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RNA甲基化与肿瘤染色质重塑

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摘要 表观遗传重编程在癌症发展中扮演着关键角色, 其中RNA的 N^6 -甲基腺嘌呤(m^6A)甲基化修饰与染色质重塑之间的相互作用对肿瘤演进具有重要影响。该文综述了RNA m^6A 甲基化与染色质重塑之间的相互作用, 包括 m^6A 写入对染色质重塑的影响、RNA m^6A 如何通过影响DNA甲基化和组蛋白修饰调控染色质状态以及RNA m^6A 对染色质三维结构的调控作用。同时, 该文探讨了染色质重塑如何调控RNA m^6A 修饰, 包括对RNA m^6A 相关因子表达及功能的影响。这些相互作用在肿瘤的发生、发展以及治疗反应中发挥着关键的调控作用。

关键词 RNA甲基化; 染色质重塑; N^6 -甲基腺嘌呤; 组蛋白修饰; DNA甲基化

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RNA Methylation and Chromatin Remodeling in Cancer

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Abstract Epigenetic reprogramming plays a crucial role in the development of cancer, where the interplay between RNA m⁶A (N⁶-methyladenosine) methylation modification and chromatin remodeling has a significant impact on tumor progression. This review summarizes the interactions between m⁶A modification and chromatin remodeling, including the impact of co-transcriptional m⁶A incorporation on chromatin remodeling, how RNA m⁶A regulates chromatin status by affecting DNA methylation and histone modifications, and the regulatory role of RNA m⁶A on the three-dimensional structure of chromatin. Additionally, it discusses how chromatin remodeling can reciprocally regulate RNA m⁶A modification, including its effects on the expression and function of RNA m⁶A-related factors. These interactions play a key regulatory role in the occurrence, development, and therapeutic response of tumors.

Keywords RNA methylation; chromatin remodeling; N⁶-methyladenosine; histone modification; DNA methylation

表观遗传重编程被认为是一种新型的癌症标志,它在驱动癌细胞恶性表型和肿瘤微环境重塑中扮演着关键角色^[1]。染色质的表观遗传修饰变化,例如DNA甲基化和组蛋白修饰,是肿瘤发展过程中的关键分子事件,在调控染色质结构及基因表达中起着至关重要的作用^[2]。N⁶-甲基腺嘌呤(N⁶-methyladenosine, m⁶A)是RNA最常见的甲基化修饰,对多种生理和病理过程中的基因表达调控有着广泛的影响^[3]。RNA m⁶A甲基化与肿瘤细胞的染色质状态之间存在密切的联系。在转录过程中, m⁶A被写入到新生的转录本中,这一过程可能通过影响DNA甲基化、组蛋白修饰以及染色质的三维结构等多维度的染色质表观遗传重塑机制,精细调控基因的转录活动,进而对肿瘤的发生和发展产生影响。本综述旨在探讨m⁶A修饰与染色质重塑之间的相互作用,涵盖它们相互作用的基础、作用方式以及分子层面的调控机制,同时讨论这些互作对肿瘤演进过程的影响。

1 RNA m⁶A修饰与染色质互作的分子基础

m⁶A修饰是指在RNA分子中腺嘌呤碱基的第6位氮原子上发生的甲基化,是真核生物mRNA上最常见的甲基化形式。这种修饰不仅存在于mRNA,还广泛存在于多种非编码RNA如转运RNA(tRNA)、

核糖体RNA(rRNA)、核仁小RNA(snoRNA)、微小RNA(miRNA)以及长链非编码RNA(lncRNA)等。多个研究表明, m⁶A修饰在调控包括癌症在内的多种生理和病理过程的生物学变化中扮演着关键角色^[4-12]。

m⁶A修饰由甲基化转移酶复合物(m⁶A methyltransferase complex, MTC)催化产生。其中,具有催化活性的甲基转移酶样3(methyltransferase like 3, METTL3)与具有支架和导向作用的甲基转移酶样14(methyltransferase like 14, METTL14)共同构成稳定复合物并促进底物结合,在细胞m⁶A修饰沉积到哺乳动物核RNA上的过程中发挥决定性作用^[13-14]。据研究报道,在HeLa细胞中, METTL3与METTL14能够以稳定二聚体的形式与1型Wilms肿瘤相关蛋白(Wilms' tumor 1 associated protein, WTAP)发生互作,三者共定位于核斑点区域^[15]。此外,在小鼠胚胎干细胞中发现,含锌指CCCH结构域蛋白13(zinc finger CCCH domain-containing protein 13, Zc3h13)是MTC发挥功能的一种新辅助因子,它可以通过固定WTAP、雄性化因子Virilizer和Hakai的细胞核定位促进m⁶A修饰的沉积。当敲除Zc3h13后,绝大多数WTAP、Virilizer(vir like m⁶A methyltransferase associated, 又称VIRMA)和Hakai转位到细胞质中,显著降低mRNA上的全局m⁶A水平^[16],提示m⁶A可能与转录过程密切相关。近期的研究表明, METTL3、METTL14、

WTAP、VIRMA、HAKAI以及Zc3h13是完整MTC的关键组分,其中VIRMA能够招募并引导甲基转移酶核心催化组分(METTL3/METTL14/WTAP)到特定的RNA区域进行m⁶A甲基化,约60%的VIRMA免疫沉淀靶mRNA在3'非翻译区(3'-untranslated region, 3'UTR)中表现出强烈的m⁶A富集^[17]。进一步分析发现, METTL3的结合位点在mRNA的编码区和3'UTR中高度富集,并且在内含子区域也观察到了其结合位点的一定分布^[18]。普遍观点认为,新生转录本特别是尚未脱离染色质的RNA中含有大量内含子序列,这些序列在随后的转录加工成熟的过程中由于剪切而迅速降解。因此, METTL3结合位点在内含子区域的富集为m⁶A修饰是在转录过程中共转录写入的假设提供了重要支持。R环(R-loop)是核内由DNA与RNA杂交形成的三链核酸结构,抑制METTL3能够促进转录终止位点处R-loop的形成,且只有具有催化活性的METTL3才能逆转这一表型,揭示了m⁶A甲基化在RNAP II转录终止位点R-loop形成中的关键作用,即在转录过程中的重要作用^[19]。此外, ZHOU等^[20]发现异质核糖核蛋白G(heterogeneous nuclear ribonucleoprotein G, hnRNP G)能够与RNA聚合酶II(RNA polymerase II, RNAP II)中磷酸化的羧基末端结构域(carboxy-terminal domain, CTD)相结合,在共转录过程中调控新生RNA的可变剪接,而这一过程受新生pre-mRNA上剪接位点附近的m⁶A修饰水平以及hnRNP G与该修饰位点结合亲和力的共同调控,进一步证实m⁶A修饰是以共转录的形式写入新生RNA上的观点。

