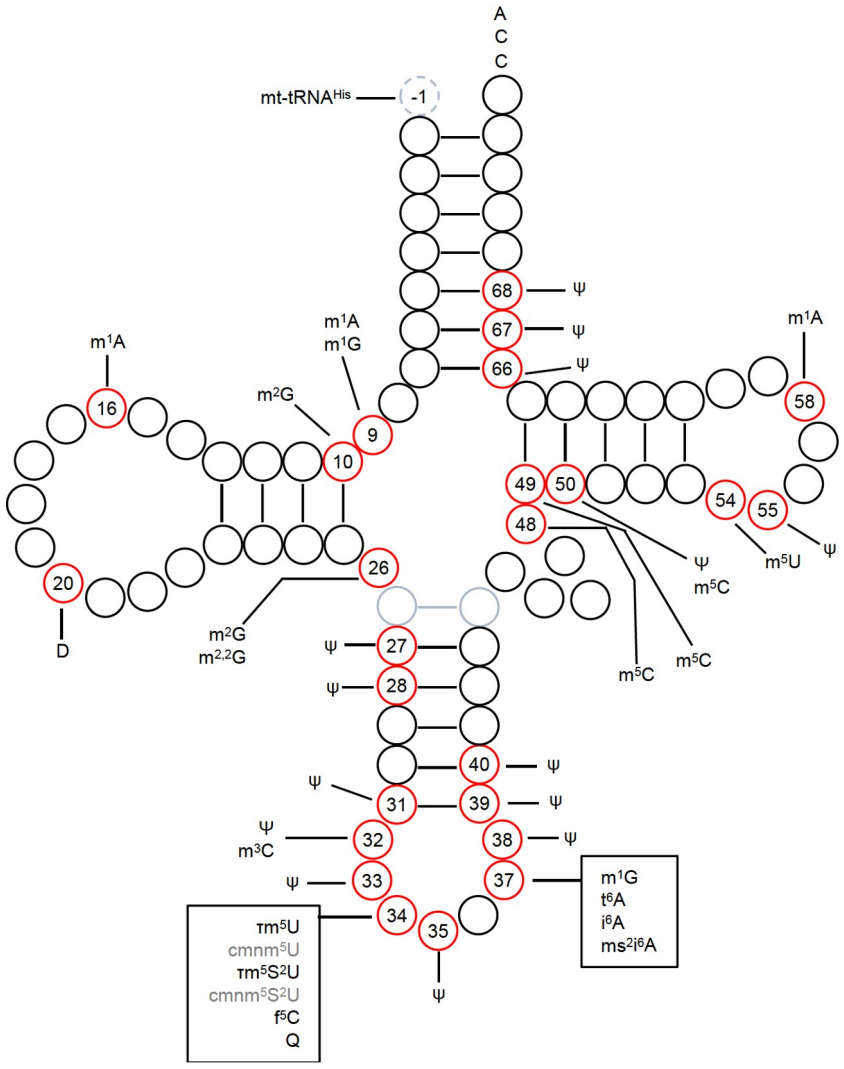


图 1 人线粒体tRNA转录后修饰. 图中红色圆圈表示核苷酸修饰位置和编号; 虚线圆圈为mt-tRNA^{His}特有; 浅灰色碱基对为mt-tRNA^{Ser}(UCN)特有

Figure 1 Post-transcriptional modifications of human mitochondrial tRNAs. Red circles indicate nucleotide modification positions and numbers. The dashed circles are specific to mt-tRNA^{His} and the light gray base pairs are specific to mt-tRNA^{Ser} (UCN)

tRNA的34位点检测到标记的τm^{5}U修饰. 早期研究发现, 在大肠杆菌中, MnmE与MnmG对于tRNA 34位的mnm^{5}U和cmnm^{5}U修饰是必需的. MnmE蛋白与MnmG蛋白分别进行二聚化, 形成两个同源二聚体, 随后两种同源二聚体相结合, 形成功能性α2/β2异四聚体复合物(MnmEG). 这种复合物催化了两种不同的GTP和FAD依赖性反应, 分别以铵和甘氨酸作为底物产生5-氨基甲基尿苷(5-methylaminomethyl uridine, mnm^{5}U)和5-羧甲基氨基-甲基尿苷(5-carboxymethylaminomethyluridine, cmnm^{5}U)[31,32]. 在酵母中, MSS1和MTO1被认为是MnmE与MnmG的同源蛋白[33], 基因缺陷时会导致线粒体tRNA代谢紊乱, 继而引发线粒体生物合成和能量代谢异常[34]. 2002年, Guan团队[35,36]成功克隆出人*GTPBP3*和*MTO1*基因, 认为两种编码蛋白分别是酵母MSS1和MTO1的结构和功能同源物. 2018年, Suzuki团队[37]利用体外实验证实了GTPBP3和MTO1能以5,5-亚甲基四氢叶酸和牛磺酸为底物催化线粒体tRNA$^{Leu(UUR)}$, tRNA^{Trp}, tRNA^{Lys}, tRNA^{Gln}和tRNA^{Glu} 34位点形成τm^{5}U, 同时详细阐述了修饰的生物发生和生理作用.

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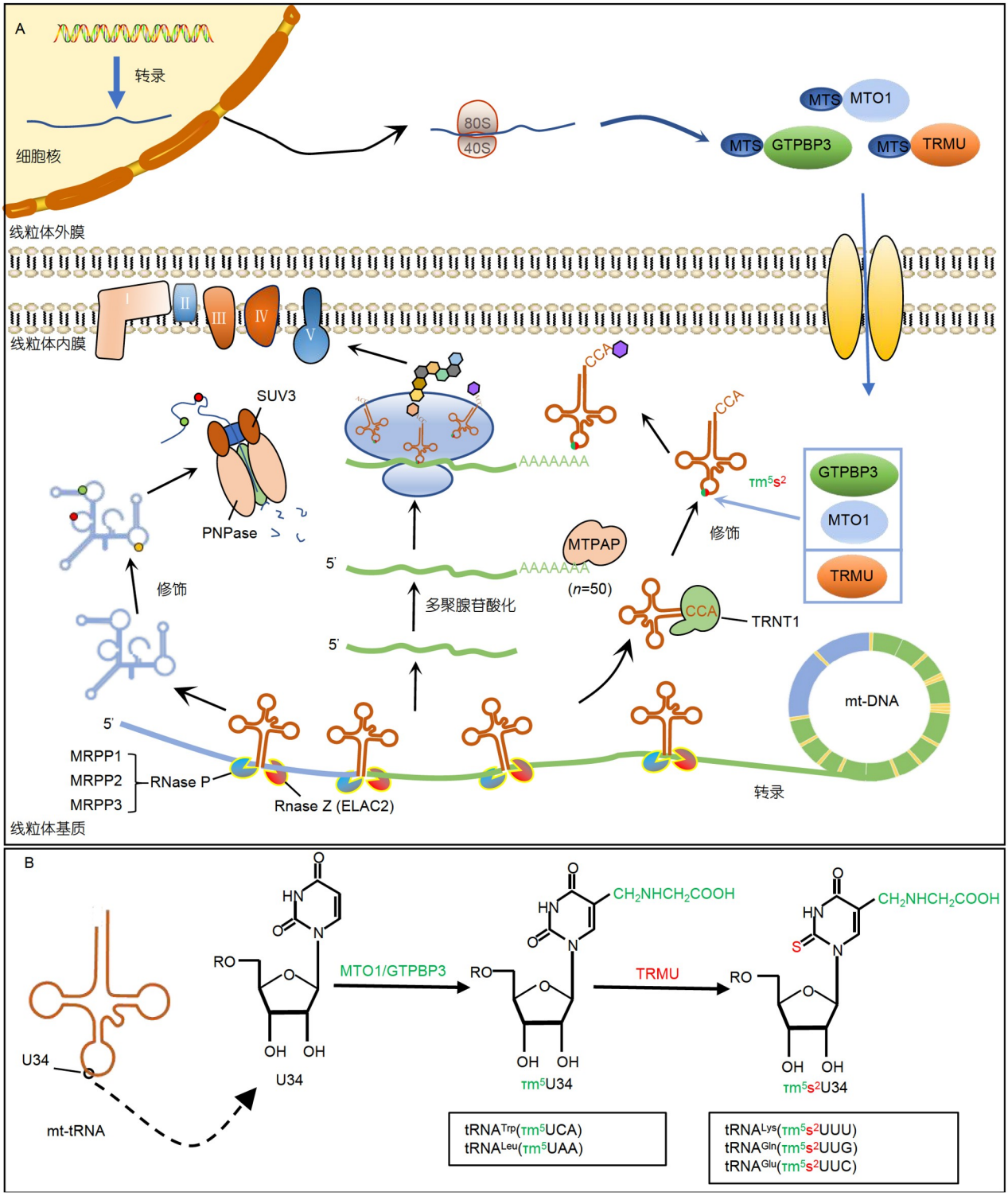


图 2　人线粒体tRNA的成熟、降解及摆动位点尿苷修饰. A: 人线粒体tRNA的成熟、降解; B: 人线粒体tRNA摆动位点尿苷修饰

Figure 2　Maturation, degradation and swing-site uridine modification of human mitochondrial tRNA. A: Maturation, degradation of human mitochondrial tRNA; B: uridine modification of human mitochondrial tRNA swing sites

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在大肠杆菌中, s^2U的硫来源于L-半胱氨酸, 其在半胱氨酸脱硫酶(cysteine desulfurase, IscS)的作用下将硫基团传递至MnmA上保守的Cys残基上[38]. 随后MnmA识别mt-tRNA, 消耗ATP活化摆动位点尿苷的C2形成腺苷酸中间体, 并催化Cys残基上结合的硫基团, 将其转移至C2形成s^2U[39]. 在酵母中, 2-硫修饰则由*TRMU*负责, 该修饰的缺陷会严重阻碍酵母的生长. 在人体中, *TRMU*为上述两种修饰基因的同源基因, 线粒体$tRNA^{Lys}$, $tRNA^{Gln}$和$tRNA^{Glu}$被TRMU催化产生s^2U修饰[26,40].

迄今为止, 已在人类线粒体tRNA的137个位置检测出18种核苷酸修饰, 这些繁杂的化学修饰在tRNA结构和功能中具有重要意义, 如mt-tRNA 34位的τm^5U与τm^5s^2U修饰能有效维持小rRNA解码位点的结构稳定, 促进tRNA氨基酰化, 提高解码准确性和保证翻译保真度, 从而保证线粒体功能的正常运行. 因此, 线粒体tRNA修饰缺陷容易引起线粒体功能障碍进而导致相关线粒体疾病的发生.

1.2 mt-tRNA摆动位点的修饰与线粒体疾病

为了更好地理解线粒体tRNA 34位修饰对线粒体功能和人类疾病发生的关系, 目前已在修饰酶鉴定和功能以及致病机制等方面进行了大量的探索. 随着二代测序的发展, 大量线粒体DNA及编码线粒体蛋白的核基因的突变被鉴定出与线粒体疾病的发病相关, 目前也已有诸多报道表明线粒体tRNA修饰酶GTPBP3, MTO1及TRMU的缺陷与耳聋、肥厚型心肌病、乳酸性酸中毒及急性肝衰竭等临床疾病密切相关[41~43].

(1) 线粒体tRNA修饰酶MTO1与相关疾病. *MTO1*的缺陷常导致线粒体tRNA修饰水平的下降, 引发线粒体tRNA代谢紊乱及线粒体蛋白质合成障碍, 最终导致线粒体功能受损进而诱发疾病的发生. 在临床上, 携带*MTO1*基因突变的患者常表现为耳聋、肥厚型心肌病、乳酸性酸中毒等.

在早期的酵母研究中发现, *Mss1*与*Mto1*突变在与线粒体15S rRNA P(R)454(在人线粒体中对应m.1555A>G突变)突变协同作用时表现出明显的线粒体功能缺陷, 且在回补人*GTPBP3*和*MTO1* cDNA后由突变导致的呼吸缺陷表型得到明显改善, 因此人们推测这两种基因可能协同作用m.1555A>G突变最终导致耳聋的产生. 后续Bykhovskaya等人[44]的研究也证实了*MTO1*与*GTPBP3*介导的tRNA修饰对于耳聋相关m.1555A>G突变表型的表达具有重要的调控作用.

携带*MTO1* c.1858dup及c.1282G>A突变的肥厚型心肌病伴乳酸性酸中毒患者的肌肉及成纤维细胞表现出显著的线粒体氧化磷酸化功能损伤[45]. 在携带*MTO1*纯合p.Ile408Phe突变的心肌肥厚伴乳酸中毒症的病例中发现, 患者成纤维细胞中线粒体编码蛋白ND1和ND5的表达水平出现显著的下降, 同时MTO1可能通过组织特异性的tRNA修饰和核糖体蛋白的表达调控来介导线粒体蛋白质的表达, 影响线粒体呼吸链复合物的组装[46].

脑病伴乳酸中毒及中风样发作(mitochondrial encephalomyopathy with lactic acidosis and stroke like episodes, MELAS)是非常严重的线粒体疾病, 主要特征为肌肉无力、中风和癫痫. 1990年, 日本的两个研究团队发现, 80%的MELAS患者都携带线粒体DNA A3243G突变, 随后进一步证实了A3243G突变是MELAS最主要的突变[47]. Yasukawa等人[48]通过分析正常人和携带3243突变MELAS患者来源细胞中的mt-tRNA修饰状态, 发现突变细胞$mt\text{-}Leu^{(UUR)}$ 34位τm^5比例远低于正常人细胞, 证明MALAS的发生与线粒体tRNA 34位点τm^5U修饰水平的下降存在密切关联.

(2) 线粒体tRNA修饰酶GTPBP3与相关疾病. *GTPBP3*作为一类核编码的线粒体tRNA修饰基因最早在酵母中被鉴定. 2002年, 人*GTPBP3*基因被克隆鉴定, 被认为参与调控线粒体A1555G和A1494T突变导致的非综合征性耳聋表型表达[35]. 2014年, 通过对790例疑似线粒体病患者进行了全外显子测序和候选基因测序, 发现自亚洲、中东和欧洲的11个家庭中的9例患者携带*GTPBP3*复合杂合突变或纯合突变, 其中8位患者骨骼肌细胞线粒体出现明显的线粒体呼吸链复合物功能受损. 这些患者表现出与MTO1突变患者相类似的临床症状, 如心肌病、乳酸酸中毒及发育迟缓等[42]. 此外, 在对三组携带*GTPBP3*突变的细胞系以及通过RNAi介导*GTPBP3*表达下调细胞系的生化检测中同样发现了线粒体蛋白合成障碍以及线粒体功能损伤. 与*MTO1*突变患者的临床症状相比, 大多数携带*GTPBP3*突变的患者同时伴随表现出明显的神经疾病表型, 核磁共振检查结果显示, 患者丘脑和脑干表现出类似Leigh综合征的状态[42]. 总体上, 研究表明, *GTPBP3*致病性突变导致了GTPBP3功能性缺陷, 致使线粒体

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tRNA修饰水平下降. 这种tRNA修饰缺陷导致了tRNA功能受损, 包括tRNA代谢水平的改变, tRNA氨酰化水平下降, tRNA结构稳定性下降以及密码子和反密码子相互作用的改变, 从而影响翻译效率的准确性, 导致线粒体蛋白翻译和呼吸链活性受损, 进而诱发疾病的产生[49].

