

组蛋白H3K36甲基化与心血管发育及疾病的关系

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摘要: 哺乳动物心脏发育受心脏特异基因转录组以空间/时间的方式精确编码调控。表观遗传因素在心肌细胞的基因转录过程中发挥重要的调控作用。在这些因素中, 组蛋白甲基化是真核生物重要的表观遗传修饰之一。其中组蛋白H3第36位赖氨酸(H3K36)的甲基化修饰与基因的转录激活相关, 标记了染色质结构中的开放区域。H3K36甲基化的稳态受多种酶和表观因子的协同影响, 包括组蛋白甲基转移酶(也称“writer”)、组蛋白去甲基化酶(也称“eraser”)和甲基化组蛋白结合因子(也称“reader”)。H3K36甲基化在神经系统、脂肪组织、心脏及其他器官的发育中起重要作用。H3K36甲基化还参与心脏纤维化、肺动脉高压、心肌肥厚和腹主动脉瘤等多种心血管疾病的发生发展进程。本文综述了组蛋白H3K36甲基化在心血管疾病发生发展中的作用, 重点介绍了H3K36甲基转移酶和H3K36去甲基化酶在心血管疾病发生发展中的作用。

关键词: H3K36; 组蛋白甲基转移酶; 组蛋白去甲基化酶; 心血管发育; 心血管疾病

Relationship between histone H3K36 methylation and cardiovascular development and diseases

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Abstract: Heart development is regulated by the transcriptome of cardiac-specific genes encoded in a spatial/temporal precise manner in mammals. Epigenetic factors play an critical role in regulating gene transcription in cardiomyocytes. Among these factors, histone methylation is one of the important epigenetic modifications in eukaryotes. Methylation of the 36th lysine of histone H3 (H3K36) is associated with the transcriptional activation of genes and marks the open regions of the chromatin. The homeostasis of H3K36 methylation is synergistically influenced by various enzymes and regulators, including histone methyltransferases (also called “writer”), histone demethylases (also called “eraser”) and methylated histone binding factors (also called “reader”). H3K36 methylation plays an essential role in the development of the nervous system, adipose tissue, heart and other organs. In recent years, numerous studies have shown that H3K36

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methylation is also involved in the development of cardiovascular diseases such as cardiac fibrosis, pulmonary hypertension, myocardial hypertrophy and abdominal aortic aneurysm. In this paper, we reviewed the role of histone H3K36 methylation in cardiovascular development and diseases, focusing on the role of H3K36 methyltransferases and demethylases in the development of cardiovascular diseases.

Key Words: H3K36; histone methyltransferases; histone demethylases; cardiovascular development; cardiovascular diseases

心血管发育异常等心血管疾病是人类生存面临的不可忽视的问题。众所周知, 环境因素和个人生活方式在心血管发育和疾病发生中起重要作用, 但这些外部风险因素与遗传学之间的联系尚不清楚。然而, 表观遗传学的提出帮助我们在一定程度上解释了在基因组DNA序列没有发生突变的情况下基因转录调控与环境因素之间的联系。多种表观遗传过程包括DNA甲基化、组蛋白修饰、非编码RNA调控和染色质重塑等, 都会影响基因的表达, 从而使细胞分化和功能发生重要变化, 进而影响生物体的健康。组蛋白修饰在心血管发育和疾病, 如先天性心脏病、肺动脉高压、心力衰竭、心肌肥厚、高血压和动脉粥样硬化等的发生过程中发挥了重要作用。

组蛋白修饰是表观遗传机制的一个重要方面。常见的组蛋白翻译后修饰(post-translational modification, PTM)包括: 甲基化(methylation)、磷酸化(phosphorylation)、乙酰化(acetylation)、糖基化(glycosylation)和泛素化(ubiquitination)等, 它们可以调节DNA和组蛋白之间的亲和力以改变染色质结构(核小体包装的松弛或紧缩)或作为基因表达的调节剂^[1]。其中, 甲基化是常见的组蛋白PTM之一。

