RNA修饰调控造血发育和白血病发生的研究进展

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摘要 胚胎造血发育和成体造血稳态维持是复杂有序、高度保守的过程, 受到诸多因素, 包括细胞内转录因子、信号通路及表观遗传修饰和细胞外造血微环境的精密调控。RNA修饰将表观修饰从转录水平提升到转录后精确调控的新层面, 参与调控多项重要生命过程, 其在血液发育及白血病发生中的调控作用已有多项研究。本文系统总结了RNA修饰在胚胎造血发育、稳态维持及白血病发生中的功能和分子机制, 不仅有助于完善目前对造血系统发育以及正常造血和恶性转化的认识, 而且为寻找潜在的白血病治疗靶点提供了新思路。

关键词 RNA修饰, RNA m⁶A甲基化, 胚胎造血发育, 造血稳态维持, 白血病发生

自1960年首次在酵母RNA中发现假尿嘧啶修饰至今, 研究人员在RNA中发现了170多种化学修饰^[1,2], 常见的RNA表观修饰包括N¹和N⁶-腺嘌呤甲基化(m¹A, m⁶A, m⁶A_m)、3-和5-胞嘧啶甲基化(m³C, m⁵C)、5-羟甲基胞嘧啶(5-hydroxymethylcytosine, hm⁵C)、假尿嘧啶(Ψ)以及RNA核糖2'-O-甲基化(2'-O-methylation, Nm)^[3]。技术的进步是人们认识RNA修饰的前提。最初, 研究人员将RNA水解为单个核苷酸, 通过色谱、光谱、质谱技术分析其化学性质, 并鉴定RNA修饰, 但是这些方法费时费力且不能准确定量^[4,5]。液相色谱-质谱/质谱联用(liquid chromatography-tandem mass spectrometry, LC-MS/MS)可以精确地定量分析特定RNA修饰, 却不能反映序列信息^[6]。自2012年开始, m⁶A测序(m⁶A-seq)、m⁶A单核苷酸分辨率交联与免疫

沉淀测序(m⁶A individual-nucleotide-resolution cross-linking and immunoprecipitation sequencing, miCLIP-seq)、RNA甲基化免疫共沉淀测序(methylated RNA immunoprecipitation sequencing, meRIP-seq)以及m⁵C重亚硫酸盐测序(Bis-seq)等深度测序技术广泛应用于RNA修饰研究中^[7-10], 这些方法能够定性并定量地检测和鉴定RNA修饰, 同时结合对修饰酶及识别蛋白的研究^[11], RNA修饰的多种生物学功能逐渐被发掘和阐明。

血液系统是机体的重要组成部分之一, 胚胎造血发育和成体造血稳态维持受到多种因素的精密调控, 调控失衡可能导致造血发育异常甚至是血细胞的恶性转化。关于血液系统的调控机制, 以往的研究多集中于转录因子、信号通路和造血微环境等因素, 表观遗传

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修饰尤其是RNA修饰在血液系统发育及白血病发生中的调控作用逐渐成为血液研究领域新的研究方向。本文系统总结了以RNA m⁶A甲基化为主的RNA修饰在胚胎造血发育、稳态维持和恶性转化过程中的功能及调控机制。

1 RNA m⁶A甲基化修饰在正常造血和白血病发生中的作用

RNA m⁶A甲基化是mRNA上分布最广泛的可逆修饰,由甲基转移酶复合体(writer)与去甲基化酶(eraser)分别催化,m⁶A经特定RNA结合蛋白(reader)识别,调控RNA命运,执行不同的生物学功能^[3,12~14]。METTL3(methyltransferase-like 3)、METTL14(methyltransferase-like 14)和WTAP(Wilms tumor 1-associated protein)是m⁶A甲基转移酶复合体的核心组分,其中METTL3和METTL14分别发挥催化和辅助RNA底物结合的作用^[15~17],WTAP可以维持复合体稳定性^[18~20]。后续有研究报道了复合体中的其他组分,包括VIRMA(KIAA1429)^[19,21],RBM15^[21],CBLL1(HAKAI)^[21,22]和ZC3H13^[21,23,24]。与甲基转移酶作用相反的是m⁶A去甲基化酶ALKBH5^[25]和FTO^[26],RNA m⁶A甲基化修饰的移除具有组织、应激和疾病特异性^[12]。m⁶A识别蛋白主要包括含有YTH结构域的YTHDC蛋白和YTHDF家族^[27~29],它们识别RNA m⁶A甲基化修饰,影响RNA代谢,进而调控特定的生物学功能,包括生殖细胞发育^[30]、胚胎发育和干细胞分化^[31]、脂肪生成^[32]、肿瘤发生^[33]以及造血系统发育和恶性转化等。