(3) 线粒体tRNA修饰酶TRMU与相关疾病. TRMU是一类进化上高度保守的线粒体tRNA修饰酶, 可催化线粒体$tRNA^{Gln}$, $tRNA^{Glu}$与$tRNA^{Lys}$ 34位反密码子摆动碱基尿嘧啶形成2-硫化修饰. 该修饰对tRNA结构稳定性、氨基酰化能力以及密码子-反密码子间的识别效率发挥关键作用[50], 因此*TRMU*突变引发的tRNA修饰异常会导致线粒体编码蛋白的合成受损和严重的线粒体呼吸链功能障碍[41,51]. 临床上, *TRMU*突变与耳聋、可逆性婴儿肝衰竭等线粒体疾病的发生高度相关[43,52].

2006年, 管敏鑫团队[41]通过对携带m.1555A>G或m.1494C>T突变的613位聋病患者(来自1个阿拉伯-以色列家系、210个意大利/西班牙家庭以及31个中国家系)进行突变筛查, 首次发现了TRMU A10S突变, 猜测m.1555A>G或m.1494C>T突变与TRMU A10S突变与耳聋高度相关. TRMU突变并不影响TRMU蛋白正常转运进入线粒体, 但会导致线粒体tRNA稳态下降和蛋白合成受损. 2016年, Meng等人[52]进一步阐述了TRMU A10S与m.1555A>G突变协同作用导致母系遗传性聋病的机制. 研究发现, TRMU A10S突变引起TRMU蛋白修饰功能下降, 导致线粒体tRNA 34位尿苷的2-硫修饰水平降低, 从而引起tRNA代谢紊乱、氧化磷酸化产能下降、ROS产生增加等系列损伤, 这些生化损伤加剧了m.1555A>G突变相关的线粒体功能缺陷, 使其低于维持细胞功能正常的阈值, 最终导致聋病表型的表达. 2018年, Zhang等人[51]从整体水平阐述了*TRMU*缺陷所引起的tRNA修饰缺陷、tRNA代谢紊乱、蛋白翻译障碍及ATP产量下降等系列线粒体功能障碍导致斑马鱼听力器官缺陷的分子机制.

2　mRNA m^6A修饰与疾病

2.1　m^6A修饰概述

N^6-甲基腺苷修饰(N^6-methyladenosine, m^6A)是真核生物mRNA上丰度最高的修饰, 参与调控几乎所有的RNA生物过程, 在多种生理和病理学过程中起关键作用.

生物体内存在多个m^6A修饰的调控因子[53](图3). 其中, 甲基转移酶复合物由核心的METTL3, METTL14以及WTAP, VIRMA, ZC3H13, HAKAI等辅助因子组成, 调控甲基生成[54~56]. 去甲基化酶(fat mass obesity-associated protein, FTO)和ALKBH5(AlkB homologue 5)实现m^6A在体内的可逆性调控[57,58]. m^6A mRNA可被甲基结合蛋白识别并调控其下游功能, 包括YTHDFs[59], IGF2BPs[60], FMRP[61]和HNRNPs[62]等. 多种结合蛋白共同发挥调控作用保证m^6A修饰复杂且重要的生理功能正常运行, 而某个调控因子异常则与人类疾病密切相关.

2.2　mRNA m^6A修饰与人类疾病

(1) m^6A修饰与肿瘤. 多项研究表明, m^6A修饰与肿瘤发生有关, 其调控因子在恶性肿瘤中起癌基因或抑癌基因的作用(表1)[63]. 下面将主要针对几种癌症类型总结m^6A修饰起到的关键作用和调控机制.

胃癌是最常见的消化道肿瘤之一, 对人体健康造成严重损害. 研究发现, 胃癌组织中的m^6A水平显著偏高. METTL3在胃癌中高表达, 通过增加甲基化HDGF或ZMYM1转录本的稳定性, 促进胃癌的生长和上皮-间质转化过程[64]. ALKBH5则被发现在胃癌中表达下调, 并通过ALKBH5-PKMYT1-IGF2BP3通路促进胃癌转移[65]. m^6A结合蛋白YTHDF1可增强*FZD7* mRNA的翻译, 激活Wnt通路进而促进胃癌的发展和转移[66]. 针对YTHDF1开发的细胞外囊泡载体能有效传递小干扰RNA, 对胃癌的增殖和转移有良好的抑制作用[67].

肝癌是造成人类死亡的第三大癌症. 研究发现, 肝母细胞瘤中METTL3高表达, 激活Wnt通路而促进其增殖、侵袭和迁移[68]. 此外, METTL3可通过下游YTHDF1促进*Snail*基因的表达, 或在YTHDF2的帮助下抑制*SOCS2*表达促进肝癌发展. 甲基转移酶成员WTAP和KIAA1429通过甲基化抑制*ETS1*或*ID2*基因的表达来促进肝癌发展[69,70]. 有趣的是, 并非所有的m^6A甲基转移酶都对肝癌起促进作用. METTL14在高转移肝癌中下调, 通过与DGCR8相互作用促进pri-miR126的成熟进而抑制肝癌的转移[71]. 去甲基化酶FTO在肝癌中也表现出双重调控作用[72,73]. m^6A在肝癌中的复杂调控作用可能与具体的遗传背景及肿瘤异质性密切

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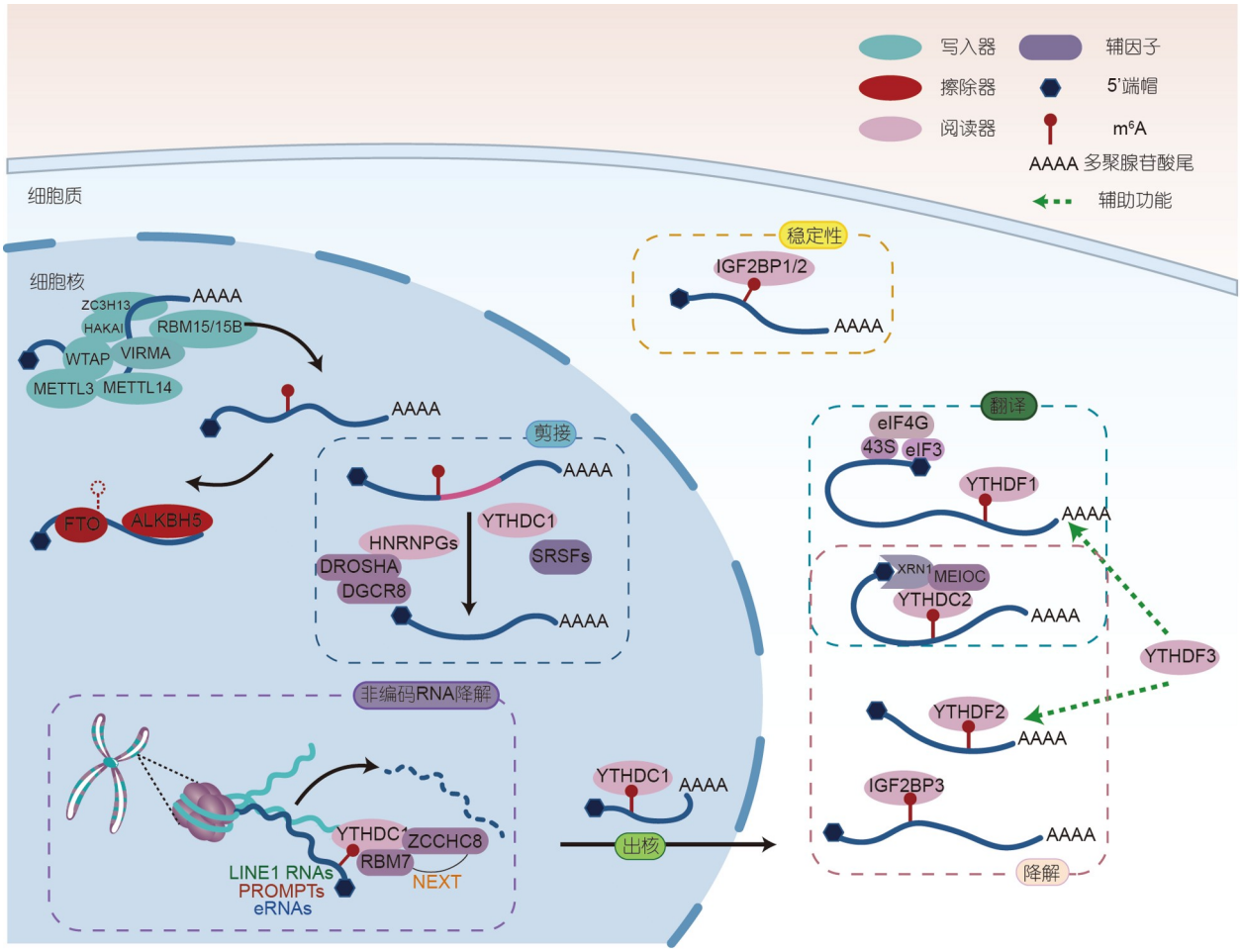


图 3 m^{6}A修饰主要的调控因子及其生物学功能. m^{6}A调节因子主要由甲基转移酶复合体(METTL3, METTL14, WTAP, VIRMA, ZC3H13, HAKAI)、去甲基化酶(FTO和ALKBH5)和阅读蛋白(YTHDFs, IGF2BPs, HNRNPs)组成. 甲基转移酶和去甲基化酶共同调节m^{6}A修饰的动态平衡. 不同的阅读蛋白可介导不同的RNA相关生物学过程. YTHDC1参与前体mRNA的剪接、成熟mRNA出核和非编码RNA的降解过程(细胞核内). YTHDF1促进翻译, YTHDF2促进mRNA降解, YTHDF3帮助YTHDF1和YTHDF2发挥相应功能, IGF2BP1/2/3促进mRNA的稳定(细胞质)

Figure 3 The main regulators of m^{6}A modification and their biological functions. The m^{6}A regulators are mainly composed of m^{6}A methyltransferase complex (METTL3, METTL14, WTAP, VIRMA, ZC3H13, HAKAI), demethylases (FTO and ALKBH5) and reading proteins (YTHDFs, IGF2BPs, HNRNPs). The methyltransferases and demethylases work together to regulate the dynamic balance of m^{6}A modifications. Various reading proteins mediate distinct RNA-related biological processes. YTHDC1 is involved in pre-mRNA splicing, nuclear export of mature mRNAs, and degradation of non-coding RNAs (nucleus). YTHDF1 facilitates translation, YTHDF2 promotes mRNA degradation, YTHDF3 assists YTHDF1 and YTHDF2 in carrying out their respective functions, and IGF2BP1/2/3 enhances mRNA stabilization (cytoplasm)

相关.

急性髓系白血病(acute myelocytic leukemia, AML)是未成熟的骨髓造血细胞的恶性肿瘤，具有高度异质性. 有研究发现, WTAP和RBM15在AML中异常表达[74]. METTL3在AML中高表达，增加甲基化*BCL2*和PTEN mRNA翻译效率，激活AKT通路并促进AML细胞的分化和自我更新[75,76]. METTL14还可通过增加*MYB*和*MYC* mRNA m^{6}A修饰水平促进AML发生[4]. 此外，YTHDC1可通过识别m^{6}A修饰并导致液-液相分离促进AML细胞存活和维持未分化状态[77]. 然而，也有研究表明，FTO和ALKBH5在AML中高表达并促进AML发展[78,79]，提示m^{6}A修饰的双向调控作用. 此外，针对METTL3和FTO开发的小分子抑制剂被证实可通过m^{6}A依赖途径抑制AML进程[80,81].

m^{6}A在结直肠癌中也起双向调控作用. METTL3在结肠癌中高表达，通过m^{6}A-CRB3-YTHDF2通路激

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表 1　m^{6}A修饰水平与相关癌症的关系

Table 1　m^{6}A modification levels of associated cancers

肿瘤类型	m^{6}A水平	m^{6}A调节因子	功能
胃癌	升高	METTL3/IGF2BP3/ALKBH5/YTHDF1	促进肿瘤发生、转移; 促进胃癌的上皮-间质转化[64~67]
肝癌	升高/降低	METTL3/WTAP/YTHDF2/KIAA1429/METTL5/METTL14/FTO	促进肿瘤增殖、侵袭和迁移/抑制转移[68~73]
结直肠癌	升高/降低	METTL3/METTL14	促进肿瘤增殖、侵袭和迁移/抑制转移[82~84]
急性髓系白血病	升高/降低	METTL3/METTL14/METTL16/ WTAP/RBM15/FTO/ALKBH5	促进AML细胞存活和维持未分化状态[4,74~81]
膀胱癌	升高	METTL3/METTL14/WTAP/YTHDF2/YTHDC1/IGF2BP1/FTO/ALKBH5	促进肿瘤发生发展、黏附、迁移和侵袭[85]
肺癌	升高	METTL3/ALKBH5/METTL7B/ALKBH5/YTHDC1/YTHDC2/YTHDF2/YTHDF3	促进肿瘤增殖、侵袭和迁移[86]
乳腺癌	升高	METTL3/METTL14/YTHDF2/YTHDF3/IGF2BP3/FTO/ALKBH5	促进肿瘤增殖、侵袭和迁移[88]
骨肉瘤	升高	METTL3/METTL14/WTAP/YTHDF1/IGF2BP1/FTO/ALKBH5	促进肿瘤增殖、侵袭和迁移[89]
皮肤癌	降低	METTL14	抑制肿瘤增殖[90]
黑色素瘤	升高/降低	METTL3/METTL14/YTHDF2/FTO	促进肿瘤增殖/抑制肿瘤增殖[91]
卵巢癌	升高	METTL3/YTHDF1/YTHDF2/IGF2BP1/FTO/ALKBH5	促进肿瘤增殖、侵袭和迁移[92]
前列腺癌	升高	METTL3/METTL14/WTAP/YTHDF2/YTHDC1/IGF2BP2/FTO	促进肿瘤增殖、侵袭和迁移[93]
神经胶质瘤	升高/降低	METTL3/FTO/ALKBH5	促进肿瘤增殖、侵袭和迁移[94]
胰腺癌	升高	METTL3/METTL14/ALKBH5/YTHDF2	促进肿瘤增殖和迁移[95]
子宫内膜癌	降低	METTL3/METTL14	促进肿瘤发生[87]

活下游Hippo通路, 或增强GLUT1翻译激活mTORC1信号通路, 促进结肠癌的发展[82,83]. METTL14在结肠癌中则显示出相反的调控作用, 其在结肠癌组织中表达下调, 通过m^{6}A-SOX4-YTHDF2通路或介导*XIST* RNA的m^{6}A修饰抑制其表达, 实现对结肠癌的抑制作用[84].

除以上几种癌症外, m^{6}A修饰也被发现在其他肿瘤中异常. METTL3在膀胱癌中高表达且与预后差高度相关[85]; 在原代肺腺癌及非小细胞肺癌中甲基化偏高且发挥致癌作用[86]; 而在子宫内膜癌中METTL3表达降低或METTL14突变导致病人m^{6}A水平偏低, 激活AKT通路进而促进癌症发展[87]. 总体而言, m^{6}A水平在乳腺癌[88]、骨肉瘤[89]、皮肤癌[90]、黑色素瘤[91]、卵巢癌[92]、前列腺癌[93]、神经胶质瘤[94]以及胰腺癌[95]中均存在异常表达(图4), 揭示其成为潜在的癌症诊断标志物或治疗靶点的可能性.