此外,另一个支持RNA修饰耦合转录的证据,是我们的研究团队及国外科研人员均发现, mRNA的转录速率影响其m⁶A修饰水平^[21-22]。具体来说,当RNAP II转录延伸速率降低或发生转录暂停时,其所在的mRNA的m⁶A修饰水平将显著提高。该结果提示RNAP II与METTL3之间存在功能性的相互作用,从而进一步证明m⁶A是在转录过程中被直接写入mRNA上的。另外,在面对复制叉压力及外界应激条件时, METTL3能够通过与RNAP II的结合定位于DNA双链断裂损伤位点,共转录修饰新生RNA,继而促进DNA双链断裂的修复过程^[23]。这些研究成果揭示了m⁶A修饰在共转录调控过程中的写入机制,为理解m⁶A修饰与染色质修饰之间的相互作用提供了关键的空间定位和分子作用理论基础,也为深入

理解肿瘤表观遗传调控的复杂性提供了新的视角。

2 RNA m⁶A调控染色质重塑

2.1 RNA m⁶A通过影响DNA甲基化调控染色质状态

DNA甲基化作为表观遗传调控系统的重要组成部分,通过多种机制对染色质的结构及基因的表达产生影响。最常见的DNA甲基化修饰形式是5-甲基胞嘧啶(5mC),这一修饰常被称为DNA的“第五个碱基”。在人体的病理生理过程中, DNA甲基化扮演着至关重要的角色,其在癌症中的失调现象被认为是肿瘤发生的关键基础之一^[24]。RNA m⁶A修饰与DNA甲基化,这两种具有广泛影响的表观遗传修饰之间是否存在相互作用并对癌症发生和发展产生影响,尚未得到明确的解答。为此,我们的研究团队就RNA m⁶A修饰如何调控DNA 5mC进行了深入研究,并为这一机制提供了解释。在食管鳞状细胞癌中,我们观察到RNA m⁶A与DNA甲基化存在互斥的现象,并且RNA m⁶A水平在肿瘤中显著升高。其中, METTL3以RNA m⁶A催化活性依赖的方式使邻近基因组DNA发生去甲基化,但METTL3并不与DNA甲基化调控相关蛋白的表达存在直接的调控关系。进一步机制探索发现,脆性X相关蛋白1(fragile X-related protein-1, FXR1)作为新型m⁶A阅读器,能够在特异性识别RNA m⁶A位点的同时,招募DNA 5-甲基胞嘧啶双加氧酶TET1(ten-eleven translocation-1)。这一现象触发了相应邻近基因座的DNA去甲基化过程,引起了染色质可及性的改变并调控了癌相关基因的表达,进而促进了癌症的发生和发展^[25]。

类似地,另一项研究发现胰岛素样生长因子2 mRNA结合蛋白(insulin-like growth factor 2 mRNA binding proteins, IGF2BPs)能够以m⁶A依赖的方式识别R-loop。这一识别过程阻碍了DNA甲基转移酶1(DNA methyltransferase-1, DNMT1)与信号素3F(semaphorin-3F, *SEMA3F*)的基因启动子的结合,从而降低了*SEMA3F*启动子的甲基化水平,提高了染色质在该区域的可及性,并促进了基因的表达。这些变化抑制了细胞的增殖和迁移,同时显著增强了前列腺癌对多西他赛化疗药物的敏感性^[26]。此外, RNA m⁶A修饰还能通过直接影响DNA甲基转移酶mRNA的稳定性,进而调控其在细胞内的表达水平,从而对DNA甲基化进行精细调控。研究发现,

DNMT1 mRNA上发生的m⁶A修饰会导致*DNMT1*转录本水平的上升。高表达的*DNMT1*通过上调DNA甲基化抑制了转录因子FOXO3a(forkhead box O3)的表达,继而降低了E-钙黏蛋白(E-cadherin)的表达水平并增加了N-钙黏蛋白(N-cadherin)的表达水平,促进了肿瘤细胞的上皮-间质转化(epithelial-mesenchymal transition, EMT),最终增强了细胞的侵袭和转移能力^[27]。上述两项研究都显示出抑制*DNMT1*在癌症治疗中的潜力,而近期的研究则发现*DNMT1*的强效抑制剂GSK3685032,能够与*DNMT1*的活性位点竞争,在体外诱导强效的DNA甲基化缺失、转录激活和癌细胞生长抑制。该药物克服了传统DNA甲基化抑制剂(如地西他滨)的细胞毒性,有着广阔的临床应用前景^[28]。综上所述,RNA m⁶A能够通过影响DNA甲基化调控染色质的开放状态,继而促进致癌基因的表达(图1)。

2.2 RNA m⁶A通过调控组蛋白修饰影响染色质开放性

组蛋白作为染色质基本单元——核小体的关键成分,与染色质的结构和功能状态紧密相关。在核小体内部,核心组蛋白展现出相似的整体结构,拥有一个球形且疏水的内部区域,这一区域构成了组蛋白的折叠核心。同时,这些组蛋白还具有从核小体中延伸出来的氨基末端,形成了灵活的突起结构。这些氨基末端(即所谓的N-端组蛋白尾部)包含大量的翻译后修饰,这些修饰在DNA复制、重组、修复和转录的调控中发挥关键作用^[29-30]。早在2015年,研究就已发现,长链非编码RNA XIST(X inactive