组蛋白(主要是H3)甲基化主要发生在赖氨酸(Lysine, K)、精氨酸(Arginine, R)和组氨酸(Histidine, H)残基的侧链上。在赖氨酸的甲基化修饰中, H3K4、H3K36和H3K79的甲基化通常与基因的转录激活相关, 而H3K9、H3K20和H3K27的甲基化一般与基因的转录抑制相关^[2]。同样的, 同一赖氨酸残基的不同甲基化状态, 如单甲基化(mono-methylation, me1)、双甲基化(di-methylation, me2)和三甲基化(tri-methylation, me3), 在染色质状态和基因转录调控方面可能扮

演不同角色。

H3K36甲基化能参与多个关键细胞生物学过程并在多种人类疾病中发挥重要作用, H3K36甲基化的调控作用逐渐引起研究者的广泛关注。H3K36单甲基化(H3K36me1)被认为是中间态, 并不发挥主要作用; 而双甲基化(H3K36me2)和三甲基化(H3K36me3)的作用和分布并不相同^[3]。H3K36me3存在于基因转录区域, 而H3K36me2存在于基因间区域, 两者均位于开放的染色质区域^[3]。目前科学家已通过对组蛋白修饰的质谱分析估算出不同器官之间的组蛋白修饰的相对丰度, 未甲基化或双甲基化的H3K36丰度最高, 可达到H3总量的50%左右, 而H3K36me1和H3K36me3只有5%左右^[3,4]。虽然已有大量文章阐明了参与H3K36甲基化的特定分子, 但是关于H3K36甲基化的生成、移除、下游调控的分子机制及生物学意义仍亟待解答。本文将概述最近的研究发现并介绍H3K36甲基化修饰在调控心血管发育和疾病发生发展中的重要作用。

1 H3K36甲基化的调控酶和识别因子

H3K36甲基化受不同类型酶和表观因子的调控, 根据其发挥的不同作用可将其分为“writer”“eraser”和“reader”。所谓的“writer”是催化组蛋白甲基化反应的酶, 即组蛋白甲基转移酶(histone methyltransferase, HMTs), 而“eraser”则执行相反的功能, 介导甲基的去除, 即组蛋白去甲基化酶(histone demethylases, HDMs), 它们共同维持组蛋白甲基化和非甲基化状态之间的动态平衡(图1)。第三类“reader”蛋白, 即甲基化组蛋白结合因子, 能够识别特定的修饰并与之相互作用。