(2) m^{6}A与免疫系统疾病. 研究表明, m^{6}A修饰调节因子与免疫及炎症反应的调控过程密切相关[96,97]. 类风湿性关节炎(rheumatoid arthritis, RA)患者的ALKBH5, FTO和YTHDF2表达明显下降, 外周血中m^{6}A水平偏高且与FTO表达量减少呈负相关[98]. 而METTL3可通过NF-κB途径激活成纤维细胞样滑膜细胞, 促进炎症反应而加速RA的发展[99]. METTL3或METTL14的缺失导致Th17和Treg细胞之间的不平衡, 进而诱发自发性结肠炎[100]. 另外, 在系统性红斑狼疮患者的外周血中观察到m^{6}A调节因子表达水平的变化[101], 多发性硬化症患者的脑脊髓液样本中多种m^{6}A修饰调节因子表达上调, 均提示m^{6}A修饰在相应发病机制中可能发挥关键作用.

(3) m^{6}A与心血管疾病. 心血管疾病是一类发病率和死亡率逐年上升的慢性疾病. 越来越多的研究表明, m^{6}A修饰在其疾病进展中发挥重要作用[102]. 在血管硬化过程中m^{6}A修饰水平升高, 敲降*METTL14*可减少钙化并促进血管修复[103]. 另外, *METTL3*过度表达会引起心肌细胞肥大, 敲除心肌细胞中的*METTL3*诱发小

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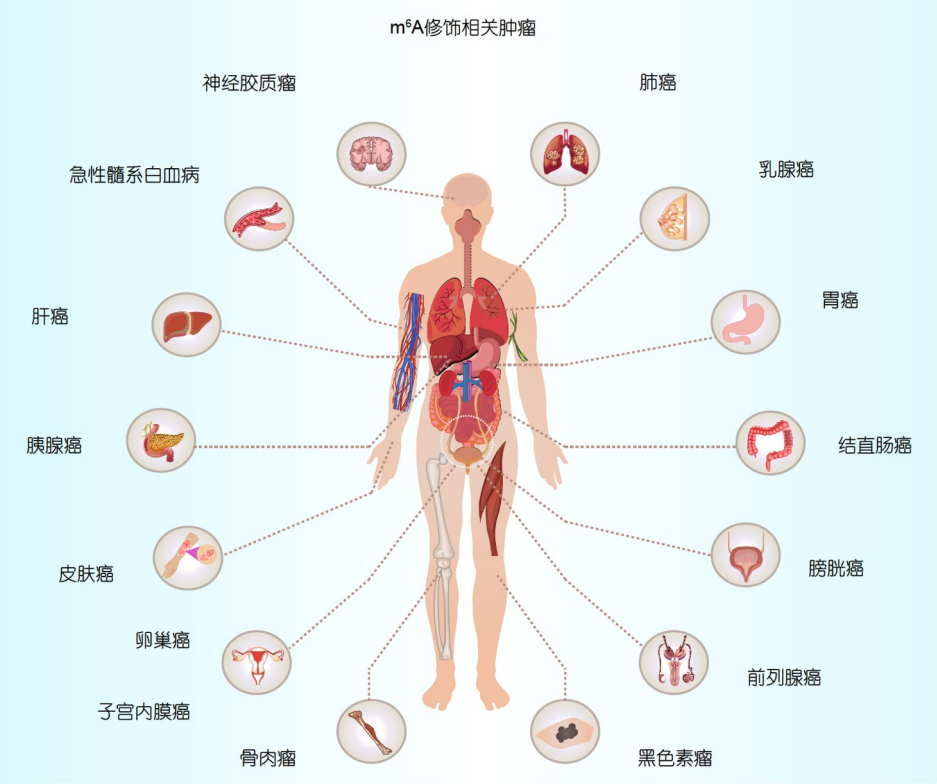


图 4 m^{6}A修饰参与多种肿瘤发展. 具体细节见表1
Figure 4 m^{6}A modifications in various cancers. More details are detailed in Table 1

鼠心脏形态和功能的变化[104]. FTO通过抑制心脏收缩相关转录本的降解保护心肌细胞收缩. 过表达FTO可减弱心肌梗死后适应不良的心脏重塑, 并减轻缺氧引起的心肌细胞功能障碍, FTO敲除会导致心脏功能受损[105]. 此外, 全基因组关联分析结果表明, 1236个m^{6}A-SNPs与高血压密切相关. 中风后的大脑组织中也观察到m^{6}A表观转录组的变化[106]. 这些研究表明, m^{6}A甲基化对维持心血管稳态起关键作用.

(4) m^{6}A与神经系统疾病. m^{6}A在神经元发育、神经胶质细胞发育、小脑发育和轴突生长过程中均起重要作用[107~109], 因此m^{6}A修饰水平异常与神经系统疾病密切相关. 研究发现, m^{6}A修饰可参与周围神经损伤修复过程, 敲除*METTL14*或*YTHDF1*使受伤背根神经节中再生相关基因的翻译降低, 从而减少了轴突生长和神经再支配, 导致功能恢复不完全, METTL3和FTO也被报道与神经损伤修复过程相关[110,111]. 在阿尔茨海默症(Alzheimer's disease, AD)患者海马体中METTL3表达上调, 与不溶性tau蛋白共定位增加, 表明二者之间可能存在相关性, 这与小鼠模型中观察到的METTL3表达上调一致[112]. IGF2BP2在AD患者的内嗅皮层、海马、后中央回和额上回中高表达也可能与AD的发生有关[113]. 帕金森病(Parkinson's disease, PD)与多巴胺神经元受损相关, 并常伴纹状体功能障碍. 有研究表明, METTL14的缺失会影响纹状体神经元的兴奋性并增加对多巴胺药物的敏感性[114]. 在6-羟基多巴胺诱导的PD大鼠模型中, 纹状体FTO表达上调且m^{6}A水平降低, 通过促进NMDA表达导致多巴胺神经元死亡[115].

3 mRNA m^{5}C修饰与人类疾病

3.1 m^{5}C修饰概述

m^{5}C修饰在20世纪70年代被发现. 近年来, m^{5}C修饰高通量测序技术得到迅速发展, 包括RNA重亚硫酸盐测序(RNA-BisSeq)、m^{5}C-RNA免疫沉淀测序(RNA m^{5}C-RIP)、甲基化-单核苷酸分辨率交联和免疫沉淀

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测序(methylation individual-nucleotide-resolution UV cross-linking and immunoprecipitation sequencing, miCLIP-seq)和5-氮杂胞苷介导的RNA免疫沉淀测序(5-azacytidine mediated RNA immunoprecipitation, Aza-IP). 测序结果表明, m^{5}C修饰同样广泛存在于mRNA上, 主要集中在翻译起始位点下游的CG富集区[116], 影响mRNA的出核[117]、稳定[118]、降解[119]、选择性剪接[120]、翻译[121]和相分离[122]等加工代谢过程, 参与调控细胞增殖、迁移[123]和疾病发生发展[124]等过程(图5).

NSUN2被发现可催化具有保守、组织特异性和动态修饰特征的mRNA m^{5}C修饰[116], 在肿瘤的发生、发展中发挥重要的作用. TRDMT1被发现影响了HEK293细胞中mRNA m^{5}C修饰, 调节了细胞的增殖和迁移[125]. NSUN6主要靶向催化mRNA 3′非翻译区(3′ untranslated region, 3′UTR)发夹结构环中CTCCA基序的m^{5}C修饰, 影响被修饰mRNA的翻译终止过程, 在人类肿瘤样本中下调, 与肿瘤预后相关[126]. 近期, TET2作为首个mRNA m^{5}C去甲基化酶被鉴定[127], 在调节白血病干细胞的归巢和干性维持中发挥重要的调节作用, 通过催化m^{5}C的擦除影响靶基因的稳定性, 从而调控趋化因子受体的活化影响白血病进程.

ALYREF作为首个mRNA m^{5}C识别蛋白, 被发现可通过K171特异性结合mRNA m^{5}C[116], 参与调节癌症进程[128]. YBX1通过冷休克结构域中W65的吲哚环识别mRNA m^{5}C[124]. YBX2识别mRNA m^{5}C, 并介导mRNA的相分离过程[122].

3.2　mRNA m^{5}C修饰与疾病

(1) m^{5}C修饰与肿瘤. 已有研究表明, 在胃癌组织中NSUN2表达上调, SUMO化修饰会影响其稳定性和核转运[129], 而长链非编码RNA(long non-coding RNA, lncRNA)*DIAPH2-AS1*通过掩蔽其K577和K579可免受泛素蛋白酶体降解[130], 催化*PIK3R1*, *PCYT1A*和*NEN1* mRNA m^{5}C修饰, 影响mRNA代谢和稳定性, 促进胃癌的增殖、迁移和侵袭[129]. lncRNA *FOXC2-AS1*在胃癌中表达显著升高, 可募集NSUN2催化*FOXC2* mRNA m^{5}C修饰, 由YBX1识别并增强其稳定性, 与胃癌的发展和生存预后相关[131].

在食管鳞状细胞癌样本中, 转录因子E2F1可调控NSUN2高表达, 催化*GRB2* mRNA m^{5}C修饰, 被LIN28B识别增强其稳定性[132]. 此外, 遗传变异也可导致NSUN2活性增加, 催化癌症相关mRNA m^{5}C修饰并影响其转录和翻译过程[133], 促进食管鳞状细胞癌的发展进程, 影响放化疗效果. NSUN2在下咽鳞状细胞癌中存在高表达, 以m^{5}C依赖的方式上调转录因子*TEAD1*, 促进其增殖和侵袭[134].

尿路上皮细胞癌(uroepithelial cell carcinoma, UCB)单碱基分辨率的mRNA m^{5}C修饰图谱, 鉴定了许多致癌基因存在较高m^{5}C修饰, 揭示了NSUN2通过催化*HDGF* mRNA 3′UTR m^{5}C修饰, YBX1识别并招募*ELAVAL1*维持其稳定性, 驱动了UCB的发病机制[124]. 此外, ALYREF通过结合*PKM2*[135], *RABL6*和*TK1*[136] mRNA m^{5}C位点, 影响其稳定性和剪接, 促进UCB的增殖和进展.

卵巢癌是最致命的妇科恶性肿瘤. 上皮性卵巢癌约占卵巢癌的90%, 化疗耐药发生率高, 预后较差. YBX1 lys304可被SIAH1泛素化, 导致其靶向m^{5}C-mRNA不稳定[137]. 宫颈癌(cervical cancer, CC)组织中, NSUN2可催化*KRT13*和*LRRC8A* mRNA m^{5}C, YBX1识别并增强其稳定性, 促进CC的增殖和迁移[138]. 与正常妊娠相比, 先兆子痫患者(preeclampsia, PE)胎盘存在异常的mRNA m^{5}C, 呈现编码区终止密码子前富集特征, 高甲基化修饰且上调的基因富集在炎症和免疫通路参与PE的致病机制[139].

研究发现, NSUN2在前列腺癌(prostate cancer, PCa)中高表达, 催化雄激素受体的m^{5}C修饰, 以YBX1依赖的方式增强其稳定性, 促进PCa的发生, 而雄激素受体抑制剂恩扎鲁胺可抑制NSUN2在PCa细胞中的表达, 并降低m^{5}C水平[140].

纤维化肺组织存在异常mRNA m^{5}C修饰, 获得性m^{5}C修饰与氧化应激反应、炎症和免疫通路有关[141]. 在肺腺癌组织中, NSUN2催化*YAP* 3′UTR m^{5}C修饰, 由ALYREF识别并提高其稳定性, 增强外泌体分泌, 促进肺腺癌的恶性表型和对AZD9291(第三代EGFR-TKI)的耐药性[142].

除以上几种癌症外, m^{5}C修饰也被发现在其他肿瘤中异常. 肝癌与癌旁组织之间差异的m^{5}C修饰, 与广泛的细胞功能相关[143]. NSUN2[144]和ALYREF[128]在肝癌组织中高表达, 介导了其靶mRNA m^{5}C和代谢, 通过Ras信号通路调节肝癌对索拉非尼的敏感性, 参与肝癌进展. 在Pdx1驱动自发性胰腺癌小鼠模型中, NSUN2在癌灶中的表达显著上调, 介导靶m^{5}C-mRNA和代谢,

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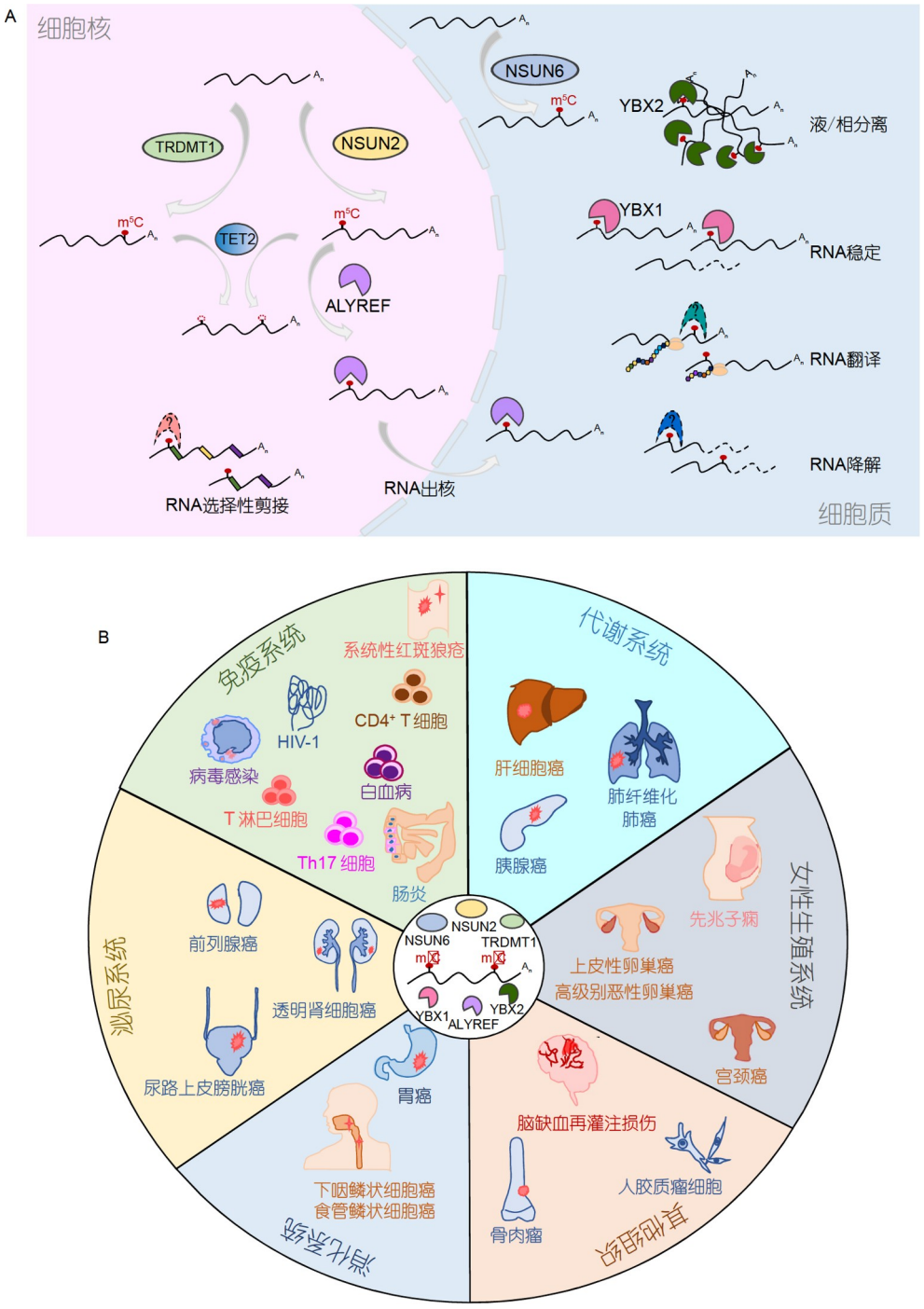


图 5 mRNA m^{5}C修饰相关蛋白及其调控RNA代谢功能. A: mRNA m^{5}C相关蛋白定位及功能; B: mRNA m^{5}C参与调控相关疾病进程

Figure 5 mRNA m^{5}C-related proteins and their roles in RNA metabolism. A: The location and functions of mRNA m^{5}C related proteins; B: the roles of mRNA m^{5}C in human diseases

调控癌症进展并抑制导管上皮细胞的分化[145]. NSUN2在骨肉瘤(osteosarcoma, OS)组织和细胞中高表达, 通过催化*FABP5* mRNA m^{5}C增强其稳定性, 提高OS细胞中的脂肪酸代谢, 促进OS发展[146].