specific transcript)的m⁶A修饰在诱导组蛋白修饰过程中发挥着至关重要的作用^[31],提示RNA m⁶A与组蛋白修饰之间可能存在相互作用。近年来,随着表观遗传学研究技术的革新及分子机制探索的不断深入,RNA m⁶A与组蛋白修饰之间的相互作用机制逐渐被揭示。研究发现,在包括超级增强子产生的RNA(super-enhancer RNA, seRNA)在内的多种染色质相关RNA(chromatin-associated RNA, caRNA)上,存在广泛的RNA m⁶A修饰。然而,这些具有m⁶A修饰的caRNA是否在染色质重塑的过程中发挥重要调控作用,以及该作用对肿瘤发生发展所产生的影响,尚未被完全阐明。为此我们的研究团队进行了深入研究,发现胰腺癌细胞通过高表达RNA甲基转移酶METTL3的协同因子CFL1(cofilin-1),特异性促进seRNA m⁶A修饰的产生;同时,m⁶A读码蛋白YTHDC2(YTH domain containing 2)特异识别seRNA m⁶A修饰并招募组蛋白甲基转移酶MLL1(mixed lineage leukemia protein-1),促进相应染色质区域发生组蛋白H3第4位赖氨酸甲基化修饰。该研究揭示了CFL1-YTHDC2-MLL1表观调控轴在促进肿瘤染色质开放和癌基因表达中的重要作用^[32]。

此外,研究发现RNA m⁶A能够通过调节组蛋白修饰相关酶的mRNA稳定性来影响组蛋白修饰^[33-34]。RNA m⁶A阅读器YTHDC1在识别含m⁶A修饰的RNA后,能够共转录性地增强组蛋白去甲基化酶KDM3B(lysine demethylase 3B)的活性^[35]。另一项研究发现,YTHDC1(YTH domain containing 1)在识别m⁶A修饰的转座子RNA

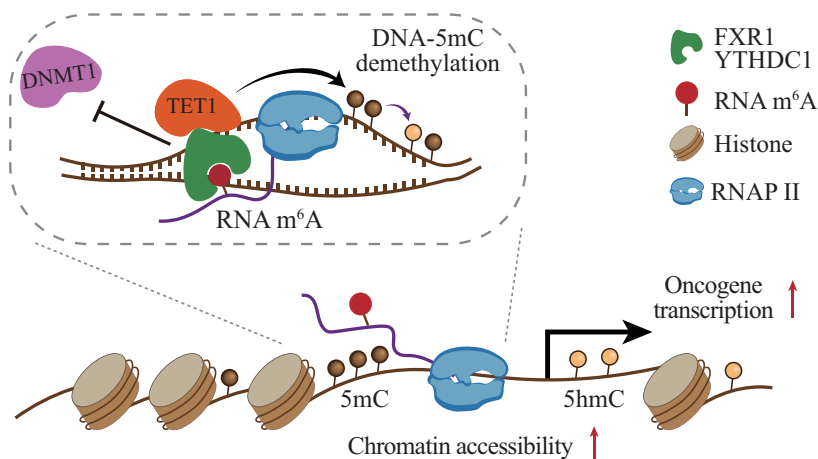


图1 RNA m⁶A通过影响DNA甲基化调控染色质状态

Fig.1 RNA m⁶A modulates chromatin status via affecting DNA methylation

IAP(intracisternal type A particle)衍生转录本时,能够促进METTL3与染色质的结合,进而影响组蛋白修饰。METTL3进一步与组蛋白H3第9位赖氨酸的三甲基化修饰(histone H3 trimethylation at Lys9, H3K9me3)的甲基转移酶SETDB1(SET domain bifurcated histone lysine methyltransferase 1)及其辅助因子TRIM28(tripartite motif containing 28)发生物理相互作用,协助其在IAP区域的定位,促进IAP区域异染色质标志物的沉积,从而维持IAP异染色质的完整性^[36]。

值得注意的是, RNA m⁶A在调控组蛋白修饰以及在多种癌症类型的发生和发展中扮演着关键角色,逐渐在广泛的研究中得到证实。RNA m⁶A甲基化酶METTL3通过介导组蛋白甲基转移酶SETMAR(SET domain and mariner transposase fusion gene) mRNA的m⁶A修饰,以IGF2BP3依赖的方式维持SETMAR mRNA的稳定性,从而调节SETMAR的表达水平。SETMAR通过在SWI/SNF相关、基质相关、肌动蛋白依赖的染色质调节因子亚家族A成员2(SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 2, SMARCA2)的启动子区域甲基化H3K36位点,增强SMARCA2的转录活性,进而促进甲状腺分化转录因子PAX8(paired box 8)和FOXE1(forkhead box E1)的表达,诱导癌细胞分化并降低其恶性程度。同时,相关科研人员利用METTL3-14-WTAP激活剂,成功增强了SETMAR和SMARCA2的表达,促进了甲状腺肿瘤细胞再分化,抑制了其侵袭和迁移,并在临床前模型中验证了该激活剂在甲状腺癌中的抗肿瘤活性^[37]。在胃癌中, METTL3介导的m⁶A修饰通过IGF2BP2依赖的方式增强了组蛋白甲基转移酶SUV39H2(suppressor of variegation 3-9 homolog 2)的mRNA稳定性,进而促进了SUV39H2的表达。SUV39H2通过抑制DUSP6(dual specificity phosphatase 6)的转录来提高DNA损伤修复关键激酶ATM(ataxia telangiectasia mutated)的磷酸化水平,从而抑制胃癌细胞对顺铂的化学敏感性^[38]。在非小细胞肺癌(non-small cell lung cancer, NSCLC)细胞中, METTL3通过甲基化KAT2A(lysine acetyltransferase 2A) mRNA的3'UTR中的m⁶A位点来稳定其表达,从而促进NSCLC细胞的恶性表型^[39]。类似地,在肺腺癌中, METTL也发挥着促癌作用。

METTL3的敲除减少了肺腺癌(lung adenocarcinoma, LUAD)细胞中SETD2(主要的H3K36me3甲基转移酶) mRNA的3'UTR中m⁶A位点的甲基化,从而增强了其RNA稳定性并上调了SETD2的表达水平。这导致VGF(VGF nerve growth factor inducible)基因启动子处H3K36me3修饰丰度的增加并导致VGF表达量减少,最终通过抑制PI3K/AKT/mTOR通路来抑制LUAD细胞的恶性表型^[40]。鉴于METTL3可以在多个方面影响肺癌的发展,近期的研究也聚焦到新型METTL3抑制剂STM2457上,发现在小细胞肺癌和非小细胞肺癌中, STM2457均可以增强癌症的化疗敏感性^[41-42], 同时还可以协同蛋氨酸腺苷转移酶2A(methionine adenosyltransferase 2A, MAT2A)抑制剂和免疫抑制治疗增强抗肿瘤作用^[43-44]。