1.1 “Writer”蛋白

H3K36的HMTs包含一个SET结构域, 并通过

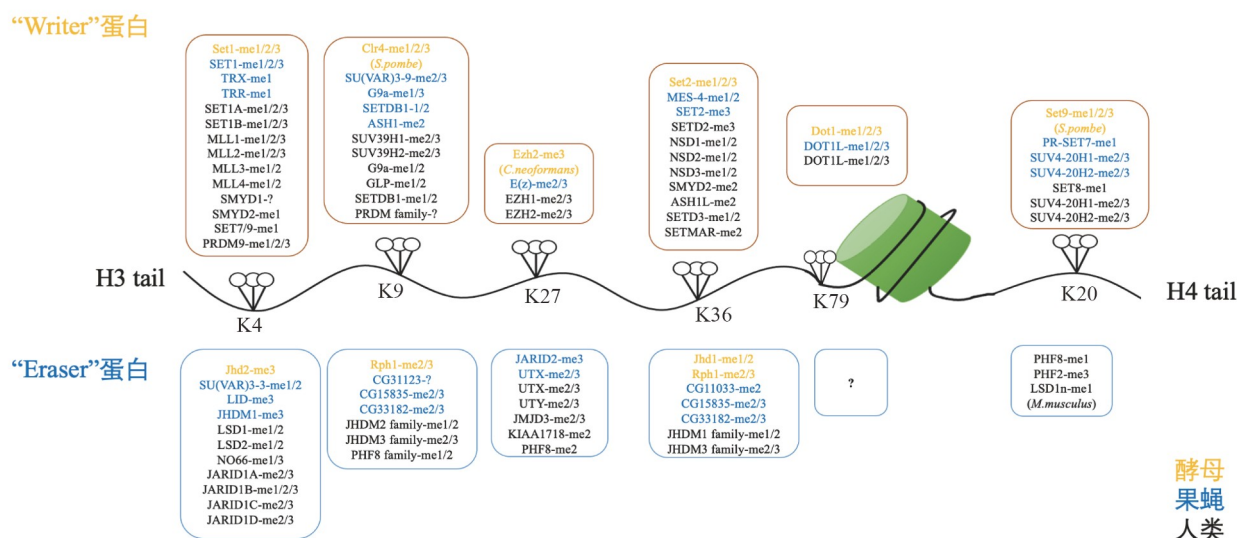


图1 组蛋白H3和H4上的主要赖氨酸甲基化位点及其HMTs和HDMs^[5]

S-腺苷甲硫氨酸向组蛋白H3K36位点转移甲基^[6]。H3K36甲基化是一个进化保守的过程。在酵母中,只有一种H3K36甲基转移酶,即SET2,能够参与从单甲基化到三甲基化的每个步骤^[7,8];在哺乳动物中,不同甲基化状态需要多种HMTs的参与。大部分的HMTs能够催化H3K36甲基化直至达到双甲基化状态,而其中只有一个成员能够催化最后一步使其达到三甲基化状态。

核受体结合SET结构域蛋白1(nuclear receptor binding SET domain protein 1, NSD1)最初被定义为类固醇激素核受体^[9],且已被证实具有催化H3K36和H4K20甲基化的作用^[10]。NSD1对H3K36具有特定的单甲基和双甲基化酶活性,产生H3K36me1和H3K36me2^[11-13]。在其他酶催化的H3K36三甲基化过程中也证实NSD1的活性具有关键作用,可能因为NSD1可以为后续反应提供适量的底物^[14]。然而,当使用组蛋白八聚体作为底物时,NSD1还可以使组蛋白H4以及组蛋白H2A和H2B发生甲基化^[15]。

在同一家族中,核受体结合SET结构域蛋白2[nuclear receptor binding SET domain protein 2, NSD2,又称为MMSET(multiple myeloma SET domain)、WHSC1(Wolf-Hirschhorn syndrome candidate 1)]突变会引起Wolf-Hirschhorn综合征(WHS)^[16],因参与WHS的染色体缺失而首次被鉴定。NSD2存在两种主要亚型,一种具有催化活

性,另一种由于没有SET结构域,导致该亚型无甲基转移酶活性^[17]。与NSD1类似,NSD2也可以催化H3K36的单甲基化和双甲基化^[10,18],然而最近的研究表明,它更倾向于催化双甲基化而不是单甲基化的发生^[19,20]。除此之外,NSD2还靶向H4K20、H3K4和H3K27^[10,21-24]。这些差异可能是由于实验中使用的底物蛋白不同造成的。如NSD2与核小体相互作用时主要充当H3K36双甲基化酶,而与组蛋白八聚体共同作用时,NSD2优先对H4K44进行双甲基化^[6]。NSD2与核小体的结合会使核小体出口处连接区域的DNA打开,从而使NSD2的催化结构域插入到开放的DNA区域与组蛋白八聚体之间^[14]。NSD2的作用机制是通过识别H3K36的PWWP(Pro-Trp-Trp-Pro)结构域中已经被双甲基化的区域来发挥作用的。这种结构域不仅能稳定染色质上的蛋白质,还能将甲基转移酶引导到染色质的相邻区域并发挥作用。因此,与NSD1相比,NSD2更多的是发挥将甲基化延展的功能,而不是介导从头开始甲基化的甲基转移酶^[10,25,26]。