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脑缺血再灌注损伤(ischemia-reperfusion injury, IRI)可引起广泛的组织器官损伤. 在诱导脑损伤模型中m^{5}C升高, 高甲基化且上调的mRNA与应激反应和细胞凋亡等过程显著相关, 参与调控IRI[147]. NSUN2被发现催化了人胶质细胞瘤细胞U87中*ATX* 3′UTR C2765修饰, ALYREF识别并促进其出核, 增强U87的迁移[123].

(2) m^{5}C修饰与免疫性疾病. 白细胞介素-17A(interleukin-17A, IL-17A)已被证明参与了各种自身免疫性疾病的过程[148]. NSUN2通过催化*IL-17A* mRNA C466的m^{5}C修饰, 促进了*IL-17A*在T淋巴细胞中的翻译, 介导高同型半胱氨酸血症(hyperhomocysteinemia, HHcy)诱导T淋巴细胞活化[149]. Nsun2与RoRγt偶联, 特异性催化Th17细胞关键细胞因子mRNA m^{5}C修饰的形成, 增强其靶基因的稳定性, 诱导肠炎发生[150]. NSUN2在系统性红斑狼疮患者的CD4$^+$ T细胞中低表达, m^{5}C-mRNA数目升高但修饰水平降低, 高甲基化的m^{5}C-mRNA与炎症和免疫通路显著相关[151]. 在SARS-CoV-2和各种病毒感染的细胞中, NSUN2被发现特异性介导*IRF3* mRNA m^{5}C修饰并加速其降解, 导致IRF3和下游IFN-β的分泌降低, 负调节Ⅰ型干扰素反应, 而内源性NSUN2水平在感染期间降低, 以增强细胞抗病毒反应, 有效消除病毒[119]. 此外, NSUN2被发现可催化HIV-1 RNA上的m^{5}C修饰, 调控了HIV-1 RNA的选择性剪接和翻译过程[120]. 近期研究首次发现, 在白血病干细胞的归巢和干性维持方面, TET2作为首个mRNA m^{5}C去甲基化酶被发现, 通过调控靶基因mRNA的稳定性(如*TSPAN13*), 进而影响了趋化因子受体4(C-X-C motif chemokine receptor 4, CXCR4)的活化[127].

综上所述, mRNA m^{5}C及其结合蛋白已被证明参与包括免疫、代谢、生殖等系统性疾病的发生和发展. 通过影响其靶向修饰mRNA的代谢过程, 在疾病的发生发展过程中发挥了重要的调控作用. 但其调控作用具有多向性, 参与通路也具有多样性, 所以对于确定mRNA m^{5}C修饰的选择性对其调控作用和功能解析具有十分重要的意义. 此外, mRNA上的多种修饰对于同种或不同种疾病都存在同向或异向的调控作用, 如果能在同一体系和条件下, 将不同种修饰的协同作用统筹解析, 将极大推动RNA表观遗传领域在生理及病理生物学过程中的拓展.

4　mRNA m^{1}A修饰与疾病

4.1　m^{1}A修饰概述

m^{1}A修饰是发生在腺嘌呤N^1位上的一种动态可逆的甲基化修饰, 于1961年被首次发现[152]. m^{1}A修饰会影响RNA的二级结构以及RNA与蛋白质之间的相互作用[153]. m^{1}A修饰在tRNA和rRNA中的含量较高, 而在mRNA中含量较低[154,155]. 在tRNA中, m^{1}A修饰存在于细菌、古生菌和真核生物tRNA的第9, 14, 16, 22, 57和58位点上[156]. 在rRNA中, m^{1}A修饰存在于芽殖酵母和人类保守的28S rRNA的第1322位点上[157,158], 以及脊椎动物线粒体16S rRNA的第947位点上[159]. 在来源于哺乳动物细胞系的mRNA中, 携带m^{1}A修饰的腺嘌呤约占所有腺嘌呤的0.015%~0.054%; 而在哺乳动物组织中则约占0.05%~0.16%[160].

m^{1}A修饰由甲基转移酶(写入器)催化产生, 并能在去甲基化酶的作用下被移除[161](图6). tRNA甲基转移酶家族蛋白TRMTs负责将*S*-腺苷甲硫氨酸(*S*-adenosyl methionine, SAM)中的甲基基团转移给RNA中腺嘌呤碱基的1号氮原子[162]. TRMT6/61A复合物可以识别mRNA中的GUUCRA(R=A, G)基序和类似于tRNA上的T-loop结构, 以催化m^{1}A修饰的产生[154]. 而Fe(II)/α-酮戊二酸依赖的双加氧酶家族蛋白ALKBHs则发挥去除m^{1}A修饰的功能, 例如, mRNA中的m^{1}A修饰可被ALKBH3介导的去甲基化反应移除[160]. RNA结合蛋白(RNA binding proteins, RBPs)广泛参与RNA命运和功能的调控, 有研究提示, YTHDF1, YTHDF2和HRSP12能与携带m^{1}A修饰的mRNA结合, 可能是m^{1}A修饰阅读器[163].

4.2　mRNA m^{1}A修饰的功能

m^{1}A修饰可通过影响mRNA代谢, 包括mRNA的结构、稳定性和翻译过程, 参与基因表达和细胞行为的调控[164]. 由于带有额外的甲基基团, m^{1}A修饰可在空间上改变RNA的二级结构[165]. m^{1}A修饰对mRNA稳定性的调控主要由m^{1}A修饰阅读器介导, 如敲除YTHDF2会上调部分携带m^{1}A修饰的mRNA的丰度[166]. m^{1}A修饰位点通常位于mRNA的翻译起始位点和第一个剪切位点附近, 与编码转录本的翻译有关[167], mRNA上携带的m^{1}A修饰可阻断其与tRNA上尿嘧啶的Watson-Crick碱基配对, 进而抑制翻译过

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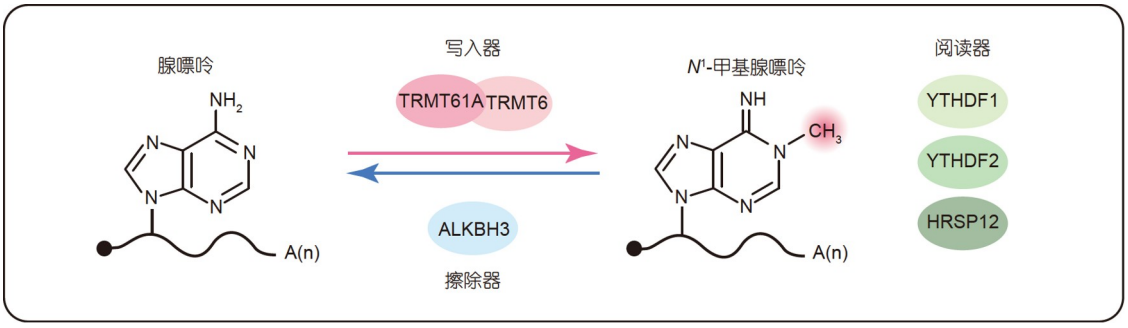


图 6 mRNA m^{1}A修饰的可逆调控. 写入器TRMT6/61A复合物催化mRNA腺嘌呤的第1位氮原子上产生甲基化修饰. 阅读器YTHDF1, YTHDF2和HRSP12能够识别和结合带有m^{1}A修饰的mRNA. 擦除器ALKBH3则介导mRNA m^{1}A修饰的去除

Figure 6 Reversible regulation of m^{1}A modification in mRNA. TRMT6/61A methyltransferase complex catalyzes the methylation on the ^{1}N of mRNA adenine. The RNA-binding proteins YTHDF1, YTHDF2 and HRSP12 recognize and bind mRNAs with m^{1}A modifications. The demethylase ALKBH3 mediates the removal of mRNA m^{1}A modifications

程[168].

越来越多的研究发现, m^{1}A修饰在多种生物学过程中扮演重要角色. 例如, 在细胞热休克应激过程中, m^{1}A修饰的mRNA相较于无修饰的mRNA优先聚集在应激颗粒中, 促进mRNA在应激恢复过程中启动翻译状态[169]. 在烷化剂造成RNA烷基化损伤的过程中, RNA上的m^{1}A修饰增高, 招募损伤修复复合物ASCC (activating signal cointegrator 1 complex)来诱导翻译抑制[170]. m^{1}A修饰写入器TRMT61A可以催化矮牵牛花mRNA中的m^{1}A修饰, 敲降TRMT61A会降低矮牵牛花的叶绿素含量, 改变叶子的形态[171]. 此外, 研究发现, m^{1}A修饰擦除器ALKBH3通过去除细胞初级纤毛负调控因子*Aurora A* mRNA上的m^{1}A修饰位点, 增强mRNA的稳定性和翻译效率, 促进细胞纤毛的解聚, 从而参与斑马鱼的纤毛相关发育过程[172].

4.3 mRNA m^{1}A修饰的测序方法

高通量测序技术的发展使得能够更全面地了解mRNA中m^{1}A修饰的分布和动态变化. m^{1}A-MeRIP-seq利用m^{1}A抗体对携带有m^{1}A修饰的RNA片段进行免疫沉淀和高通量测序, 是目前使用最为广泛的测序方法. 随后开发的m^{1}A-seq, 则先对m^{1}A抗体富集的RNA片段进行Dimroth重排反应(碱性条件下诱导m^{1}A修饰转变成m^{6}A修饰), 然后通过分析m^{1}A修饰引起的碱基错配情况来鉴定m^{1}A修饰位点[153]. 另外, 研究者利用一些逆转录酶在逆转录过程中遇到m^{1}A修饰位点会发生截断或突变等特性, 开发了一些测序方法. 例如, m^{1}A-ID-seq将m^{1}A抗体免疫沉淀获得的RNA片段, 使用AMV (avian myeloblastosis virus)逆转录酶进行cDNA合成, 根据cDNA的截断位置来识别转录本中m^{1}A修饰的信息[160]. m^{1}A-seq-TGIRT和m^{1}A-seq-SS分别利用逆转录酶TGIRT(thermostable group II intron reverse transcriptase)和Super Script III能够精确地在m^{1}A修饰位点产生碱基错配和截断的原理, 可在单碱基分辨率上绘制m^{1}A修饰图谱[154]. m^{1}A-quant-seq利用RT-1306(一种工程改造的HIV-1逆转录酶)在逆转录过程中遇到m^{1}A修饰位点会发生碱基错配的原理, 可用于绘制单碱基分辨率的m^{1}A修饰图谱[155]; 同时, 该方法能进一步结合m^{1}A抗体免疫沉淀以及大肠杆菌AlkB处理, 识别出人类细胞mRNA上的215个m^{1}A修饰位点[155]. m^{1}A-MAP综合应用AlkB去除m^{1}A修饰和TGIRT介导的逆转录错配, 在人类细胞mRNA中共检测出740个m^{1}A修饰位点[173].

4.4 mRNA m^{1}A修饰与疾病

尽管人们发现人类细胞mRNA上存在m^{1}A修饰, 但对其在疾病发生发展中作用的认知还十分有限. mRNA m^{1}A修饰的相关酶在多种肿瘤组织中异常表达, 参与肿瘤发生发展的调控[174]. 研究显示, CSF1 (colony-stimulating factor 1)与乳腺癌及卵巢癌的不良预后显著相关, ALKBH3可去除*CSF1* mRNA翻译起始位点附近的m^{1}A修饰, 提高*CSF1* mRNA的稳定性, 从而增强癌细胞的侵袭能力[174]. 在霍奇金淋巴瘤患者中, ALKBH3启动子CpG岛存在明显的超甲基化水平和转录抑制, 其高甲基化水平与患者较低的生存周期相关, ALKBH3的转录抑制会导致mRNA m^{1}A修饰的

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水平升高, 细胞基因表达谱发生改变, 进而影响霍奇金淋巴瘤的进展[175]. ATP5D是线粒体ATP合成酶的一个关键亚基, 去除*ATP5A* mRNA上的m^1A修饰能够提升其翻译效率, 从而促进肿瘤的发展[176].

5 mRNA A-to-I编辑

5.1 mRNA A-to-I编辑概述

腺苷到肌苷(adenosine to inosine, A-to-I)和胞苷到尿苷(cytidine to uridine, C-to-U)是主要的RNA编辑类型[177,178], mRNA A-to-I编辑是mRNA分子转录和转录后调控的机制之一. 这种调控机制由RNA腺嘌呤脱氨酶(adenosine deaminases acting on RNA, ADARs)介导[179]. ADARs能够催化腺嘌呤(A)转化为肌苷(I), 在后续的生物学过程中, 肌苷(I)被识别为鸟苷(G). ADAR蛋白家族是一个高度保守的蛋白家族[180], 由N端的双链RNA结合结构域(double-stranded RNA binding domain, dsRBD)与C端的脱氨酶结构域(deaminase domain, DD)组成. RNA结合结构域介导RNA序列及结构的识别, 脱氨酶结构域介导脱氨反应. 在哺乳动物中有三个ADAR家族成员(ADAR1, ADAR2和ADAR3), 其中ADAR3缺乏编辑活性, 可能与其他ADAR竞争性结合dsRNAs, 降低这些酶的效率[181]; ADAR1在N端还有可以结合Z-DNA和Z-RNA的Zα结构域(图7). A-to-I RNA编辑位点在mRNA上广泛分布, 此外其分布在不同生物和不同组织中有所不同. 例如, 在人类中, RNA编辑位点主要分布在内含子和非翻译区, 此外还有一些编辑位点分布在编码区域和miRNA区域[182].