除METTL3外, RNA m⁶A去甲基化酶同样在癌症细胞中对组蛋白修饰起到重要作用。在骨肉瘤中, ALKBH5(AlkB homolog 5)介导的m⁶A修饰水平降低引起去泛素化酶USP22(ubiquitin-specific peptidase 22)和RNF40(ring finger protein 40)的表达水平增加。这些蛋白通过与以DDB1-CUL4为主体的泛素E3连接酶复合物相互作用,影响其稳定性,抑制癌症细胞中H2A的泛素化并诱导关键原癌基因的表达,最终导致细胞增殖失控^[45]。类似地,在缺氧条件下, ALKBH5催化组蛋白去乙酰化酶HDAC4(histone deacetylase 4) mRNA的m⁶A修饰水平下降,导致m⁶A阅读器YTHDF2诱导的HDAC4 mRNA降解减少, HDAC4水平升高,最终促进胰腺癌细胞的增殖和迁移^[46]。目前已有使用小分子ALKBH5抑制剂辅助癌症治疗的相关实验报道^[47],但是该抑制剂在表观遗传网络中的具体作用和影响仍有待进一步探索。在宫颈癌中, FZD6(frizzled class receptor 6)在调控癌细胞的增殖、侵袭以及EMT过程中扮演着关键角色。RNA m⁶A去甲基化酶FTO(fat mass and obesity associated)减少了HOXC13反义RNA(HOXC13 antisense RNA, HOXC13-AS)上的m⁶A修饰,增强了其稳定性同时提高了其细胞内表达水平。HOXC13-AS通过cAMP反应元件结合蛋白(cAMP-response element binding protein-binding protein, CBP)调控组蛋白H3第27位赖氨酸乙酰化(H3K27ac), 增强FZD6的表达,从而增强癌细胞的恶性表型^[48]。综上所述, RNA m⁶A在读码蛋白的介导下能够影响多种组蛋

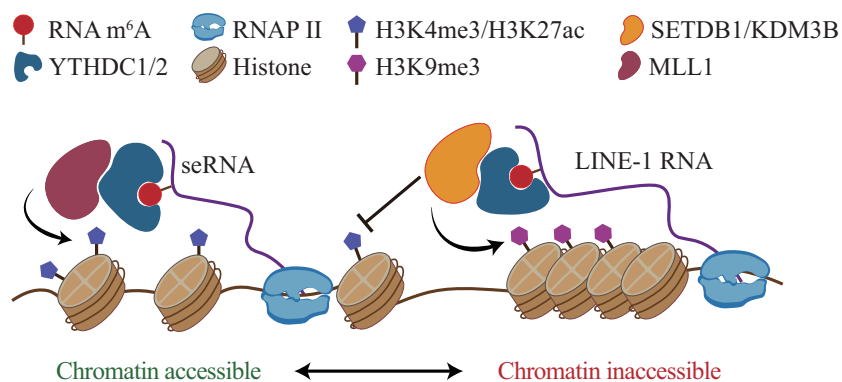


图2 RNA m⁶A通过影响组蛋白修饰调控染色质状态

Fig.2 RNA m⁶A modulates chromatin status via affecting histone modification

白修饰,继而调控肿瘤染色质的开放状态,最终影响癌症的发生与发展(图2)。

2.3 RNA m⁶A通过影响染色质三维结构调控染色质状态

染色质的三维结构是通过DNA和蛋白质复合物的多层次折叠实现的,这一结构不仅使庞大的DNA信息链得以压缩在狭小的细胞核内,还能通过形成拓扑相关结构域(topologically associating domains, TADs)将染色质划分为多个内部相互作用的区域。这种分区限制了顺式作用元件的调控范围,优化了基因表达的调控^[49]。染色质结构是动态变化的,能够持续影响调节因子与DNA之间的相互作用,并在表观遗传层面调节基因表达^[50]。染色质三维结构的调控受到多种因素(包括上文讨论的DNA甲基化、组蛋白修饰和长染色质环的形成等)的影响。因此, RNA m⁶A能直接或间接调控染色质三维结构的变化,进一步影响基因表达,并参与生理和病理过程。

R-loop是一种由DNA与RNA杂交形成的三链核酸结构。在这一结构中, RNA与DNA模板链配对,导致非模板DNA链被置换并形成环状结构。R-loop在基因表达调控、染色质结构维持、DNA复制及修复等关键生物学过程中扮演着重要角色^[51]。近期的研究通过R-loop印迹结合DNA测序方法揭示了细胞中大多数的RNA-DNA杂合物以R-loop的形式存在,说明了R-loop是DNA共转录过程中的重要结构^[52]。在基因组中, R-loop可以调节染色质修饰,如DNA去甲基化和组蛋白修饰,有助于其作为调控结构在正常基因表达中发挥作用。同时, R-loop已被证明可以通过稳定转录起始位点来调节基因表达,在具有

富含鸟嘌呤核苷酸的启动子序列的基因中更为明显^[53]。不仅如此, R-loop还在活化诱导胞苷脱氨酶(activation-induced cytidine deaminase, AID)介导的免疫球蛋白同种型转换中发挥作用^[54]。

研究报道指出, RNA m⁶A通过调节R-loop的积累影响染色质结构,其中, METTL3的下调会增加转录终止位点处R-loop的形成。值得注意的是,只有具有催化活性的METTL3才能逆转这一表型,揭示了m⁶A甲基化在RNAP II转录终止位点R-loop形成中的关键作用^[19]。此外, m⁶A阅读器YTHDF2与细胞分裂期间R-loop丰富的位点相互作用,促进其降解。YTHDF2的缺失会导致哺乳动物细胞中R-loop水平升高、细胞生长减缓,以及DNA双链断裂标记物 γ -H2AX累积^[55]。相反地, m⁶A修饰也可能稳定R-loop。含端粒重复序列RNA(telomeric repeat-containing RNA, TERRA)是一种从端粒区域转录的长非编码RNA,它能够通过与端粒DNA的嵌入式结合形成R-loop。研究发现, TERRA上的m⁶A修饰由METTL3催化,并由m⁶A阅读器YTHDC1识别和稳定。敲除METTL3或YTHDC1均能促进TERRA的降解。经m⁶A修饰的TERRA会形成R-loop,并促进同源重组,这对于癌细胞中的端粒替代性延长途径至关重要;而敲降METTL3则会导致R-loop减少、端粒缩短和基因组不稳定^[56]。另外, R-loop的形成也能够影响其他染色质调控途径。生长停滞和DNA损伤蛋白45A(growth arrest and DNA damage protein 45A, GADD45A)作为R-loop的阅读器,能够识别肿瘤抑制因子TCF21(transcription factor 21)基因启动子区域的R-loop,并招募去甲基化酶TET1到CpG岛区域,引发局部DNA去甲基化并促进TCF21的转录。值得