1.1 RNA m⁶A甲基化修饰在胚胎期造血及成体造血稳态维持中的功能

造血干祖细胞(hematopoietic stem and progenitor cell, HSPC)最早产生于胚胎期主动脉-性腺-中肾(aorta-gonad-mesonephros, AGM)区域,由特化的动脉内皮细胞——生血内皮,经过内皮生血转化(endothelial-to-hematopoietic transition, EHT)而来^[34~36]。在斑马鱼胚胎中,抑制内源mettl3,降低m⁶A水平,可影响内皮生血转化过程,导致HSPC产生减少。机制研究证明,m⁶A修饰通过Ythdf2介导notch1a mRNA稳定性,以维持内皮生血转化过程中内皮细胞和造血细胞基因表达的平衡,进而调控造血干细胞命运决定^[37]。同样的机制在

小鼠中得到验证,特异性敲除内皮细胞Mettl3导致HSPCs产生数量减少、功能削弱^[38]。这两项研究证明了在脊椎动物胚胎期造血发生过程中m⁶A甲基化对HSPC命运决定发挥关键作用(图1)。

m⁶A甲基化在成体造血中发挥至关重要的调控作用。小鼠成体HSCs中高表达Mettl3和Mettl14^[39~41]。几项独立研究显示,Mxl-Cre; Mettl3^{fl/fl}条件性敲除鼠的m⁶A水平降低,HSCs比例增高^[41~43],HSC自我更新相关基因表达下降,而细胞周期相关基因表达增加,促使其进入细胞周期,进而导致HSCs聚集^[42,43]。除了影响HSCs静息状态,敲除Mettl3或Mettl14都能使HSCs的移植重建功能受损^[42,43]。条件性敲除Ythdf2后,m⁶A修饰的mRNA稳定性增加,如Tall1, Gata2等,它们与HSC自我更新相关,可促进HSCs扩增,但不影响其分化和移植能力^[44]。m⁶A水平变化会影响HSC分化能力。敲除Mettl3后,Myc mRNA稳定性及翻译水平降低,导致造血干细胞或多能祖细胞分化受到阻滞,髓系分化能力削弱,终末分化细胞减少^[41,43]。同时,m⁶A修饰对各系血细胞行使不同的功能。应用Lysm-Cre特异敲除髓系细胞Mettl3,髓系细胞的数量和功能不受影响^[41]。m⁶A甲基化的水平改变能影响淋巴细胞的增殖和分化,特异性敲除初始T细胞中Mettl3会导致SOCS家族基因的mRNA和蛋白水平增加,抑制STAT5激活及T细胞增殖和分化^[45]。综上,mRNA m⁶A甲基化修饰影响成体HSCs的静息状态、移植重建、扩增和分化能力(图1)。

以人脐带血来源的HSPCs为对象进行的研究也证明了m⁶A甲基化修饰对造血稳态维持的重要性,然而体外实验与在体实验的结论却不尽相同。在人脐血HSPCs中敲低METTL3降低m⁶A水平,HSPCs的增殖能力受抑制,髓系分化能力增强^[39];同样,敲低METTL14也会促进人脐血HSPCs向髓系分化^[40];而抑制YTHDF2表达,则能促进HSPCs扩增且不影响其功能^[44]。这与上文提到应用Mxl-Cre/loxP系统在小鼠体内敲除Mettl3或Mettl14,HSCs比例增加、髓系分化能力削弱的表型相反。以上表型差异可能是由于在体内生理条件下,HSPCs稳态维持与微环境因素密切相关。Mxl-Cre虽然是一种造血研究常用的Cre转基因鼠,但Mxl-Cre不仅在造血干祖细胞中表达,还可在间充质干细胞、NESTIN⁺细胞、血管周细胞中诱导而发挥作用^[46];并且最近研究证明,METTL3能调节间充质干细

图 1 RNA m^6A 甲基化修饰调控胚胎造血发育、稳态维持及恶性转化

Figure 1 RNA m^6A methylation in the regulation of embryonic hematopoiesis, homeostatic maintenance and leukemogenesis

细胞的命运^[47]，因此这些基质细胞有可能受到 m^6A 甲基化水平变化的影响，从而影响HSPCs稳态；同时，体外培养的HSPCs对细胞因子反应性更强，而对基质细胞依赖性更小^[48]。因此，体内外实验结果的差异可能与HSPCs所处微环境不同有关。