通过搜索与NSD1和NSD2具有序列相似性的基因,Angrand等^[27]发现了核受体结合SET结构域蛋白3(nuclear receptor binding SET domain protein 3, NSD3)。有研究结果证实,NSD3在脑、心脏和骨骼肌中均有表达,而在肝和肺中表达较低^[28,29]。NSD3有长型、短型和WHISTLE三种亚型。长(1 437 aa)、短(645 aa)两种亚型具有相同的

氨基末端序列, 但短型缺乏具有催化活性的SET结构域, 只能结合已发生甲基化的H3K36^[30,31]。WHISTLE是包含一个活跃的SET结构域的最短亚型(506 aa), WHISTLE的活性与H3K4和H3K27的甲基化及转录抑制相关^[10,31]。

除NSD家族外, 还有其他一些酶可以将H3K36催化为H3K36me1或/及H3K36me2。目前已经发现, 缺失、小的、同源异形1蛋白(absent, small or homeotic 1-like, ASH1L), SET结构域和mariner转座酶融合基因(SET domain and mariner transposase fusion gene, SETMAR), SET结构域蛋白3(SET domain-containing protein 3, SETD3)和SET-MYND结构域蛋白2(SET and MYND domain containing protein 2, SMYD2)都是调控H3K36甲基化的甲基转移酶。这些酶对于H3K36不具有高特异性, 可以识别其他底物^[32-35]。例如ASH1L对H3K36me2具有甲基转移酶活性^[36], 但是ASH1L也靶向H3K4并定位在活跃靶基因的转录区域内^[37]。在没有ASH1L的情况下, 生物体内H3K4me3水平降低。同时也有研究表明, ASH1L还针对H4K20^[38]。除了靶向H3K36外, SMYD2还可以催化H3K4和非组蛋白底物(如p53和视网膜母细胞瘤蛋白)的甲基化^[39,40]。尽管SMYD2主要在大脑和心脏中表达, 但是在小鼠心肌细胞中条件性敲除SMYD2并不影响心脏发育, 同时它的缺失对H3K36甲基化整体水平没有任何影响^[41]。

到目前为止, SET结构域蛋白2(SET domain-containing protein 2, SETD2)是哺乳动物细胞中已知的唯一H3K36三甲基转移酶^[42]。类似于其酵母同源物SET2, SETD2包含一个SET结构域, 并保留了与RNA聚合酶II(RNA polymerase II, Pol II)大亚基B1的相互作用^[43]。在转录延伸过程中, SETD2能够通过结合Pol II的CTD“尾巴”被招募到染色质上, 在核小体H3K36位点产生三甲基化修饰(H3K36me3)。这一修饰通过招募下游的效应因子在转录延伸、RNA剪接、DNA甲基化以及DNA损伤修复等过程中发挥关键调节作用^[44]。SETD2通过与Pol II的大亚基RBP1的相互作用将H3K36me3与转录延伸结合起来^[45,46]。细胞体外和动物体内实验敲低SETD2都表明仅H3K36me3水平发生下降^[47,48]。

1.2 “Eraser” 蛋白

生物体为了在组蛋白甲基化和未甲基化状态之间保持平衡, 必须具有可以从特定残基上去除甲基化修饰的酶, 即HDMs。这些酶属于Jumonji家族, 因为它们含有必需的JmjC(Jumonji C)催化结构域^[49,50]。

酵母中有两种能够去除H3K36甲基化的赖氨酸去甲基化酶(Lysine demethylase, KDM)——KDM2(JHD1)和KDM4(RPH1)^[49,50]; 而在哺乳动物中, H3K36的去甲基化是通过两个蛋白质家族——JHD1的直系同源物KDM2A/2B和RPH1的直系同源物KDM4A/4B/4D实现的。KDM2A位于细胞核中, 通过ZF-CXXC结构域与未甲基化的CpG DNA结合^[51], 对于维持异染色质稳态至关重要^[52]。作为一种表观遗传调节因子, KDM2A的表达和生物学功能受多种外部因素的影响。p300可以在K409位直接乙酰化KDM2A, 进而减少H3K36me2的去甲基化并增强p21和PUMA的转录, 从而抑制骨肉瘤的生长和转移^[53]。轻度葡萄糖饥饿通过AMPK信号通路诱导KDM2A介导的H3K36me2去甲基化, 从而降低rRNA转录和乳腺癌细胞的增殖^[54]。H3K36me1/me2去甲基化由KDM2A/2B完成^[52], 而KDM4A/4B/4D对H3K36me3具有特异性, 但也能够作用于H3K9me2/me3^[55,56]。