注意的是, 胚胎干细胞的基因组图谱揭示了CpG岛上上千个R-loop依赖的TET1结合位点^[57], 为探索TET1介导R-loop依赖的DNA甲基化重编程提供了重要的基因组学基础。除了在癌症发生和发展中的作用外, YTHDC1的缺失还能促进依赖于R-loop的RNA损伤诱导的DNA断裂(RNA damage-induced DNA breaks, RDIBs), 增加肿瘤对破坏核酸的化疗药物(如烷化剂)的敏感性^[58]。种种证据均提示我们R-loop是RNA m⁶A影响肿瘤病理的重要一环, 但目前其影响尚未被完全阐明, 有待后续进一步的探索补充。

2.4 RNA m⁶A间接影响染色质

RNA甲基化修饰广泛存在于细胞的众多转录本中, 并对多样的生理过程及信号转导路径产生重要影响^[3]。RNA甲基化复合体不仅直接参与染色质重塑, 还通过作用于caRNA等因子和信号通路, 间接调控染色质的结构与功能。这一过程进一步实现了染色质重塑, 精细调控了基因表达, 并在生理和病理过程中扮演着关键角色, 尤其是在癌症的发生与发展中。研究发现, 在小鼠胚胎干细胞中, 包括LINE-1在内的caRNA发生m⁶A修饰后被YTHDC1识别, 后者通过招募核外切体靶向(nuclear exosome targeting, NEXT)复合物, 促进靶caRNA降解, 导致染色质开放性受限并抑制转录^[59]。此外, YTHDC1与其靶标RNA结合后还可以作用于SETDB1, 促进组蛋白H3第9位赖氨酸三甲基化(H3K9me3)修饰, 从而抑制逆转录元件和双细胞期(2C)样程序的主诱导因子Dux的激活^[60]。

在癌症中对类似的间接调控也有相应的研究, 例如上文提到的FTO通过HOCX13-AS1间接调节组蛋白乙酰化, 促进癌症恶性表型。在口腔上皮细胞癌中, RNA甲基转移酶METTL14表达上调, 提高非编码RNA MALAT1的m⁶A修饰丰度并使其细胞内水平上调; MALAT1与miR-224-5p结合增强组蛋白赖氨酸脱甲基酶2A(histone lysine demethylase 2A, KDM2A)的mRNA稳定性, 从而实现了RNA甲基化对染色质的间接调控, 促进了肿瘤细胞的增殖^[61]。SUCLG2-AS1是一种与鼻咽癌转移和放射敏感性相关的长非编码RNA, 研究人员发现RNA m⁶A也可通过影响SUCLG2-AS1间接改变染色质结构, 具体的机制是: METTL3介导m⁶A修饰以IGF2BP3依赖的方式增强SUCLG2-AS1的稳定性, 提高其表达水平, 随后SUCLG2-AS1通过形成SOX2(SRY-box transcrip-

tion factor 2)基因的增强子和启动子区域之间的长距离染色质环介导CTCF(CCCTC-binding factor)对SOX2增强子和启动子区域的占据, 从而调控SOX2的表达, 促进NPC转移和放疗抵抗^[62]。

值得注意的是, RNA甲基化改变到染色质重塑这个调控通路的中间环节还涉及代谢的重编程。在肾透明细胞癌(clear cell renal cell carcinoma, ccRCC)中, TCF7L2是转录因子HIF2 α 介导的经典Wnt通路发挥调控作用不可或缺的关键基因, 可以促进ccRCC的转移, HIF2 α 通过转录调控METTL3的表达, 继而以m⁶A依赖的方法增强TCF7L2 mRNA的稳定性。TCF7L2的上调增强了细胞脂肪酸氧化, 最终促进了组蛋白乙酰化, 增强了ccRCC的侵袭性^[63]。无独有偶, 在肺癌中, m⁶A修饰的SLC7A5 mRNA以依赖m⁶A阅读器IGF2BP2的方式增强自身稳定性和翻译活性, 导致SLC7A5介导的蛋氨酸转运增强以产生S-腺苷甲硫氨酸, 这通过进一步增加组蛋白H3第4位赖氨酸三甲基(H3K4me3)修饰来影响IGF2BP2启动子区域, 上调IGF2BP2表达, 形成正反馈环路, 最终通过AKT/mTOR通路引起肺癌放射治疗抵抗^[64]。还有一种有趣的间接调节方式是m⁶A-eRNA在增强子中招募核m⁶A阅读器YTHDC1, 使其相分离成液态凝结物, 随后与溴结构域蛋白4(bromodomain-containing protein 4, BRD4)辅激活剂凝聚物共同混合, 促进基因的激活, YTHDC1的缺失导致增强子位点的BRD4减少, 影响增强子位点的染色质结构和转录调控^[65]。

总之, 上述这些研究为我们揭示了RNA m⁶A如何通过细胞庞大生理运行网络与染色质重塑相联系(表1), 为我们认识细胞复杂生理调控提供了新的角度。同时我们也需要更多研究去深入地探索相应的潜在机制, 尤其是癌症发生发展的相关分子机制方面, 以帮助我们更好地精细了解癌症失调的细胞调控网络。

3 染色质调控RNA m⁶A修饰

m⁶A被写入到新生的转录本中, 这一过程可能通过DNA甲基化、组蛋白修饰以及染色质的三维结构等多维度表观调控方式影响染色质重塑。然而RNA m⁶A修饰与染色质的相互作用并不是单向的, 染色质也能够通过调控RNA m⁶A相关因子的表达及功能作用影响m⁶A(表2)。