1.2 RNA m^6A 甲基化修饰在白血病发生中的作用

恶性造血表现为造血细胞异常发育、异常增殖以及分化阻滞。RNA m^6A 甲基化修饰不仅在HSC产生、增殖和血细胞分化中起调节作用，其在白血病的发生发展中也扮演重要的角色。目前有关 m^6A 甲基化修饰在恶性造血中的研究主要集中于急性髓系白血病 (acute myeloid leukemia, AML)。

与健康人 $CD34^+$ HSPCs及肾透明细胞癌等其他系统肿瘤相比，*METTL3*在AML细胞系和病人样本中的表达丰度高^[39]。两项独立实验表明，*METTL3*介导的 m^6A 甲基化在AML中起作用^[39,49]。在小鼠AML细胞、不同人AML亚型细胞系及AML病人样本中，应用RNA干扰或CRISPR/Cas9(clustered regularly interspaced

short palindromic repeats/CRISPR-associated protein 9) 方法下调*METTL3*的表达，能够抑制AML细胞的生长增殖，诱导其向髓系分化；将敲除或敲低*Mettl3*的白血病细胞移植到免疫缺陷鼠时，小鼠生存时间延长^[39,49]。同时，敲低*Mettl3*会导致白血病细胞凋亡增加，却不影响正常HSCs生存，暗示了 m^6A 修饰可以作为AML的治疗靶点^[39]。机制研究中，Kharas研究组^[39]揭示了在人白血病细胞系MOLM-13转录组中 m^6A 修饰富集于凋亡及MYC相关基因的转录本中。 m^6A 水平降低能抑制*c-MYC*、*BCL2*和*PTEN* mRNA的翻译，导致AML细胞凋亡；同时PTEN表达降低导致AKT磷酸化水平升高，这可能是AML细胞向髓系分化增强的原因。而Kouzarides研究组^[49]发现，CEBPZ结合蛋白招募*METTL3*到靶基因转录起始位点，介导靶基因(如原癌基因*SP1*、*SP2*)mRNA m^6A 修饰，增强其翻译效率，促进AML发生(图1)。*METTL14*同样高表达于多种亚型AML病人骨髓以及AML细胞系中，敲低*METTL14*能促进白血病细胞的髓系分化能力，延缓受体鼠白血病发病时间并提高生存率。机制上，髓系分化关键因子SPI1负向调控

METTL14,而在AML细胞中*SPI1*表达下调、*METTL14*表达升高,通过下游*MYC*和*MYB*介导AML细胞增殖和自我更新,而敲低*METTL14*导致*MYC*和*MYB* mRNA的稳定性和翻译受损,影响AML细胞的生存^[40](图1)。以上三项研究显示,m⁶A修饰影响AML的病理过程,但相应的机制却不同。究其原因,一方面可能是研究所用的RNA修饰测序技术、抗体、实验条件不同^[50,51];另一方面,m⁶A修饰变化涉及到整个转录组,并不可尽数归因于特定转录本,例如m⁶A甲基化修饰调控原癌基因*SPI1*,*MYC*,*MYB*转录本的翻译,而*MYB*和*MYC*同时又是*SPI1*的下游靶基因^[52,53],所以下调m⁶A所导致的AML表型可能是原癌基因表达直接变化和上下游基因调控的综合结果^[49]。因此,进行转录组与表观转录组学的整合分析是必要的。

YTHDF2的相关研究同样说明,白血病发生发展与RNA m⁶A甲基化修饰相关。Paris等人^[54]发现,YTHDF2高表达于多种AML细胞中,且能促进AML发生发展,YTHDF2通过识别RNA m⁶A修饰介导mRNA降解,负向调控肿瘤坏死因子*TNFRSF1B*,敲除YTHDF2则引起肿瘤坏死因子表达水平上升,促使白血病前体细胞响应凋亡信号,从而导致AML细胞减少,同时研究发现敲低YTHDF2促进正常HSC扩增。因此,在AML中靶向YTHDF2促进白血病干细胞凋亡的同时,不影响甚至增强了正常HSCs的增殖能力,可作为AML治疗的理想方案^[54](图1)。