组蛋白甲基化的“writer”蛋白和“eraser”蛋白共同维持组蛋白甲基化和非甲基化两种状态之间的动态平衡。图2描述了不同甲基化状态之间的转换, 突出显示了在哺乳动物中发现的能够催化和去除H3K36甲基化的酶。

1.3 “Reader” 蛋白

H3K36甲基化对于协调染色质中的核小体功能至关重要。为了执行这个功能, H3K36信号必须被染色质辅因子识别。在H3K36甲基化的情况下, “reader”蛋白通常包含以下结构域之一: Tudor、PWWP或Chromo结构域^[4]。如前所述, 许多表观遗传修饰的“writer”蛋白也可以作为“reader”蛋白来确保特异赖氨酸残基甲基化的完成。

除了已经提到的NSD家族外, 还有许多其他蛋白质, 包括DNA甲基化转移酶(DNA methyltransferase, DNMT) DNMT3A和DNMT3B、

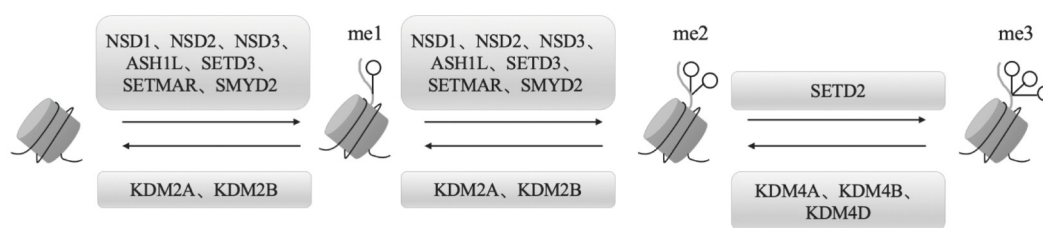


图2 哺乳动物中催化和去除H3K36甲基化的酶^[57]

参与DNA修复的酶(mutS homolog 6, MSH6和lens epithelium-derived growth factor, LEDGF)和H3.3K36me3特异性“reader”蛋白——含MYND结构域11锌指蛋白(zinc finger mynd-type containing 11, ZMYND11)都包含PWWP结构域^[4]。植物同源结构域指蛋白1(plant homeodomain finger 1, PHF1)和植物同源结构域指蛋白19(plant homeodomain finger 19, PHF19)都包含Tudor结构域,与多梳抑制复合物2(polycomb repressed complex 2, PRC2)相互作用促进H3K36me3^[58,59]。H3K36me3的“reader”蛋白MORF相关基因15(MORF-related gene 15, *MRG15*)包含一个Chromo结构域,可以与其他表观遗传修饰因子(如KDM5A)相互作用,以维持转录区域H3K4甲基化的减少,确保转录延伸状态^[60]。

SET结构域蛋白7(SET domain-containing protein 7, SETD7)最初被鉴定为H3K4me1的甲基转移酶^[61],可参与调节DNA甲基化、染色质重塑和基因转录等多个过程^[61-63]。SETD7通过介导各种蛋白质赖氨酸残基甲基化调节细胞生长、凋亡和氧化应激。但近期研究表明,SETD7与H3K36me3、H3K36me2、H3K36me1、H3K36ac和未修饰的H3K36具有较强的亲和力^[64]。此外,心肌细胞中的免疫共沉淀实验证实了SETD7和H3K36me3之间的相互作用,表明心肌细胞中的SETD7和H3K36me3之间存在强相互作用^[64]。