5.2 mRNA A-to-I编辑与疾病

非编码区A-to-I编辑是RNA编辑最重要的功能之一. ADAR1介导的非编码区RNA编辑, 特别是重复区RNA编辑可用于标记“自我”和“非我”RNA, 使MDA5一类的RNA传感器对内源性dsRNA不产生反应[183], 在先天免疫系统中起着重要的负调控作用. ADAR1敲除会导致小鼠干扰素和dsRNA传感信号通路激活, 从而胚胎致死[183~186]. 与之相吻合地, A-to-I RNA编辑可以影响自身免疫疾病的发生和发展, 如多发性硬化、类风湿性关节炎、自身免疫性脑脊髓炎和系统性红斑狼疮等疾病[187~190]. 同时, 越来越多的研究表明, 人类重复区A-to-I编辑在肿瘤的发生和发展中发挥着重要作用. ADAR1的免疫负调节功能广泛地被肿瘤利用, 很多肿瘤类型高表达ADAR1, 帮助癌细胞逃避先天免疫反应[191]. 抑制肿瘤细胞的ADAR1 RNA编辑活性可以激活肿瘤免疫反应, 从而增强机体对肿瘤的杀伤力[192,193]. ADAR1已经成为肿瘤免疫治疗领域最热门的靶点之一[194]. 此外, 缺乏ADAR1和MDA5或ADAR1和MAVS的小鼠在出生后的第一周仍然会死亡[184,195,196], 说明ADAR1还能限制其他免疫传感器的自发激活. 研究表明, ADAR1能通过Zα结构域促进内源反向配对Alu元件的RNA编辑, 以防止dsRNA形成, 否则将诱导ZBP1(Z-DNA binding protein 1)激活. ZBP1激活则会引发caspase-8依赖性细胞凋亡和MLKL(mixed-lineage kinase domain-like)介导的坏死性凋亡, 进而导致ADAR1基因敲除小鼠的死亡[197].

与非编码区的RNA编辑不同, 编码区域A-to-I编辑最重要的功能是通过改变RNA序列进而改变氨基酸, 从而影响蛋白功能[198]. 成人大脑中的大多数AMPA受体(α-氨基-3-羟基-5-甲基-4-异恶唑丙酸受体, AMPAR)含有谷氨酸受体2(glutamate receptors2, GluA2)亚基, 可以由ADAR2介导的特定碱基处进行RNA编辑(Q607R 位点), 把谷氨酰胺转变为精氨酸. 精氨酸与不带电的谷氨酰胺相比带正电荷, 使离子通道的关键特征发生改变. 未编辑的GluA2亚基是钙离子可渗透的, 而编辑后的GluA2亚基是钙离子不可渗透的, 这对正常AMPA受体功能很重要[199~203]. Q/R编辑在神经发育和神经元细胞成熟期间逐渐增加, 并且所有GluA2亚基在成人大脑中都是编辑后的[202,204~210]. 除了决定AMPA受体的钙离子通透性外, GluA2的Q/R编辑还影响其他电生理特性. 含有编辑后的GluA2的AMPA受体显示出线性的电流-电压关系, 而含有未经编辑的GluA2的AMPA受体具有向内整流的电流-电压关系[199,200,211]. 此外, 编辑过的GluA2的单通道电导率比未编辑的GluA2低. 当小鼠的GluA2未编辑时会发生严重的癫痫和过早死亡[212,213]. 同时, 在表达不同水平的未编辑的GluA2的小鼠中会产生从嗜睡到过度兴奋等不同的神经功能障碍[207]. 另外, 前脑中引起癫痫的电路改变可能与含有未编辑的GluA2亚基的受体钙离子内流升高有关[213~215]. 根据对散发性肌萎缩性侧索硬化症患者的尸检分析, Q/R位点编辑不足的GluA2在引起运动神经元细胞死亡中具有的潜在作用[216].

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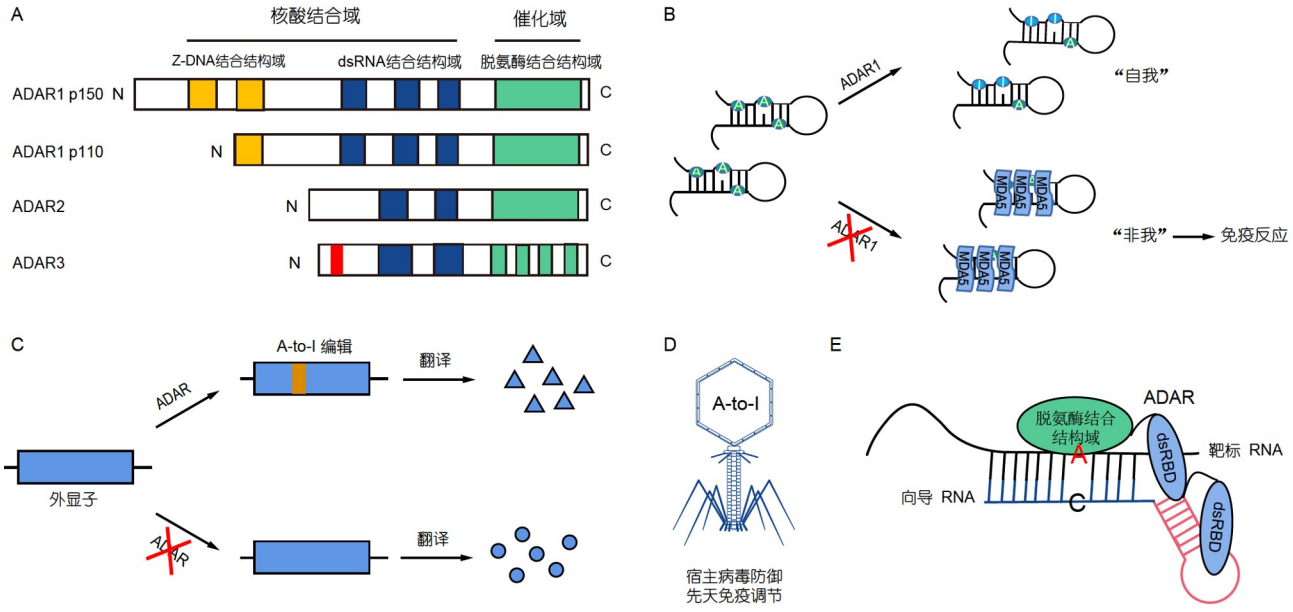


图 7 RNA编辑系统及其功能和应用. A: 哺乳动物中有三个高保守的ADAR家族成员: ADAR1, ADAR2和ADAR3. ADAR含有一个催化脱氨酶结构域和两个或三个双链RNA结合结构域, ADAR1还有可以结合Z-DNA和Z-RNA的Zα结构域. 除了组成型ADAR1 p110亚型, 哺乳动物ADAR1基因还表达干扰素诱导的p150亚型; B: ADAR1介导的重复区RNA编辑可用于标记“自我”和“非我”RNA, 使MDA5一类的RNA传感器对内源性双链RNA不产生免疫反应; C: 编码区域RNA A-to-I编辑通过改变RNA序列达到改变氨基酸的目的, 从而影响蛋白的功能; D: 病毒RNA的A-to-I编辑会影响宿主对病毒的防御能力以及宿主本身的先天免疫调节; E: 利用具有目标序列结合序列和ADAR募集序列的gRNA募集内源性ADAR酶进行定点RNA编辑

Figure 7 RNA editing systems and its functions and applications. A: There are three highly conserved members of the ADAR family: ADAR1, ADAR2 and ADAR3. ADARs contain a catalytic deaminase domain and two or three double-stranded RNA-binding domains, and ADAR1 also has a Zα domain that binds Z-DNA and Z-RNA. In addition to the constitutive ADAR1 p110 isoform, the mammalian ADAR1 gene also expresses p150 isoform. ADAR3 contains an arginine-rich domain, shown in red, which binds single-stranded RNA; B: ADAR1-mediated repeat region RNA editing can be used to label “self” and “non-self” RNAs so that RNA sensors such as MDA5 do not produce an immune response to endogenous double-stranded RNAs; C: coding region RNA A-to-I editing changes the amino acid sequence by changing the RNA sequence, thus affecting the function of the protein; D: A-to-I editing of viral RNA affects the host’s defenses against the virus and the host’s own innate immune regulation; E: gRNA with target sequence binding domain and ADAR recruitment domain can be used to recruit ADAR enzymes for site-specific RNA editing

不同的病毒中鉴定出许多宿主介导的RNA编辑[217]. RNA编辑可以通过促进或抑制病毒复制、蛋白质合成等诱导促病毒或抗病毒作用[218]. 一方面, ADAR可以通过对病毒RNA的编辑破坏其结构或者蛋白编码序列, 抑制病毒的复制; 另一方面, 病毒RNA可以利用A-to-I RNA编辑加速其进化[219]. 此外, 病毒RNA编辑可以促进宿主丧失区分内源性宿主RNA和外源性病毒RNA的能力, 有利于病毒免疫逃逸.

RNA编辑可在RNA层面改变遗传信息. 可利用这一机制靶向修复或改变自身mRNA, 达到疾病治疗的目的. 通过设计一条单链RNA与携带拟编辑位点的mRNA形成碱基互补配对, 招募ADAR进行定点RNA编辑[220]. 相比于DNA编辑, 定点RNA编辑具有可控和可逆性, 不影响基因组稳定性, 在临床上具有一系列优势. 特别是基于内源ADAR酶的编辑方式可以只导入一段向导RNA而非蛋白, 不会诱导免疫反应. 此外, RNA编辑天然底物的发现和不同ADAR对二级结构的偏好性有助于设计新型向导RNA, 进一步提高靶向RNA编辑的效率[221]. 最后, 相对应的核酸递送技术也在不断突破[222]. 这些前沿技术的不断突破会推进将RNA编辑作为纠正致病突变、调节基因表达和蛋白质功能的治疗手段的临床应用.

6 总结与展望

mRNA的表观遗传学修饰已成为基因表达调控的新模式, 其可逆性和动态性使RNA能够实现快速和上下游特异性的基因调控. 根据修饰酶及其亚细胞定位以及阅读器的不同, 修饰会导致转录物翻译效率的

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改变，调节其在细胞质和细胞核之间的运输，影响其稳定性或招募剪接因子，从而决定剪接变异. RNA修饰作为一类新的表观转录生物标志物，在肿瘤、神经系统疾病、免疫性疾病等多种疾病的诊断和治疗中受到广泛关注. 尽管目前的研究证明了RNA修饰在生长发育过程中的作用，但是在复杂生物体内这些相关效应蛋白与其他细胞成分之间的相互作用仍需进一步探索.

生物化学工具与高通量测序相结合的技术已经在分析RNA修饰的定位和功能方面取得了快速进展，只是目前关于修饰的确切频率与基因调控的因果关系和功能的认识有限. 并且目前大多数工作都集中在确定单个修饰类型上，虽然有研究发现m^5C和m^6A彼此靠近并共同调节转录本的命运[223]. 然而，单个转录本上的修饰之间的协同作用对mRNA的稳定性或翻译机制尚不清楚. 多种RNA修饰之间的组合调控作用以及它们在特定细胞环境中与基因调控的相关性亟待研究. 因此，未来的研究应采用整体方法来了解它们导致协同作用或竞争的相互作用.

质谱检测技术的进步使线粒体tRNA转录后修饰图谱被快速解析，但部分修饰所对应的修饰蛋白、修饰酶结构及修饰机制还有待解析，其在人体内的功能及作用机制还未得到全面系统的阐述. 此外，线粒体疾病中的组织特异性问题仍是致病机制研究的焦点. 因此，深入研究个体内不同组织和发育阶段下线粒体tRNA修饰水平及修饰蛋白表达水平的时空调节变化，有助于更好地把握疾病的关键节点，阐明致病机制，进而筛选潜在的药物靶点. 临床上，利用患者有限组织样本进行快速、准确、全面的修饰水平检测对于线粒体tRNA修饰酶突变导致的线粒体疾病的诊断、治疗具有重要指导意义.

随着表观遗传学的快速发展，最近的研究已经将表观遗传学相关蛋白的重编程与人类疾病联系起来，发展产生了大量用于检测RNA修饰的功能表征的策略. 通过调控m^6A，m^1A，m^5C增加或减少它们与目标mRNA上特定序列基序的结合亲和力，实现转录激活或抑制改变调控基因的水平. 这可能是基于表观遗传的药物开发的关键点. RNA编辑可以为识别新的疾病生物标志物提供方法，为开发潜在的新型非侵入性诊断生物标记物和靶向RNA修饰的新型药物的开发提供了另一种途径，并为各种疾病提供更个性化的治疗和监测工具.

参考文献

1 Hendra C, Pratanwanich P N, Wan Y K, et al. Detection of m^6A from direct RNA sequencing using a multiple instance learning framework. Nat Methods, 2022, 19: 1590–1598

2 Boccaletto P, Stefaniak F, Ray A, et al. MODOMICS: a database of RNA modification pathways. 2021 update. Nucl Acids Res, 2022, 50: D231–D235

3 Song J H, Peng J Y, Yin C Q. Chemical modifications in eukaryotic mRNA regulate gene expression (in Chinese). Sci Sin Vitae, 2021, 51: 479–495 [宋靖慧, 彭金英, 伊成器. 真核生物mRNA上的化学修饰和基因表达调控. 中国科学: 生命科学, 2021, 51: 479–495]

4 Weng H, Huang H, Wu H, et al. METTL14 inhibits hematopoietic stem/progenitor differentiation and promotes leukemogenesis via mRNA m^6A modification. Cell Stem Cell, 2018, 22: 191–205.e9

5 Zhou K I, Shi H, Lyu R, et al. Regulation of co-transcriptional pre-mRNA splicing by m^6A through the low-complexity protein hnRNPG. Mol Cell, 2019, 76: 70–81.e9

6 Mao Y, Dong L, Liu X M, et al. m^6A in mRNA coding regions promotes translation via the RNA helicase-containing YTHDC2. Nat Commun, 2019, 10: 5332

7 Hsu P J, Shi H, Zhu A C, et al. The RNA-binding protein FMRP facilitates the nuclear export of N^6-methyladenosine-containing mRNAs. J Biol Chem, 2019, 294: 19889–19895

8 Wang X, Lu Z, Gomez A, et al. N^6-methyladenosine-dependent regulation of messenger RNA stability. Nature, 2014, 505: 117–120

9 Kouzarides T. Chromatin modifications and their function. Cell, 2007, 128: 693–705

10 Kadumuri R V, Janga S C. Epitranscriptomic code and its alterations in human disease. Trends Mol Med, 2018, 24: 886–903

11 Wang Y, Li Y, Toth J I, et al. N^6-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. Nat Cell Biol, 2014, 16: 191–198

https://engine.scichina.com/doi/10.1360/SSV-2023-0127

12 Pan J, Huang Z, Xu Y. m^{5}C RNA methylation regulators predict prognosis and regulate the immune microenvironment in lung squamous cell carcinoma. Front Oncol, 2021, 11: 657466