m⁶A去甲基化酶FTO和ALKBH5对白血病发生的影响比较复杂。首先,CRISPR遗传筛选显示,敲除ALKBH5和FTO对多种白血病细胞生存没有明显影响^[39]。后续有报道证明,FTO仅在一些特殊类型的AML细胞中高表达,尤其是发生t(11q23)/MLL重排的AML细胞,从而促进白血病发生^[55]。另一项研究中,添加FTO抑制剂——R-2-羟基戊二酸(R-2-hydroxyglutarate, R-2-HG)可增加白血病细胞中m⁶A修饰的整体水平,降低致癌基因*MYC/CEBPA*转录本的稳定性,从而抑制白血病细胞的生存、增殖能力,发挥抗白血病作用^[56]。其次,近来也有报道阐明,FTO可介导mRNA 5'端2'-O-二甲基腺嘌呤(m⁶A_m)的去甲基化,而对m⁶A的作用很微弱^[57],那么之前观察到FTO促进AML发生的表型可能不只依赖于m⁶A修饰,其对m⁶A_m修饰的调控也可能参与其中,这些还需要进一步实验证明。之前关于ALKBH5在AML中的作用研究较少,而最近的两

项报道阐明了ALKBH5作为m⁶A去甲基化酶特异性调控AML发生发展的作用机制。两项研究均发现,ALKBH5在多种不同亚型的AML患者骨髓样本以及白血病干细胞中高表达,且其表达水平与病人不良预后呈正相关,敲低或敲除ALKBH5后,显著抑制了AML发生发展,同时白血病干细胞功能受损,而正常造血过程却不受影响^[58,59]。机制方面,研究发现白血病干细胞中ALKBH5编码区域染色体开放程度显著增加,表达水平上调,并通过影响下游*AXL* m⁶A修饰调控白血病干细胞功能^[58]。另一项研究鉴定出ALKBH5主要影响下游*TACC3* mRNA的稳定性,调控AML发生发展^[59](图1)。

2 其他RNA修饰与白血病发生

在正常和恶性造血领域,RNA m⁶A甲基化修饰的作用得到了较为广泛的研究,而关于其他种类RNA修饰的报道较少,且相对集中于构成亚细胞结构的非编码RNA,如rRNA的假尿嘧啶修饰、tRNA的m⁵C甲基化修饰。针对每一种特定修饰的研究都有助于整体理解RNA表观修饰在血液系统的作用。

假尿嘧啶修饰是最丰富的RNA修饰^[60,61],真核rRNA假尿嘧啶修饰几乎都是通过box H/ACA RNPs催化而来^[60,61],其中催化组件CBF5(也称作DKC1)发生突变会导致X染色体连锁的先天性角化不良疾病^[62],患者肿瘤易感性增加,并出现造血衰竭的症状^[63,64]。DKC1突变通过影响rRNAs假尿嘧啶修饰导致患者CD34⁺造血干细胞分化能力受损^[65],同时小鼠研究证实Dkc1突变影响核糖体进入位点(internal ribosome entry site, IRES),从而干扰抑癌蛋白p27、抗凋亡蛋白XIAP和Bcl-xl的翻译,表现出造血衰竭表型^[66]。

m⁵C修饰存在于多种类型的RNA,如tRNA, rRNA, mRNA等^[67]。已报道的m⁵C甲基转移酶包括DNMT2^[68]和NOL1/NOP2/sun 1-7(NSUN1-7)家族成员^[69],其中DNMT2介导Asp, Gly, Val tRNA C38的m⁵C修饰,其作用与翻译的准确性有关,影响造血干祖细胞的数量和分化能力^[68]。5-氮杂胞苷(5-azacitidine, 5-AZA)作为一种去甲基化药物,被广泛应用于血液肿瘤治疗中。最近研究发现,5-AZA耐受的AML患者、骨髓增生异常综合征(myelodysplastic syndromes, MDS)患者骨髓样本中mRNA m⁵C水平高于敏感患者;

进一步研究发现, NSUN1与BRD4结合会形成对5-氮杂胞苷耐受的染色质结构. BRD4抑制剂以及下调NSUN1的表达能逆转白血病细胞对5-AZA的耐药性, 但该研究中并没有证据显示NSUN1对染色质结构的影响是m⁵C修饰介导的^[70].

3 结论和展望

在造血发育和其他多种生物学过程中, 基因表达调控机制的研究已经拓展到表观转录组层面. 以m⁶A甲基化为主的RNA修饰, 通过影响关键基因mRNA稳定性或翻译效率等方式, 调控脊椎动物胚胎期造血、成体造血稳态以及白血病发生发展. 相关研究进一步丰富了造血系统发育、血细胞恶性转化的机制. 但由于RNA类型及修饰种类繁多, 血液系统中转录后修饰的研究还处于起步阶段, 仍有许多空白等待填补.