2 H3K36甲基化与心脏发育

心脏是胚胎发生过程中最早形成和发挥功能的器官之一,它的发育形成涉及复杂的生物学过程^[65,66]。心脏发育包括心肌细胞特化、分化和心脏形态发生,这些过程由一组核心心脏转录因子以时间和空间的方式进行调节。心脏转录因子和组

蛋白甲基化修饰酶之间的相互作用在心脏发育过程中起重要作用。发育期和成年期组蛋白甲基化修饰酶的异常表达或突变可导致胚胎死亡或先天性心脏病,并影响成年心脏对病理性应激的反应。现阶段多数研究主要围绕H3K36 HMTs和HDMs对心脏发育的影响展开,因此本文综述了部分与HMTs和HDMs相关的心脏发育缺陷研究。

2.1 HMTs与心脏发育

NSD2在H3K36甲基化过程中发挥多重功能,既可以介导H3K36me2促进转录激活,还可以通过H3K36me3调节RNA切割修饰和DNA损伤修复^[20]。在生物学功能上,NSD2通过H3K36me3调控的下游基因 $NKX2.5$ 调控心脏发育和心肌细胞分化^[67]。WHS是由4号染色体短臂部分缺失引起的,其特征是面部畸形、先天性心脏缺陷、智力发育障碍和先天性膈疝(congenital diaphragmatic hernia, CDH)风险增加^[68,69]。其中,先天性心脏缺陷包括房间隔和心室中隔缺损(atrial septal defect/ventricular septal defect, ASD/VSD)^[69],主要由NSD2的缺失引起^[68,69]。 $NSD2^{-/-}$ 小鼠在胚胎期第15.5天可检测到H3K36me3水平降低,小鼠表现出生长迟缓并在10天内死亡,伴有ASD和VSD的发生^[70]。这些结果表明,NSD2缺失会导致多种间隔缺陷,包括WHS中出现的心脏病变,并导致小鼠在出生后死亡^[67]。除此之外,NSD2介导的H3K36甲基化在脂肪组织发育和功能中也起关键作用。Zhuang等^[71]的研究虽然没有探明NSD2或H3K36甲基化对心脏发育的影响,但其文章中使用的H3.3K36M突变体小鼠可作为研究H3K36甲基化的重要工具。

已有文献报道,全身敲除SETD2小鼠在胚胎期第10.5天左右胚胎致死,但SETD2在心脏中的作用尚不明确。Chen等^[72]利用Mesp1-Cre对多种心血管细胞谱系,如心肌细胞、血管内皮细胞和心内

膜细胞特异性地敲除心血管系统中的*SETD2*基因。实验结果表明, 敲除*SETD2*显著降低了胚胎心脏中H3K36me3的水平, 但不降低H3K36me1和H3K36me2的水平。同时, *SETD2*缺乏会严重阻碍冠状血管形成并导致心肌致密化不全, 最终导致胚胎致死。通过染色质免疫共沉淀后测序(chromatin immunoprecipitation-seq, ChIP-seq)和mRNA-seq转录组测序表明, 心脏相关基因R-脊椎蛋白3(recombinant R-spondin 3, *RSPO3*)和富含纤维连接蛋白亮氨酸跨膜蛋白2(fibronectin leucine rich transmembrane protein 2, *FLRT2*)的表达会因为*SETD2*缺失而下调^[72]。总之, 该研究证明, *SETD2*是胚胎心脏中H3K36me3的关键调节因子, 并通过调节冠状血管形成在心脏发育中发挥关键作用。