13 Han D, Xu M M. RNA modification in the immune system. Annu Rev Immunol, 2023, 41: 73–98

14 Cox D B T, Gootenberg J S, Abudayyeh O O, et al. RNA editing with CRISPR-Cas13. Science, 2017, 358: 1019–1027

15 Zhang H, Fu Q, Shi X, et al. Human A-to-I RNA editing SNP loci are enriched in GWAS signals for autoimmune diseases and under balancing selection. Genome Biol, 2020, 21: 288

16 Orellana E A, Siegal E, Gregory R I. tRNA dysregulation and disease. Nat Rev Genet, 2022, 23: 651–664

17 Smith A C, Blackshaw J A, Robinson A J. MitoMiner: a data warehouse for mitochondrial proteomics data. Nucl Acids Res, 2012, 40: D1160–D1167

18 Suzuki T, Nagao A, Suzuki T. Human mitochondrial tRNAs: biogenesis, function, structural aspects, and diseases. Annu Rev Genet, 2011, 45: 299–329

19 Liu Y, Chen Y. Mitochondrial tRNA mutations associated with essential hypertension: from molecular genetics to function. Front Cell Dev Biol, 2021, 8: 634137

20 Suzuki T, Yashiro Y, Kikuchi I, et al. Complete chemical structures of human mitochondrial tRNAs. Nat Commun, 2020, 11: 4269

21 Armengod M E, Moukadiri I, Prado S, et al. Enzymology of tRNA modification in the bacterial MnmEG pathway. Biochimie, 2012, 94: 1510–1520

22 Ji Y, Nie Z, Meng F, et al. Mechanistic insights into mitochondrial tRNA^{Ala} 3′-end metabolism deficiency. J Biol Chem, 2021, 297: 100816

23 Nagaike T, Suzuki T, Tomari Y, et al. Identification and characterization of mammalian mitochondrial tRNA nucleotidyltransferases. J Biol Chem, 2001, 276: 40041–40049

24 Crick F H. Codon—anticodon pairing: the wobble hypothesis. J Mol Biol, 1966, 19: 548–555

25 Harada F, Nishimura S. Possible anticodon sequences of tRNAHis, tRNAAsn, and tRNAAsp from *Escherichia coli*. universal presence of nucleoside O in the first position of the anticodons of these transfer ribonucleic acid. Biochemistry, 1972, 11: 301–308

26 Suzuki T, Suzuki T. A complete landscape of post-transcriptional modifications in mammalian mitochondrial tRNAs. Nucl Acids Res, 2014, 42: 7346–7357

27 Moriya J, Yokogawa T, Wakita K, et al. A novel modified nucleoside found at the first position of the anticodon of methionine tRNA from bovine liver mitochondria. Biochemistry, 1994, 33: 2234–2239

28 Takemoto C, Spremulli L L, Benkowski L A, et al. Unconventional decoding of the AUA codon as methionine by mitochondrial tRNA ^{Met} with the anticodon f ^{5} CAU as revealed with a mitochondrial *in vitro* translation system. Nucl Acids Res, 2009, 37: 1616–1627

29 Sokołowski M, Klassen R, Bruch A, et al. Cooperativity between different tRNA modifications and their modification pathways. Biochim Biophys Acta, 2018, 1861: 409–418

30 Suzuki T, Suzuki T, Wada T, et al. Taurine as a constituent of mitochondrial tRNAs: new insights into the functions of taurine and human mitochondrial diseases. EMBO J, 2002, 21: 6581–6589

31 Yim L, Martínez-Vicente M, Villarroya M, et al. The GTPase activity and C-terminal cysteine of the *Escherichia coli* MnmE protein are essential for its tRNA modifying function. J Biol Chem, 2003, 278: 28378–28387

32 Moukadiri I, Prado S, Piera J, et al. Evolutionarily conserved proteins MnmE and GidA catalyze the formation of two methyluridine derivatives at tRNA wobble positions. Nucl Acids Res, 2009, 37: 7177–7193

33 Umeda N, Suzuki T, Yukawa M, et al. Mitochondria-specific RNA-modifying enzymes responsible for the biosynthesis of the wobble base in mitochondrial tRNAs—implications for the molecular pathogenesis of human mitochondrial diseases. J Biol Chem, 2005, 280: 1613–1624

34 Wang X, Yan Q, Guan M X. Combination of the loss of cmnm^{5}U$_{34}$ with the lack of s^{2}U$_{34}$ modifications of tRNA^{Lys}, tRNA^{Glu}, and tRNA^{Gln} altered mitochondrial biogenesis and respiration. J Mol Biol, 2010, 395: 1038–1048

35 Li X, Guan M X. A human mitochondrial GTP binding protein related to tRNA modification may modulate phenotypic expression of the deafness-associated mitochondrial 12S rRNA mutation. Mol Cell Biol, 2002, 22: 7701–7711

36 Li X, Li R, Lin X, et al. Isolation and characterization of the putative nuclear modifier gene *MTO1* involved in the pathogenesis of deafness-associated mitochondrial 12 S rRNA A1555G mutation. J Biol Chem, 2002, 277: 27256–27264

37 Asano K, Suzuki T, Saito A, et al. Metabolic and chemical regulation of tRNA modification associated with taurine deficiency and human disease. Nucl Acids Res, 2018, 46: 1565–1583

https://engine.scichina.com/doi/10.1360/SSV-2023-0127

38 Mueller E G. Trafficking in persulfides: delivering sulfur in biosynthetic pathways. Nat Chem Biol, 2006, 2: 185–194

39 Ikeuchi Y, Shigi N, Kato J, et al. Mechanistic insights into sulfur relay by multiple sulfur mediators involved in thiouridine biosynthesis at tRNA wobble positions. Mol Cell, 2006, 21: 97–108

40 Suzuki T, Nagao A, Suzuki T. Human mitochondrial diseases caused by lack of taurine modification in mitochondrial tRNAs. WIREs RNA, 2011, 2: 376–386

41 Guan M X, Yan Q, Li X, et al. Mutation in *TRMU* related to transfer RNA modification modulates the phenotypic expression of the deafness-associated mitochondrial 12S ribosomal RNA mutations. Am J Hum Genet, 2006, 79: 291–302

42 Kopajtich R, Nicholls T J, Rorbach J, et al. Mutations in *GTPBP3* cause a mitochondrial translation defect associated with hypertrophic cardiomyopathy, lactic acidosis, and encephalopathy. Am J Hum Genet, 2014, 95: 708–720

43 Zeharia A, Shaag A, Pappo O, et al. Acute infantile liver failure due to mutations in the *TRMU* gene. Am J Hum Genet, 2009, 85: 401–407

44 Bykhovskaya Y, Mengesha E, Wang D, et al. Phenotype of non-syndromic deafness associated with the mitochondrial A1555G mutation is modulated by mitochondrial RNA modifying enzymes MTO1 and GTPBP3. Mol Genet Metab, 2004, 83: 199–206

45 Baruffini E, Dallabona C, Invernizzi F, et al. *MTO1* mutations are associated with hypertrophic cardiomyopathy and lactic acidosis and cause respiratory chain deficiency in humans and yeast. Hum Mutat, 2013, 34: 1501–1509

46 Tischner C, Hofer A, Wulff V, et al. MTO1 mediates tissue specificity of OXPHOS defects via tRNA modification and translation optimization, which can be bypassed by dietary intervention. Hum Mol Genet, 2015, 24: 2247–2266

47 Goto Y I, Nonaka I, Horai S. A mutation in the $tRNA^{Leu(UUR)}$ gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature, 1990, 348: 651–653

48 Yasukawa T, Suzuki T, Suzuki T, et al. Modification defect at anticodon wobble nucleotide of mitochondrial $tRNAs^{Leu}$(UUR) with pathogenic mutations of mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes. J Biol Chem, 2000, 275: 4251–4257

49 Peng G X, Zhang Y, Wang Q Q, et al. The human tRNA taurine modification enzyme GTPBP3 is an active GTPase linked to mitochondrial diseases. Nucl Acids Res, 2021, 49: 2816–2834

50 Kazuhito T, Wei F Y. Posttranscriptional modifications in mitochondrial tRNA and its implication in mitochondrial translation and disease. J Biochem, 2020, 168: 435–444

51 Zhang Q, Zhang L, Chen D, et al. Deletion of Mtu1 (Trmu) in zebrafish revealed the essential role of tRNA modification in mitochondrial biogenesis and hearing function. Nucl Acids Res, 2018, 46: 10930–10945

52 Meng F, Cang X, Peng Y, et al. Biochemical evidence for a nuclear modifier Allele (A10S) in TRMU (methylaminomethyl-2-thiouridylate-methyltransferase) related to mitochondrial tRNA modification in the phenotypic manifestation of deafness-associated 12S rRNA mutation. J Biol Chem, 2017, 292: 2881–2892

53 Shi H, Wei J, He C. Where, when, and how: context-dependent functions of rna methylation writers, readers, and erasers. Mol Cell, 2019, 74: 640–650

54 Śledź P, Jinek M. Structural insights into the molecular mechanism of the m^6A writer complex. eLife, 2016, 5: e18434

55 Patil D P, Chen C K, Pickering B F, et al. m^6A RNA methylation promotes *XIST*-mediated transcriptional repression. Nature, 2016, 537: 369–373

56 Schwartz S, Mumbach M R, Jovanovic M, et al. Perturbation of m6A writers reveals two distinct classes of mrna methylation at internal and 5′ sites. Cell Rep, 2014, 8: 284–296

57 Jia G, Fu Y, Zhao X, et al. N^6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol, 2011, 7: 885–887

58 Zheng G, Dahl J A, Niu Y, et al. ALKBH5 is a mammalian rna demethylase that impacts RNA metabolism and mouse fertility. Mol Cell, 2013, 49: 18–29

59 Wang X, Zhao B S, Roundtree I A, et al. N^6-methyladenosine modulates messenger RNA translation efficiency. Cell, 2015, 161: 1388–1399

60 Huang H, Weng H, Sun W, et al. Recognition of RNA N^6-methyladenosine by IGF2BP proteins enhances mRNA stability and translation. Nat Cell Biol, 2018, 20: 285–295

61 Zhang F, Kang Y, Wang M, et al. Fragile X mental retardation protein modulates the stability of its m^6A-marked messenger RNA targets. Hum Mol Genet, 2018, 27: 3936–3950

62 Liu N, Zhou K I, Parisien M, et al. N^6-methyladenosine alters RNA structure to regulate binding of a low-complexity protein. Nucl Acids Res,

https://engine.scichina.com/doi/10.1360/SSV-2023-0127

2017, 45: 6051–6063
63 Liu J, Harada B T, He C. Regulation of gene expression by N^6-methyladenosine in cancer. Trends Cell Biol, 2019, 29: 487–499
64 Yue B, Song C, Yang L, et al. METTL3-mediated N^6-methyladenosine modification is critical for epithelial-mesenchymal transition and metastasis of gastric cancer. Mol Cancer, 2019, 18: 142
65 Hu Y, Gong C, Li Z, et al. Demethylase ALKBH5 suppresses invasion of gastric cancer via PKMYT1 m^6A modification. Mol Cancer, 2022, 21: 34
66 Pi J, Wang W, Ji M, et al. YTHDF1 promotes gastric carcinogenesis by controlling translation of *FZD7*. Cancer Res, 2021, 81: 2651–2665
67 METTL3 Promotes mRNA Translation to Drive Tumorigenesis. Cancer Disco, 2018, 8: 1346
68 Liu L, Wang J, Sun G, et al. m^6A mRNA methylation regulates CTNNB1 to promote the proliferation of hepatoblastoma. Mol Cancer, 2019, 18: 188
69 Chen Y, Peng C, Chen J, et al. WTAP facilitates progression of hepatocellular carcinoma via m^6A-HuR-dependent epigenetic silencing of ETS1. Mol Cancer, 2019, 18: 127
70 Cheng X, Li M, Rao X, et al. KIAA1429 regulates the migration and invasion of hepatocellular carcinoma by altering m^6A modification of ID2 mRNA. Onco Targets Ther, 2019, Volume 12: 3421–3428
71 Ma J Z, Yang F, Zhou C C, et al. METTL14 suppresses the metastatic potential of hepatocellular carcinoma by modulating N^6-methyladenosine-dependent primary MicroRNA processing. Hepatology, 2017, 65: 529–543
72 Liu X, Liu J, Xiao W, et al. SIRT1 regulates N^6-methyladenosine RNA modification in hepatocarcinogenesis by inducing RANBP2-dependent FTO SUMOylation. Hepatology, 2020, 72: 2029–2050
73 Mittenbühler M J, Saedler K, Nolte H, et al. Hepatic FTO is dispensable for the regulation of metabolism but counteracts HCC development *in vivo*. Mol Metab, 2020, 42: 101085
74 Bansal H, Yihua Q, Iyer S P, et al. WTAP is a novel oncogenic protein in acute myeloid leukemia. Leukemia, 2014, 28: 1171–1174
75 Vu L P, Pickering B F, Cheng Y, et al. The N^6-methyladenosine (m^6A)-forming enzyme METTL3 controls myeloid differentiation of normal hematopoietic and leukemia cells. Nat Med, 2017, 23: 1369–1376
76 Liu F, Wang L. RNA modifications in the regulation of hematopoiesis and leukemogenesis (in Chinese). Sci Sin Vitae, 2020, 50: 827–835 [刘帆, 王璐. RNA修饰调控造血发育和白血病发生的研究进展. 中国科学: 生命科学, 2020, 50: 827–835]
77 Cheng Y, Xie W, Pickering B F, et al. N^6-methyladenosine on mRNA facilitates a phase-separated nuclear body that suppresses myeloid leukemic differentiation. Cancer Cell, 2021, 39: 958–972.e8
78 Shen C, Sheng Y, Zhu A C, et al. RNA demethylase ALKBH5 selectively promotes tumorigenesis and cancer stem cell self-renewal in acute myeloid leukemia. Cell Stem Cell, 2020, 27: 64–80.e9
79 Li Z, Weng H, Su R, et al. FTO plays an oncogenic role in acute myeloid leukemia as a N^6-methyladenosine RNA demethylase. Cancer Cell, 2017, 31: 127–141
80 Huang Y, Su R, Sheng Y, et al. Small-molecule targeting of oncogenic FTO demethylase in acute myeloid leukemia. Cancer Cell, 2019, 35: 677–691.e10
81 Yankova E, Blackaby W, Albertella M, et al. Small-molecule inhibition of METTL3 as a strategy against myeloid leukaemia. Nature, 2021, 593: 597–601
82 Pan J, Liu F, Xiao X, et al. METTL3 promotes colorectal carcinoma progression by regulating the m^6A-CRB3-Hippo axis. J Exp Clin Cancer Res, 2022, 41: 19
83 Chen H, Gao S, Liu W, et al. RNA N^6-methyladenosine methyltransferase METTL3 facilitates colorectal cancer by activating the m^6A-GLUT1-mTORC1 axis and is a therapeutic target. Gastroenterology, 2021, 160: 1284–1300.e16
84 Chen X, Xu M, Xu X, et al. METTL14-mediated N^6-methyladenosine modification of SOX4 mRNA inhibits tumor metastasis in colorectal cancer. Mol Cancer, 2020, 19: 106
85 Yang F, Jin H, Que B, et al. Dynamic m^6A mRNA methylation reveals the role of METTL3-m^6A-CDCP1 signaling axis in chemical carcinogenesis. Oncogene, 2019, 38: 4755–4772
86 Liu Z, Wang T, She Y, et al. N^6-methyladenosine-modified circIGF2BP3 inhibits $CD8^+$ T-cell responses to facilitate tumor immune evasion by promoting the deubiquitination of PD-L1 in non-small cell lung cancer. Mol Cancer, 2021, 20: 105
87 Liu J, Eckert M A, Harada B T, et al. m^6A mRNA methylation regulates AKT activity to promote the proliferation and tumorigenicity of