血液系统恶性肿瘤类型较多, 目前有关RNA m⁶A修饰调控恶性血液系统疾病发生发展的研究集中于AML, 未见其影响其他类型白血病的报道, 这可能与m⁶A相关修饰酶在AML白血病细胞中特异性高表达有关^[39,40]. 弥漫大B细胞淋巴瘤中METTL3的表达水平较高^[39], 且有生物信息分析研究根据风险比将10个m⁶A调控因子(YTHDF3, METTL3, FTO等)分为套细胞淋巴瘤有利基因和有害基因两类, 并将两类基因表达水平的比值定义为m⁶A指数, 分析发现低m⁶A指数与套细胞淋巴瘤患者的不良生存呈正相关^[71], 但并没有研究探索m⁶A修饰调控淋巴瘤的机制. 关于其他类型RNA修饰调控异常造血过程的研究也较少, 因此RNA修饰对其他恶性血液系统疾病发生的影响亟待探索.

尽管目前尚未有报道介绍其他类型mRNA修饰与造血调控的关系, 但这些修饰的其他重要生物学功能逐渐被发掘. 例如, NSUN2催化的mRNA m⁵C修饰不仅影响细胞周期相关蛋白(P27, CDK1)的翻译, 参与细

胞生长^[72]和复制性衰老等过程^[73], 还能通过促进ICAM-1的翻译进而影响血管内皮细胞的炎症反应^[74]; mRNA m⁵C识别蛋白YBX1(也称作YPS), 在斑马鱼和果蝇中分别参与母源合子转换和生殖细胞发育过程^[75-77]. 在mRNA中, 假尿嘧啶修饰能使终止密码子(UAA, UGA, UAG)转变为有义密码子^[78], 并且在热休克处理或营养不足等条件下, 假尿嘧啶修饰的变化会影响mRNA的稳定性^[60,79]. 前体mRNA(pre-mRNA)的核糖Nm修饰可影响其剪切过程^[80], Nm修饰影响mRNA与tRNA的相互作用进而调控翻译过程^[81]; 而在mRNA 5'端附近的Nm修饰是“自我”的标志, 一些病毒通过此方式参与免疫逃避^[82]. 这些mRNA修饰能否参与造血调控是值得系统研究的问题.

目前绝大多数关于RNA修饰生物学功能的研究均是以特定类型RNA上的某种化学修饰为对象, 那么不同种类核酸之间的修饰是否存在协同调控呢? 最新一项研究发现, TRMT10A可以协同调控mRNA和tRNA甲基化, 其通过与FTO相互作用影响mRNA m⁶A水平, 并且甲基化的tRNA能优先作用于TRMT10A-FTO依赖的mRNA, 从而发挥功能^[83]. 这一发现证明, 不仅某一种RNA修饰水平的变化会影响下游调控网络, 不同种类核酸之间的协同调控也具有重要的生物学功能. 生物体中是否存在更多种类的协同调节, 以及不同类型RNA修饰的协同调节机制能否调控造血过程, 都为造血领域的研究留出了无限探索的空间.

在应用基础研究中, 已经有报道表明, 靶向FTO去甲基化酶的小分子抑制剂FB23-2在体外白血病细胞系、体内小鼠模型中均显示出抗白血病作用^[84], 这暗示有关RNA修饰调控造血的分子机制具有向临床转化的潜能. 以RNA修饰酶、识别蛋白为靶点, 探索促使HSC体外扩增、抑制白血病细胞增殖和降低白血病细胞耐药性的机制, 将成为转化医学的新着眼点.

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RNA modifications in the regulation of hematopoiesis and leukemogenesis

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Embryonic and adult hematopoiesis is an intricate and highly conserved process, which is regulated by cell-intrinsic transcription factors, signal pathways and epigenetic modifications, and cell-extrinsic factors in hematopoietic microenvironment. RNA modification has been identified as a new layer of epigenetics via post-transcriptional regulation and is required for pivotal biological functions. Recently, growing number of studies have demonstrated that RNA modification acts as a key regulator in hematopoietic processes. In this review, we summarize the functions and underlying mechanisms of RNA modifications in the regulation of embryonic hematopoiesis, homeostatic maintenance and leukemogenesis at the epi-transcriptome level, which will not only shed new light on our understanding of developmental hematopoiesis and the relationship between normal and malignant hematopoiesis, but also provide potential therapeutic targets for leukemia treatment.

RNA modification, RNA m⁶A methylation, embryonic hematopoiesis, hematopoietic homeostasis, leukemogenesis

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