表1 RNA m⁶A对肿瘤染色质重塑的调控作用Table 1 Regulatory function of RNA m⁶A modification on chromatin remodeling in cancer

调控方式	因子	机制	对染色质的影响	在肿瘤中的作用	文献
Regulatory approach	Factor	Mechanisms	Impact on chromatin	Role in tumors	Reference
DNA methylation	TET1	Upon recognizing RNA m ⁶ A, m ⁶ A reader FXR1 recruits TET1 to genomic loci to demethylate DNA	Open	Promote proliferation and migration	[25]
	DNMT1	The m ⁶ A reader IGF2BPs prevents DNMT1 from binding to the promoters of target genes and inhibits DNA methylation	Open	Inhibit proliferation and migration, and enhance chemosensitivity	[26]
	DNMT1	METTL3 mediates the m ⁶ A methylation of <i>DNMT1</i> mRNA and facilitates DNA methylation	Closure	Increase activity and invasiveness	[27]
Histone modification	SETMAR	The m ⁶ A reader IGF2BP3 augments the expression of <i>SETMAR</i> mRNA, leading to the methylation of histone H3K36	Open	Promote cell differentiation	[37]
	SUV39H2	IGF2BP2 stabilizes <i>SUV39H2</i> mRNA, therefore increases ATM phosphorylation by transcriptional repression of <i>DUSP6</i>	Closure	Reduce cisplatin sensitivity	[38]
	KAT2A	METTL3 stabilizes KAT2A expression by catalyzing the m ⁶ A site in the 3'UTR of <i>KAT2A</i> mRNA	Open	Promote malignant phenotypes	[39]
	SETD2	METTL3 methylates the m ⁶ A site within the 3'UTR of <i>SETD2</i> mRNA, leading to downregulation of SETD2. This, in turn, decreases H3K36me3 modification at the <i>VGF</i> promoter and ultimately promotes VGF expression	Open	Promote malignant phenotypes	[40]
	DDB1-CUL4	ALKBH5-mediated m ⁶ A deficiency results in elevated expression of USP22 and RNF40, impacts the stability of the DDB1-CUL4-based ubiquitin E3 ligase complex, and suppresses H2A ubiquitination	Open	Promote proliferation	[45]
	HDAC4	ALKBH5 mediates the reduction of m ⁶ A modification in <i>HDAC4</i> mRNA, which lessens the degradation induced by m ⁶ A reader YTHDF2 and subsequently decreases histone acetylation levels	Closure	Promote proliferation, migration	[46]
	CBP	FTO increases the intracellular level of HOXC13-AS by downregulating its m ⁶ A modification. Meanwhile, HOXC13-AS1 enhances FZD expression through the regulation of histone H3K27ac by CBP	Open	Promote malignant phenotypes	[48]
	MLL1	YTHDC2 identifies the m ⁶ A methylation sites on seRNA and recruits MLL1 to co-transcriptionally facilitate the modification of H3K4me3	Open	Promote the occurrence and development	[32]
Three-dimensional structure	TERRA	YTHDC1 can recognize and stabilize m ⁶ A-modified TERRA, triggering R-loop formation and facilitates homologous recombination repair	Stabilization	Increase activity	[56]
	TET1	Upon recognizing the R-loop at the promoter site of <i>TCF21</i> , the R-loop reader GADD45A recruits TET1 to the CpG island region, leading to local DNA demethylation and upregulation of TCF21	Open	Suppress malignant phenotypes	[57]
	DNMT1	IGF2BPs recognize the R-loop in an m ⁶ A-dependent manner. Therefore, they impede DNMT1 from binding to the <i>SEMA3F</i> promoter, which in turn blocks DNA methylation	Open	Inhibit proliferation and migration, enhance chemosensitivity	[26]
	THOC	The m ⁶ A reader YTHDC1 inhibits R-loop-dependent RNA damage-induced DNA breaks	Stabilization	Reduce chemosensitivity	[58]

续表1

调控方式	因子	机制	对染色质的影响	在肿瘤中的作用	文献
Regulatory approach	Factor	Mechanisms	Impact on chromatin	Role in tumors	Reference
Indirect regulation	SMARCA2	m ⁶ A modification stabilizes <i>SETMAR</i> mRNA. The methylation of the <i>SMARCA2</i> promoter by SETMAR promotes SMARCA2 transcription and augments the function of the chromatin remodeling SWI/SNF complex where SMARCA2 is involved	Open	Promote cell differentiation	[37]
	KDM2A	METTL14 mediates m ⁶ A modification of MALAT1, leading to upregulation of MALAT1. Subsequently, MALAT1 binds to miR-224-5p to promote KDM2A transcription and histone demethylation	Open	Promote proliferation	[61]
	SUCLG2-AS1	IGF2BP3 promotes the stability of SUCLG2-AS1 in an m ⁶ A-dependent manner. Meanwhile, SUCLG2-AS1 enables CTCF to occupy the enhancer and promoter regions of <i>SOX2</i> by forming long-distance chromatin loops between these regions	Open	Promote metastasis and radioresistance	[62]
	Acetyl-CoA	<i>TCF7L2</i> mRNA stability is enhanced in an m ⁶ A-dependent manner. Its upregulation boosts fatty acid oxidation and promotes histone acetylation	Open	Increase invasiveness	[63]
	SAM	<i>SLC7A5</i> mRNA enhances its stability and translational activity relying on m ⁶ A reader IGF2BP2. It mediates increased methionine transport to generate SAM, thereby augmenting H3K4me3 modification	Open	Promote radioresistance	[64]