https://engine.scichina.com/doi/10.1360/SSV-2023-0127

endometrial cancer. Nat Cell Biol, 2018, 20: 1074–1083
88 Xu Y, Song M, Hong Z, et al. The N^6-methyladenosine METTL3 regulates tumorigenesis and glycolysis by mediating m^{6}A methylation of the tumor suppressor LATS1 in breast cancer. J Exp Clin Cancer Res, 2023, 42: 10
89 Li H B, Huang G, Tu J, et al. METTL14-mediated epitranscriptome modification of MN1 mRNA promote tumorigenicity and all-trans-retinoic acid resistance in osteosarcoma. Ebiomedicine, 2022, 82: 104142
90 Yang Z, Yang S, Cui Y H, et al. METTL14 facilitates global genome repair and suppresses skin tumorigenesis. Proc Natl Acad Sci USA, 2021, 118: e2025948118
91 Yu J, Chai P, Xie M, et al. Histone lactylation drives oncogenesis by facilitating m^{6}A reader protein YTHDF2 expression in ocular melanoma. Genome Biol, 2021, 22: 85
92 Xu F, Li J, Ni M, et al. FBW7 suppresses ovarian cancer development by targeting the N^6-methyladenosine binding protein YTHDF2. Mol Cancer, 2021, 20: 45
93 Wen S, Wei Y, Zen C, et al. Long non-coding RNA *NEAT1* promotes bone metastasis of prostate cancer through N^6-methyladenosine. Mol Cancer, 2020, 19: 171
94 Dixit D, Prager B C, Gimple R C, et al. The RNA m^{6}A reader YTHDF2 maintains oncogene expression and is a targetable dependency in glioblastoma stem cells. Cancer Discov, 2021, 11: 480–499
95 Wang M, Liu J, Zhao Y, et al. Upregulation of METTL14 mediates the elevation of *PERP* mRNA N^6 adenosine methylation promoting the growth and metastasis of pancreatic cancer. Mol Cancer, 2020, 19: 130
96 Wang H, Hu X, Huang M, et al. Mettl3-mediated mRNA m^{6}A methylation promotes dendritic cell activation. Nat Commun, 2019, 10: 1898
97 Zong X, Fu J, Jin M L, et al. Progress on N^6-methyladenosine regulation of immune homeostasis (in Chinese). Sci Sin Vitae, 2023, 53: 334–346 [宗鑫, 付杰, 靳明亮, 等. RNA m^{6}A甲基化调控免疫稳态的机制及研究进展. 中国科学: 生命科学, 2023, 53: 334–346]
98 Zhou H, Mao L, Xu H, et al. The functional roles of m^{6}A modification in T lymphocyte responses and autoimmune diseases. Cytokine Growth Factor Rev, 2022, 65: 51–60
99 Shi W, Zheng Y, Luo S, et al. METTL3 promotes activation and inflammation of FLSs through the NF-κB signaling pathway in rheumatoid arthritis. Front Med, 2021, 8: 607585
100 Lu T X, Zheng Z, Zhang L, et al. A new model of spontaneous colitis in mice induced by deletion of an RNA m^{6}A methyltransferase component METTL14 in T cells. Cell Mol Gastroenterol Hepatol, 2020, 10: 747–761
101 Luo Q, Rao J, Zhang L, et al. The study of *METTL14*, *ALKBH5* , and *YTHDF2* in peripheral blood mononuclear cells from systemic lupus erythematosus. Mol Genet Genomic Med, 2020, 8: e1298
102 Roth G A, Forouzanfar M H, Moran A E, et al. Demographic and epidemiologic drivers of global cardiovascular mortality. N Engl J Med, 2015, 372: 1333–1341
103 Chen J, Ning Y, Zhang H, et al. METTL14-dependent m^{6}A regulates vascular calcification induced by indoxyl sulfate. Life Sci, 2019, 239: 117034
104 Dorn L E, Lasman L, Chen J, et al. The N^6-methyladenosine mRNA methylase METTL3 controls cardiac homeostasis and hypertrophy. Circulation, 2019, 139: 533–545
105 Berulava T, Buchholz E, Elerdashvili V, et al. Changes in m^{6}A RNA methylation contribute to heart failure progression by modulating translation. Eur J Heart Fail, 2020, 22: 54–66
106 Mo X B, Lei S F, Zhang Y H, et al. Examination of the associations between m^{6}A-associated single-nucleotide polymorphisms and blood pressure. Hypertens Res, 2019, 42: 1582–1589
107 Chen J, Zhang Y C, Huang C, et al. m^{6}A regulates neurogenesis and neuronal development by modulating histone methyltransferase Ezh2. Genom Proteom BioInf, 2019, 17: 154–168
108 Wang C X, Cui G S, Liu X, et al. METTL3-mediated m^{6}A modification is required for cerebellar development. PLoS Biol, 2018, 16: e2004880
109 Wu R, Li A, Sun B, et al. A novel m^{6}A reader Prrc2a controls oligodendroglial specification and myelination. Cell Res, 2019, 29: 23–41
110 Wang Y, Mao J, Wang X, et al. Genome-wide screening of altered m^{6}A-tagged transcript profiles in the hippocampus after traumatic brain injury in mice. Epigenomics, 2019, 11: 805–819
111 Yu J, Zhang Y, Ma H, et al. Epitranscriptomic profiling of N^6-methyladenosine-related RNA methylation in rat cerebral cortex following traumatic brain injury. Mol Brain, 2020, 13: 11

https://engine.scichina.com/doi/10.1360/SSV-2023-0127

112 Han M, Liu Z, Xu Y, et al. Abnormality of m^{6}A mRNA methylation is involved in Alzheimer's disease. Front Neurosci, 2020, 14: 98
113 Deng Y, Zhu H, Xiao L, et al. Identification of the function and mechanism of m^{6}A reader IGF2BP2 in Alzheimer's disease. Aging, 2021, 13: 24086–24100
114 Koranda J L, Dore L, Shi H, et al. Mettl14 is essential for epitranscriptomic regulation of striatal function and learning. Neuron, 2018, 99: 283–292.e5
115 Chen X, Yu C, Guo M, et al. Down-regulation of m^{6}A mRNA methylation is involved in dopaminergic neuronal death. ACS Chem Neurosci, 2019, 10: 2355–2363
116 Yang X, Yang Y, Sun B F, et al. 5-methylcytosine promotes mRNA export—NSUN2 as the methyltransferase and ALYREF as an m^{5}C reader. Cell Res, 2017, 27: 606–625
117 Liu Y, Yang Y, Wu R, et al. mRNA m^{5}C inhibits adipogenesis and promotes myogenesis by respectively facilitating *YBX2* and *SMO* mRNA export in ALYREF-m^{5}C manner. Cell Mol Life Sci, 2022, 79: 481
118 Yang Y, Wang L, Han X, et al. RNA 5-methylcytosine facilitates the maternal-to-zygotic transition by preventing maternal mRNA decay. Mol Cell, 2019, 75: 1188–1202.e11
119 Wang H, Feng J, Zeng C, et al. NSUN2-mediated m^{5}C methylation of IRF3 mRNA negatively regulates type I interferon responses during various viral infections. Emerging Microbes Infects, 2023, 12: 2178238
120 Courtney D G, Tsai K, Bogerd H P, et al. Epitranscriptomic addition of m^{5}C to HIV-1 transcripts regulates viral gene expression. Cell Host Microbe, 2019, 26: 217–227.e6
121 Xing J, Yi J, Cai X, et al. NSun2 promotes cell growth via elevating cyclin-dependent kinase 1 translation. Mol Cell Biol, 2015, 35: 4043–4052
122 Wang X, Wang M, Dai X, et al. RNA 5-methylcytosine regulates YBX2-dependent liquid-liquid phase separation. Fundamental Res, 2022, 2: 48–55
123 Xu X, Zhang Y, Zhang J, et al. NSun2 promotes cell migration through methylating autotaxin mRNA. J Biol Chem, 2020, 295: 18134–18147
124 Chen X, Li A, Sun B F, et al. 5-Methylcytosine promotes pathogenesis of bladder cancer through stabilizing mRNAs. Nat Cell Biol, 2019, 21: 978–990
125 Xue S, Xu H, Sun Z, et al. Depletion of *TRDMT1* affects 5-methylcytosine modification of mRNA and inhibits HEK293 cell proliferation and migration. Biochem Biophys Res Commun, 2019, 520: 60–66
126 Selmi T, Hussain S, Dietmann S, et al. Sequence- and structure-specific cytosine-5 mRNA methylation by NSUN6. Nucl Acids Res, 2021, 49: 1006–1022
127 Li Y, Xue M, Deng X, et al. TET2-mediated mRNA demethylation regulates leukemia stem cell homing and self-renewal. Cell Stem Cell, 2023, 30: 1072–1090.e10
128 Xue C, Gu X, Zheng Q, et al. ALYREF mediates RNA m^{5}C modification to promote hepatocellular carcinoma progression. Sig Transduct Target Ther, 2023, 8: 130
129 Hu Y, Chen C, Tong X, et al. NSUN2 modified by SUMO-2/3 promotes gastric cancer progression and regulates mRNA m^{5}C methylation. Cell Death Dis, 2021, 12: 842
130 Li Y, Xia Y, Jiang T, et al. Long noncoding RNA DIAPH2-AS1 promotes neural invasion of gastric cancer via stabilizing NSUN2 to enhance the m^{5}C modification of NTN1. Cell Death Dis, 2023, 14: 260
131 Yan J, Liu J, Huang Z, et al. FOXC2-AS1 stabilizes FOXC2 mRNA via association with NSUN2 in gastric cancer cells. Hum Cell, 2021, 34: 1755–1764
132 Su J, Wu G, Ye Y, et al. NSUN2-mediated RNA 5-methylcytosine promotes esophageal squamous cell carcinoma progression via LIN28B-dependent GRB2 mRNA stabilization. Oncogene, 2021, 40: 5814–5828
133 Niu X, Peng L, Liu W, et al. A cis-eQTL in NSUN2 promotes esophageal squamous-cell carcinoma progression and radiochemotherapy resistance by mRNA-m^{5}C methylation. Sig Transduct Target Ther, 2022, 7: 267
134 Chen L, Ding J, Wang B, et al. RNA methyltransferase NSUN2 promotes hypopharyngeal squamous cell carcinoma proliferation and migration by enhancing TEAD1 expression in an m^{5}C-dependent manner. Exp Cell Res, 2021, 404: 112664
135 Wang J Z, Zhu W, Han J, et al. The role of the HIF-1α/ALYREF/PKM2 axis in glycolysis and tumorigenesis of bladder cancer. Cancer Commun, 2021, 41: 560–575
136 Wang N, Chen R, Deng M, et al. m^{5}C-dependent cross-regulation between nuclear reader ALYREF and writer NSUN2 promotes urothelial

https://engine.scichina.com/doi/10.1360/SSV-2023-0127

bladder cancer malignancy through facilitating RABL6/TK1 mRNAs splicing and stabilization. Cell Death Dis, 2023, 14: 139

137 Gao W, Chen L, Lin L, et al. SIAH1 reverses chemoresistance in epithelial ovarian cancer via ubiquitination of YBX-1. Oncogenesis, 2022, 11: 13

138 Chen Y, Zuo X, Wei Q, et al. Upregulation of LRRC8A by m^5C modification-mediated mRNA stability suppresses apoptosis and facilitates tumorigenesis in cervical cancer. Int J Biol Sci, 2023, 19: 691–704

139 Wei X, Zhou S, Liao L, et al. Comprehensive analysis of transcriptomic profiling of 5-methylcytosin modification in placentas from preeclampsia and normotensive pregnancies. FASEB J, 2023, 37: e22751

140 Zhu W, Wan F, Xu W, et al. Positive epigenetic regulation loop between AR and NSUN2 promotes prostate cancer progression. Clin Transl Med, 2022, 12: e1028

141 Han X, Liu H, Zhang Z, et al. Epitranscriptomic 5-methylcytosine profile in $PM_{2.5}$-induced mouse pulmonary fibrosis. Genom Proteom Bioinf, 2020, 18: 41–51

142 Yu W, Zhang C, Wang Y, et al. YAP 5-methylcytosine modification increases its mRNA stability and promotes the transcription of exosome secretion-related genes in lung adenocarcinoma. Cancer Gene Ther, 2023, 30: 149–162

143 Zhang Q, Zheng Q, Yu X, et al. Overview of distinct 5-methylcytosine profiles of messenger RNA in human hepatocellular carcinoma and paired adjacent non-tumor tissues. J Transl Med, 2020, 18: 245

144 Song D, An K, Zhai W, et al. NSUN2-mediated mRNA m^5C modification regulates the progression of hepatocellular carcinoma. Genom Proteom Bioinf, 2022, S1672-0229: 00123-1

145 Chen S Y, Chen K L, Ding L Y, et al. RNA bisulfite sequencing reveals NSUN2-mediated suppression of epithelial differentiation in pancreatic cancer. Oncogene, 2022, 41: 3162–3176

146 Yang M, Wei R, Zhang S, et al. NSUN2 promotes osteosarcoma progression by enhancing the stability of FABP5 mRNA via m^5C methylation. Cell Death Dis, 2023, 14: 125

147 Jian H, Zhang C, Qi Z Y, et al. Alteration of mRNA 5-methylcytosine modification in neurons after OGD/R and potential roles in cell stress response and apoptosis. Front Genet, 2021, 12: 633681

148 Bisoendial R, Lubberts E. A mechanistic insight into the pathogenic role of interleukin 17A in systemic autoimmune diseases. Mediators Inflamm, 2022, 2022: 6600264

149 Wang N, Tang H, Wang X, et al. Homocysteine upregulates interleukin-17A expression via NSun2-mediated RNA methylation in T lymphocytes. Biochem Biophys Res Commun, 2017, 493: 94–99

150 Yang W L, Qiu W, Zhang T, et al. Nsun2 coupling with RoRγt shapes the fate of Th17 cells and promotes colitis. Nat Commun, 2023, 14: 863

151 Guo G, Wang H, Shi X, et al. Disease activity-associated alteration of mRNA m^5C methylation in $CD4^+$ T cells of systemic lupus erythematosus. Front Cell Dev Biol, 2020, 8: 430

152 DUNN D B. The occurence of 1-methyladenine in ribonucleic acid. Biochim Biophys Acta, 1961, 46: 198–200

153 Xiong X, Li X, Yi C. N^1-methyladenosine methylome in messenger RNA and non-coding RNA. Curr Opin Chem Biol, 2018, 45: 179–186

154 Safra M, Sas-Chen A, Nir R, et al. The m^1A landscape on cytosolic and mitochondrial mRNA at single-base resolution. Nature, 2017, 551: 251–255