3.1 染色质调控RNA m⁶A相关因子的表达

染色质重塑在调控多种基因表达中扮演着关键角色,其中包括那些涉及RNA m⁶A修饰的酶以及读码蛋白。在一项基于癌症基因组图谱(The Cancer Genome Atlas, TCGA)的综合性分析中发现,58个DNA甲基化探针(DNA methylation probes, DMPs)所指示的甲基化位点参与了RNA m⁶A调节因子的表达调控,其中7个位点的甲基化改变可促进肿瘤的发生和发展^[66]。另一项关于甲状腺癌的生物信息学分析也发现,RNA m⁶A甲基转移酶*FTO*的启动子DNA甲基化和拷贝数变异可能导致*FTO*水平下调,进而影响甲状腺癌的TP53通路^[67]。在*KRAS*突变、*LKB1*缺失的肺癌中,发现*LKB1*缺失会通过*ALKBH5*启动子上CTCF结合基序的DNA超甲基化上调*ALKBH5*的表达,最后通过依赖于m⁶A阅读器YTHDF2的途径稳定SOX2、SMAD7和MYC等致癌驱动因子的转录本,增强肺癌恶性表型^[68]。类似地,除DNA甲基化外,组蛋白修饰等染色质重塑途径也可以影响RNA m⁶A修饰相关酶的表达^[69-74]。组蛋白赖氨酸特异性去甲基化酶KDM5B和KDM5C均可以介导组蛋白H3第4位赖氨酸三甲基(H3K4me3)的去甲基化,从而抑制*METTL14*的转录,进而促进非小细胞肺癌和结直肠

癌的恶性表型^[69,73]。乙酰化水平也影响*METTL14*的转录,在黑色素瘤中,组蛋白去乙酰化抑制剂(histone deacetylation inhibitors, HDACi)可恢复*METTL14*的正常组蛋白乙酰化水平,从而诱导其表达;随后,*METTL14*以YTHDC1依赖的方式促进肿瘤抑制因子*FAT4*的表达,从而发挥肿瘤抑制作用^[74]。除了*METTL14*外,*METTL3*的表达同样受组蛋白修饰的调控。转录因子ETS1募集P300和WDR5,它们分别介导*METTL3*启动子中的H3K27ac和H3K4me3组蛋白修饰并诱导*METTL3*转录激活,促进宫颈癌的增殖转移^[70],人乳头瘤病毒(human papillomavirus, HPV)来源的E7癌蛋白转录因子E2F1介导的H3K27ac和H3K4me3组蛋白修饰激活*ALKBH5*的表达,使*PAK5* mRNA去甲基化,增强其稳定性,促进宫颈癌细胞的增殖转移^[71]。

影响染色质结合蛋白,也是改变染色质可及性进而影响RNA m⁶A相关因子转录的一种途径。聚腺苷二磷酸核糖聚合酶1(poly ADP-ribose polymerase 1, PARP-1)是参与DNA损伤修复和转录调节的关键酶,转录因子核因子I-C(nuclear factor I C, NFIC)和TATA结合蛋白(TATA-binding protein, TBP)依赖PARP1接近*METTL3*启动子区域激活*METTL3*的转录,辐照或者使用PARP1抑制剂后,PARP1从

表2 肿瘤染色质重塑对RNA m⁶A的调控作用

Table 2 Regulatory function of chromatin remodeling upon RNA m⁶A modification in cancer

调控方式 Regulatory approach	因子 Factor	机制 Mechanisms	对m ⁶ A的影响 Impact on m ⁶ A	在肿瘤中的作用 Role in tumors	文献 Reference
Expression of m ⁶ A methylation factors	FTO	The DNA methylation of the <i>FTO</i> promoter, along with copy number variants, results in the downregulation of FTO levels and impacts the TP53 pathway	Increased	Promote malignant phenotypes	[67]
	ALKBH5	Deletion of LKB1 leads to upregulation of ALKBH5 via DNA hypermethylation of the CTCF-binding motif within the <i>ALKBH5</i> promoter. Moreover, it stabilizes oncogenic drivers like SOX2, SMAD7 and MYC mediated by YTHDF2	Decreased	Promote malignant phenotypes	[68]
	METTL14	The demethylation of H3K4me3 mediated by KDM5C suppresses METTL14 transcription, markedly diminishes the m ⁶ A modification of <i>SOX4</i> mRNA and augments SOX4 expression	Decreased	Promote malignant processes	[69]
	METTL14	KDM5B down-regulates METTL14 expression in an H3K4me3-dependent manner and subsequently up-regulates LINC02747 levels to regulate PI3K/Akt and CDK4/Cyclin D1 signaling pathways	Decreased	Promote malignant processes	[73]
	METTL14	The histone deacetylation inhibitor (HDACi) is capable of restoring normal histone acetylation levels and triggering the expression of METTL14. Subsequently, METTL14 upregulates FAT4 in a YTHDC1-dependent manner	Increased	Inhibit malignant processes	[74]
	METTL3	The transcription factor ETS1 recruits P300 and WDR5, which mediate H3K27ac and H3K4me3 histone modifications in the <i>METTL3</i> promoter and induce METTL3 transcriptional activation	Increased	Promote proliferation and metastasis	[70]
	ALKBH5	HPV-derived E7 oncoprotein transcription factor E2F1 mediates histone modifications of H3K27ac and H3K4me3, which activate the expression of ALKBH5, demethylate <i>PAK5</i> mRNA and bolster its stability	Decreased	Promote proliferation and metastasis	[71]
	METTL3	NFIC and the TATA-binding protein TBP activate METTL3 transcription by entering the <i>METTL3</i> promoter in a PARP1-dependent manner	Increased	Promote radiore-sistance	[75]
	METTL3	Mediated by the histone modifying enzymes SMYD2 and EP300, the modifications of H3K27ac and H3K4me3 govern the overexpression of LINC01605. Thereafter, the overexpressed LINC01605 attaches to the METTL3 protein and promotes the m ⁶ A modification of <i>SPTBN2</i> mRNA	Increased	Promote carcinogenesis	[82]
Functions of m ⁶ A methylation factors	METTL3	Elevated histone H3K4me1 and H3K27ac modifications trigger the activation of LINC00969 expression. This, in turn, enhances its interaction with METTL3, and subsequently inhibits NLRP3 expression and the activation of the classical heat shock signaling pathway in an m ⁶ A-YTHDF2-dependent manner	Increased	Endow an anti-pyrototic phenotype and promoting TKI resistance	[83]

*METTL3*启动子染色质上脱离, 导致NFIC和TBP对*METTL3*启动子区域的可及性减弱, 从而抑制METTL3的表达和RNA的m⁶A甲基化, 进而影响肿瘤的发展^[75]。除影响RNA m⁶A的编写器和擦除器外, 染色质重塑也可以通过调控RNA m⁶A阅读器的表达进而