155 Zhou H, Rauch S, Dai Q, et al. Evolution of a reverse transcriptase to map N^1-methyladenosine in human messenger RNA. Nat Methods, 2019, 16: 1281–1288

156 Suzuki T. The expanding world of tRNA modifications and their disease relevance. Nat Rev Mol Cell Biol, 2021, 22: 375–392

157 Sergiev P V, Aleksashin N A, Chugunova A A, et al. Structural and evolutionary insights into ribosomal RNA methylation. Nat Chem Biol, 2018, 14: 226–235

158 Sloan K E, Warda A S, Sharma S, et al. Tuning the ribosome: The influence of rRNA modification on eukaryotic ribosome biogenesis and function. RNA Biol, 2017, 14: 1138–1152

159 Bar-Yaacov D, Frumkin I, Yashiro Y, et al. Mitochondrial 16S rRNA is methylated by tRNA methyltransferase TRMT61B in all vertebrates. Plos Biol, 2016, 14: e1002557

160 Li X, Xiong X, Wang K, et al. Transcriptome-wide mapping reveals reversible and dynamic N_1-methyladenosine methylome. Nat Chem Biol, 2016, 12: 311–316

161 Wiener D, Schwartz S. The epitranscriptome beyond m^6A. Nat Rev Genet, 2021, 22: 119–131

https://engine.scichina.com/doi/10.1360/SSV-2023-0127

162 Xiong W, Zhao Y, Wei Z, et al. N_1-methyladenosine formation, gene regulation, biological functions, and clinical relevance. Mol Ther, 2023, 31: 308–330

163 Boo S H, Ha H, Kim Y K. m^1A and m^6A modifications function cooperatively to facilitate rapid mRNA degradation. Cell Rep, 2022, 40: 111317

164 Zhao B S, Roundtree I A, He C. Post-transcriptional gene regulation by mRNA modifications. Nat Rev Mol Cell Biol, 2017, 18: 31–42

165 Zhou H, Kimsey I J, Nikolova E N, et al. m^1A and m^1G disrupt A-RNA structure through the intrinsic instability of Hoogsteen base pairs. Nat Struct Mol Biol, 2016, 23: 803–810

166 Seo K W, Kleiner R E. YTHDF2 recognition of N_1-methyladenosine (m^1A)-modified RNA is associated with transcript destabilization. ACS Chem Biol, 2020, 15: 132–139

167 Dominissini D, Nachtergaele S, Moshitch-Moshkovitz S, et al. The dynamic N^1-methyladenosine methylome in eukaryotic messenger RNA. Nature, 2016, 530: 441–446

168 Thomas E N, Kim K Q, McHugh E P, et al. Alkylative damage of mRNA leads to ribosome stalling and rescue by trans translation in bacteria. eLife, 2020, 9: e61984

169 Alriquet M, Calloni G, Martinez-Limon A, et al. The protective role of m^1A during stress-induced granulation. J Mol Cell Biol, 2021, 12: 870–880

170 Tsao N, Brickner J R, Rodell R, et al. Aberrant RNA methylation triggers recruitment of an alkylation repair complex. Mol Cell, 2021, 81: 4228–4242.e8

171 Yang W, Meng J, Liu J, et al. The N_1-methyladenosine methylome of petunia Mrna (1). Plant Physiol, 2020, 183: 1710–1724

172 Kuang W, Jin H, Yang F, et al. ALKBH3-dependent m^1A demethylation of *Aurora A* mRNA inhibits ciliogenesis. Cell Discov, 2022, 8: 25

173 Li X, Xiong X, Zhang M, et al. Base-resolution mapping reveals distinct m^1A methylome in nuclear- and mitochondrial-encoded transcripts. Mol Cell, 2017, 68: 993–1005.e9

174 Woo H H, Chambers S K. Human ALKBH3-induced m^1A demethylation increases the CSF-1 mRNA stability in breast and ovarian cancer cells. Biochim Biophys Acta, 2019, 1862: 35–46

175 Esteve-Puig R, Climent F, Piñeyro D, et al. Epigenetic loss of m^1A RNA demethylase ALKBH3 in Hodgkin lymphoma targets collagen, conferring poor clinical outcome. Blood, 2021, 137: 994–999

176 Wu Y, Chen Z, Xie G, et al. RNA m^1A methylation regulates glycolysis of cancer cells through modulating ATP5D. Proc Natl Acad Sci USA, 2022, 119: e2119038119

177 Bass B L, Weintraub H. A developmentally regulated activity that unwinds RNA duplexes. Cell, 1987, 48: 607–613

178 Smith H C, Bennett R P, Kizilyer A, et al. Functions and regulation of the APOBEC family of proteins. Semin Cell Dev Biol, 2012, 23: 258–268

179 Woolf T M, Chase J M, Stinchcomb D T. Toward the therapeutic editing of mutated RNA sequences. Proc Natl Acad Sci USA, 1995, 92: 8298–8302

180 Nishikura K. Functions and regulation of RNA editing by ADAR deaminases. Annu Rev Biochem, 2010, 79: 321–349

181 Tan M H, Li Q, Shanmugam R, et al. Dynamic landscape and regulation of RNA editing in mammals. Nature, 2017, 550: 249–254

182 Li L, Song Y, Shi X, et al. The landscape of miRNA editing in animals and its impact on miRNA biogenesis and targeting. Genome Res, 2018, 28: 132–143

183 Liddicoat B J, Piskol R, Chalk A M, et al. RNA editing by ADAR1 prevents MDA5 sensing of endogenous dsRNA as nonself. Science, 2015, 349: 1115–1120

184 Pestal K, Funk C C, Snyder J M, et al. Isoforms of RNA-editing enzyme ADAR1 independently control nucleic acid sensor MDA5-driven autoimmunity and multi-organ development. Immunity, 2015, 43: 933–944

185 Wang Q, Miyakoda M, Yang W, et al. Stress-induced apoptosis associated with null mutation of *ADAR1* RNA editing deaminase gene. J Biol Chem, 2004, 279: 4952–4961

186 Hartner J C, Schmittwolf C, Kispert A, et al. Liver disintegration in the mouse embryo caused by deficiency in the RNA-editing enzyme ADAR1. J Biol Chem, 2004, 279: 4894–4902

187 Roth S H, Danan-Gotthold M, Ben-Izhak M, et al. Increased RNA editing may provide a source for autoantigens in systemic lupus erythematosus. Cell Rep, 2018, 23: 50–57

188 Nakahama T, Kato Y, Kim J I, et al. ADAR1-mediated RNA editing is required for thymic self-tolerance and inhibition of autoimmunity. EMBO Rep, 2018, 19: e46303

https://engine.scichina.com/doi/10.1360/SSV-2023-0127

189 Chung H, Rice C M. T time for ADAR: ADAR1 is required for T cell self-tolerance. EMBO Rep, 2018, 19: e47237

190 Li Q, Gloudemans M J, Geisinger J M, et al. RNA editing underlies genetic risk of common inflammatory diseases. Nature, 2022, 608: 569–577

191 Eisenberg E, Levanon E Y. A-to-I RNA editing—immune protector and transcriptome diversifier. Nat Rev Genet, 2018, 19: 473–490

192 Ishizuka J J, Manguso R T, Cheruiyot C K, et al. Loss of ADAR1 in tumours overcomes resistance to immune checkpoint blockade. Nature, 2019, 565: 43–48

193 Liu H, Golji J, Brodeur L K, et al. Tumor-derived IFN triggers chronic pathway agonism and sensitivity to ADAR loss. Nat Med, 2019, 25: 95–102

194 Mehdipour P, Marhon S A, Ettayebi I, et al. Epigenetic therapy induces transcription of inverted SINEs and ADAR1 dependency. Nature, 2020, 588: 169–173

195 Mannion N M, Greenwood S M, Young R, et al. The RNA-editing enzyme ADAR1 controls innate immune responses to RNA. Cell Rep, 2014, 9: 1482–1494

196 Bajad P, Ebner F, Amman F, et al. An internal deletion of ADAR rescued by MAVS deficiency leads to a minute phenotype. Nucl Acids Res, 2020, 48: 3286–3303

197 Jiao H, Wachsmuth L, Wolf S, et al. ADAR1 averts fatal type I interferon induction by ZBP1. Nature, 2022, 607: 776–783

198 Daniel C, Wahlstedt H, Ohlson J, et al. Adenosine-to-inosine RNA editing affects trafficking of the γ-aminobutyric acid type A ($GABA_A$) receptor. J Biol Chem, 2011, 286: 2031–2040

199 Isaac J T R, Ashby M C, McBain C J. The role of the GluR2 subunit in AMPA receptor function and synaptic plasticity. Neuron, 2007, 54: 859–871

200 Liu S J, Zukin R S. Ca^{2+}-permeable AMPA receptors in synaptic plasticity and neuronal death. Trends Neuroscis, 2007, 30: 126–134

201 Hume R I, Dingledine R, Heinemann S F. Identification of a site in glutamate receptor subunits that controls calcium permeability. Science, 1991, 253: 1028–1031

202 Burnashev N, Monyer H, Seeburg P H, et al. Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit. Neuron, 1992, 8: 189–198

203 Lomeli H, Mosbacher J, Melcher T, et al. Control of kinetic properties of AMPA receptor channels by nuclear RNA editing. Science, 1994, 266: 1709–1713

204 Barbon A, Fumagalli F, Caracciolo L, et al. Acute spinal cord injury persistently reduces R/G RNA editing of AMPA receptors. J Neurochem, 2010, 114: 397–407

205 Pachernegg S, Münster Y, Muth-Köhne E, et al. GluA2 is rapidly edited at the Q/R site during neural differentiation *in vitro*. Front Cell Neurosci, 2015, 9: 69

206 Higuchi M, Single F N, Köhler M, et al. RNA editing of AMPA receptor subunit GluR-B: a base-paired intron-exon structure determines position and efficiency. Cell, 1993, 75: 1361–1370

207 Feldmeyer D, Kask K, Brusa R, et al. Neurological dysfunctions in mice expressing different levels of the Q/R site-unedited AMPAR subunit GluR-B. Nat Neurosci, 1999, 2: 57–64

208 Carlson N G, Howard J, Gahring L C, et al. RNA editing (Q/R site) and flop/flip splicing of AMPA receptor transcripts in young and old brains. Neurobiol Aging, 2000, 21: 599–606

209 Kawahara Y, Ito K, Sun H, et al. Low editing efficiency of GluR2 mRNA is associated with a low relative abundance of ADAR2 mRNA in white matter of normal human brain. Eur J Neurosci, 2003, 18: 23–33

210 Kawahara Y, Ito K, Sun H, et al. Glutamate receptors: RNA editing and death of motor neurons. Nature, 2004, 427: 801

211 Liu S J, Savtchouk I. Ca^{2+} permeable AMPA receptors switch allegiances: mechanisms and consequences. J Physiol, 2012, 590: 13–20

212 Brusa R, Zimmermann F, Koh D S, et al. Early-onset epilepsy and postnatal lethality associated with an editing-deficient *GluR-B* allele in mice. Science, 1995, 270: 1677–1680

213 Higuchi M, Maas S, Single F N, et al. Point mutation in an AMPA receptor gene rescues lethality in mice deficient in the RNA-editing enzyme ADAR2. Nature, 2000, 406: 78–81

214 Kortenbruck G, Berger E, Speckmann E J, et al. RNA editing at the Q/R site for the glutamate receptor subunits GLUR2, GLUR5, and GLUR6 in hippocampus and temporal cortex from epileptic patients. Neurobiol Dis, 2001, 8: 459–468

215 Zhang J, Abdullah J M. The role of GluA1 in central nervous system disorders. Rev Neuroscis, 2013, 24: 499–505

https://engine.scichina.com/doi/10.1360/SSV-2023-0127

216 Hideyama T, Yamashita T, Suzuki T, et al. Induced loss of ADAR2 engenders slow death of motor neurons from Q/R site-unedited GluR2. J Neurosci, 2010, 30: 11917–11925
217 Pfaller C K, Donohue R C, Nersisyan S, et al. Extensive editing of cellular and viral double-stranded RNA structures accounts for innate immunity suppression and the proviral activity of ADAR1^{p150}. PLoS Biol, 2018, 16: e2006577
218 Samuel C E. ADARs: viruses and innate immunity. Curr Top Microbiol Immunol, 2012, 353: 163–195
219 Taylor D R, Puig M, Darnell M E R, et al. New antiviral pathway that mediates hepatitis C virus replicon interferon sensitivity through ADAR1. J Virol, 2005, 79: 6291–6298
220 Qu L, Yi Z, Zhu S, et al. Programmable RNA editing by recruiting endogenous ADAR using engineered RNAs. Nat Biotechnol, 2019, 37: 1059–1069
221 Nose K, Hidaka K, Yano T, et al. Short-chain guide RNA for site-directed A-to-I RNA editing. Nucl Acid Ther, 2021, 31: 58–67
222 Paunovska K, Loughrey D, Dahlman J E. Drug delivery systems for RNA therapeutics. Nat Rev Genet, 2022, 23: 265–280
223 Li Q, Li X, Tang H, et al. NSUN2-mediated m^{5}C methylation and METTL3/METTL14-mediated m6A methylation cooperatively enhance p21 translation. J Cell Biochem, 2017, 118: 2587–2598

Research progress of RNA modification and its association with human diseases

GAO ChunChun[1], GUAN MinXin[2], HE Xiao[2], JIN Hao[3], JIN YongFeng[5], LIU Jun[6], LUO Hui[4], QU HuiRu[5], XIE ShanShan[3], YANG YunGui[1], ZHANG Rui[4], ZHANG XinNing[6], ZHANG YiFan[6] & ZHOU TianHua[3]

1 Beijing Institute of Genomics, Chinese Academy of Sciences, China National Center for Bioinformation, Beijing 100101, China;
2 Zhejiang Provincial Key Laboratory of Genetic & Developmental Disorders, Center for Mitochondrial Biomedicine, the Fourth Affiliated Hospital, Zhejiang University School of Medicine, Institute of Genetics, School of Medicine, Zhejiang University, Hangzhou 310058, China;
3 Zhejiang University School of Medicine, Hangzhou 310058, China;
4 School of Life Science, Sun Yat-sen University, Guangzhou 510275, China;
5 National Key Laboratory of Advanced Drug Delivery and Release Systems, College of Life Science, Zhejiang University, Hangzhou 310058, China;
6 State Key Laboratory of Protein and Plant Gene Research, School of Life Sciences, Peking-Tsinghua Center for Life Sciences, Peking University, Beijing 100871, China

Post-transcriptional chemical modifications are prevalent in various RNA molecules and play a profound role in biological functions, including the regulation of RNA splicing, translation, transport, localization, and stability. Recent advancements in high-throughput sequencing and chemical modification detection technologies have facilitated significant breakthroughs in accurately identifying and localizing different RNA modifications, such as m^{6}A, m^{5}C, and m^{1}A. As a novel category of epigenetic transcriptional biomarkers, RNA modifications have garnered considerable attention in the diagnosis and treatment of numerous diseases, including tumors, neurological diseases and immunological diseases. This review aims to provide a comprehensive overview of the biological roles of RNA modification and editing, the underlying molecular mechanisms regulating protein expression and further highlight the progress in the application of RNA modification in disease diagnosis and treatment.

RNA modifications, RNA editing, epigenetics, disease, biomarker

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