影响RNA m⁶A修饰后续作用的发挥, 在头颈部鳞状细胞癌中, YTHDF1的染色质区域开放性明显增加, 可能通过诱导SOX2、TP63的结合而促进*YTHDF1*的转录, 进而影响该m⁶A阅读器的作用, 从而影响肿瘤微环境中的免疫细胞浸润^[76]。需要注意的是, 有

些改变染色质状态的酶或蛋白质可能通过非经典途径,在不影响染色质的情况下调节RNA甲基化。研究显示RNA去甲基化酶FTO可以通过减少胃癌细胞中MYC的m⁶A甲基化稳定MYC mRNA,增强胃癌细胞的迁移和侵袭活性;转录因子FOXA2与FTO基因启动子结合并降低了其表达水平,而组蛋白去乙酰化酶3(histone deacetylase 3, HDAC3)则通过降解FOXA2维持FTO-MYC mRNA及其后续信号转导通路的稳定,增强胃癌细胞的恶性表型^[77]。

3.2 染色质影响RNA m⁶A相关因子的功能

m⁶A甲基化转移酶复合体在许多启动子和增强子位点附近催化RNA m⁶A修饰的写入活动,通过招募m⁶A阅读蛋白YTHDC1来抑制Integrator复合体与新生RNA的结合,保护新生RNA免受Integrator复合体介导的终止^[78],而染色质对此具有调节作用。另一项研究发现,组蛋白H3K36me3促进m⁶A甲基化酶复合体结合到相邻的RNAP II,将m⁶A甲基化酶复合体传递给活跃转录的新生RNA以共转录地写入m⁶A修饰,促进肝癌细胞的恶性表型^[79]。此外,补体反应性增强子结合蛋白(tonicity-responsive enhancer binding protein, TonEBP)能识别由紫外线或喜树碱(camptothecin, CPT)等DNA损伤剂产生的R-loop,将METTL3募集到R-loop上促进RNA m⁶A甲基化^[80]。无独有偶, RNA螺旋酶DDX21也可通过识别R-loop,从而募集METTL3到染色质促进RNA中m⁶A沉积^[81]。

除此之外,组蛋白还可以通过调控非编码RNA的表达来调节RNA甲基化酶的活性,并且这与各型癌症的发生发展以及耐药有着密切关系。据报道,组蛋白修饰酶SMYD2-EP300介导的H3K27ac和H3K4me3修饰调控LINC01605的过表达,随后LINC01605与METTL3蛋白结合,促进SPTBN2 mRNA的m⁶A修饰,从而促进结直肠癌的发生^[82]。组蛋白H3K4me1和H3K27ac修饰水平的升高可激活LINC00969的表达,增强其与METTL3的相互作用,继而METTL3通过m⁶A-YTHDF2依赖的方式抑制NLRP3的表达,抑制NLRP3/caspase-1/GSDMD相关的经典热休克信号通路的激活,从而赋予肺癌抗热休克表型并促进TKI耐药^[83]。

4 总结与展望

综上所述,越来越多的研究证据表明,RNA

m⁶A与染色质之间存在密切的联系。RNA m⁶A与转录的耦合能够通过影响DNA甲基化、组蛋白修饰以及染色质的空间结构等重塑染色质的开放性;同时染色质状态的改变也能通过影响RNA修饰酶、读码蛋白的表达及活性等调控RNA m⁶A。在肿瘤的发生、进展及治疗反应中,RNA m⁶A与染色质的互作发挥着至关重要的作用。在肿瘤起始阶段,它可能通过精细调控关键基因的表达,为肿瘤细胞的增殖与存活奠定基础;在肿瘤进展过程中,影响细胞的侵袭与转移能力,助力肿瘤的扩散;在治疗阶段,通过改变肿瘤细胞对药物的敏感性,对治疗效果产生决定性影响。然而,这种互作是否仅作为一种相对固定的作用模式,还是在不同肿瘤背景、发展阶段及外界刺激条件下,存在差异化的机制,仍是一个亟待解答的科学问题。不同肿瘤类型可能因基因突变、微环境差异等因素,导致RNA m⁶A与染色质的互作模式各异;在肿瘤的早期与晚期阶段,由于细胞状态、代谢活动等变化,互作机制也可能发生转变;外界刺激如药物、辐射等,通过改变细胞内的信号通路,可能重塑RNA m⁶A与染色质的互作关系,进而影响肿瘤细胞的生物学行为。靶向调控RNA m⁶A与染色质互作的关键蛋白,为肿瘤治疗提供了新的研究方向。理论上,精准干预这些关键蛋白,有望打破RNA m⁶A与染色质之间的不良互作,抑制肿瘤的恶化与发展,提升治疗效果。但在实际应用中,仍面临诸多挑战。基础研究需深入挖掘关键蛋白的具体作用机制,明确其在不同肿瘤类型和阶段中的功能差异;临床实践则需开展严谨的试验,评估靶向药物的安全性、有效性和可行性。在表观遗传调控的复杂网络中,关键酶如METTL3的双重身份也增加了研究的复杂性。METTL3在肿瘤中的作用并非一成不变,其调控的基因表达具有多样性,可能在某些情况下促进肿瘤发展,而在另一些情况下发挥抑制作用。如何精准调节其活性,克服耐药性等问题,仍需要科研人员的持续探索。因此,拓展对各癌种中RNA m⁶A与染色质互作的认识,深入探究其在不同情境下的具体作用和机制,对于寻找精准靶点、指导临床治疗具有重要意义。这不仅有助于提升治疗效果,还能为癌症患者带来更个性化的治疗方案,提高生存率和生活质量。

尽管RNA m⁶A甲基化修饰与染色质之间的双向调控及其对肿瘤的影响已现端倪,但在表观遗传

调控网络的复杂背景下,仍存在广阔的未知领域,亟待研究人员探索。表观遗传网络涵盖DNA甲基化、组蛋白修饰、RNA修饰、染色质重塑等多元调控机制,它们彼此交织、协同运作,维系基因表达的动态平衡。RNA m⁶A与染色质的互作只是这一网络中的一个环节,其他潜在的调控机制和相互作用亟待发掘。深入研究表观遗传网络,将为癌症的诊疗提供更全面的理论支持,助力发现新的标志物、开发靶向药物、制定联合治疗方案,推动癌症研究迈向新台阶,为战胜癌症带来新的希望。

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