在心肌细胞分化过程中, *SETD7*通过靶向不同的基因集驱动其阶段特异性表达。*SETD7*在这些阶段与不同的辅助因子有关, 如在中胚层形成过程中与SWI/SNF染色质重塑因子和心脏祖细胞中的转录因子*NKX2.5*共同驱动其分化^[64]。RNA-seq中的基因分类(gene ontology, GO)分析结果也显示, *SETD7*下调与早期心脏发育途径相关, 如心室心肌组织发育和心室心肌组织形态发生^[64]。同时, *SETD7*在其靶基因内部结合甲基化的H3K36, 以促进Pol II转录^[64]。异常的*SETD7*表达损害了终末分化的心肌细胞功能属性^[64]。*SETD7*和H3K36me3在基因组重叠区域与心脏分化过程中的基因激活高度相关, 表明*SETD7*识别H3K36甲基化并调节靶基因的转录, 但敲低或敲除*SETD7*对其靶基因的H3K36me3水平没有影响, 说明*SETD7*将H3K36残基识别为结合靶标, 但不识别为甲基化的底物蛋白。

2.2 HDMs与心脏发育

F-box和富含亮氨酸的重复蛋白11(F-box and leucine-rich repeat protein 11, *FBXL11*)也称为KDM2A或JHDM1A(JmjC-domain-containing histone demethylase), 是富含JmjC结构域的HDMs。*FBXL11*与*FBXL10*具有相似的结构, 对H3K36me1和H3K36me2具有组蛋白去甲基化酶活性。*FBXL11*^{-/-}小鼠在胚胎期第10.5~12.5天时部分出现胚胎致死, 且存活胎鼠伴有严重的生长迟缓。

解剖学结果发现, *FBXL11*^{-/-}小鼠胚胎心脏虽然形成了正常结构, 但心脏形态较小并出现心脏循环缺陷^[73]。

含有JmjC结构域的核仁蛋白质66(nucleolar protein 66, NO66)也是一种HDMs, 且对H3K4和H3K36具有特异性。NO66对小鼠发育过程中的成骨以及成年小鼠的骨稳态起重要作用, 其通过调节骨骼发育过程、控制前成骨细胞/成骨细胞的数量以及骨形成相关基因的表达来调节骨形成。在Prx1-Cre;*No66*^{fl/fl}小鼠中, H3K4me3和H3K36me3水平增加, 但是鉴于H3K4和H3K36甲基化水平可能受多种其他HDMs的影响, 现在仍不确定NO66是否影响其他HDMs, 也不确定NO66缺失介导的H3K4me3和H3K36me3增加是否是细胞自主改变的^[74]。心肌是一类特殊的骨骼肌, 虽然作者并未在文中探究NO66对心脏发育的影响, 但其研究结果对后续研究NO66与心脏发育之间的关系具有重要的意义。

3 H3K36甲基化与血管发育

由动脉、静脉和相互连接的毛细血管网组成的血管系统是在胚胎发生过程中通过血管生成或血管再生形成的。维持血管生物稳态对机体健康至关重要, 一旦这种平衡被破坏, 就会导致心血管系统发育不良或心血管疾病的发生^[75-80]。表观遗传机制在这些过程中发挥了不可或缺的作用, 一些已发表的综述总结了部分与血管生物学相关的表观遗传调控, 包括非编码RNA、DNA甲基化、蛋白质乙酰化和磷酸化^[81,82]。在本部分中, 我们着重关注H3K36的HMTs与血管发育之间的联系。

胚胎血管的形成是哺乳动物胚胎发育最重要的过程之一, 这一过程出错将引起生长迟缓、心包肿胀等现象, 并导致胎儿在早期器官形成期间在胚胎内死亡^[83]。亨廷顿酵母伴侣B(huntingtin yeast partner B, HYPB)也被称为SETD2、HSET2或KMT3A, 是一种H3K36特异性甲基转移酶^[84]。由于其介导H3K36me3(而不是H3K36me1和H3K36me2)受损, 阻碍了毛细血管组织重塑以及进一步的血管发育, 导致胚胎、卵黄囊和胎盘中出现严重的血管发育缺陷, *HYPB*^{-/-}小鼠在胚胎期第10.5~11.5天时出现胚胎致死^[58]。

目前关于HDMs与血管发育关系的研究比HMTs少,深入探讨HDMs与血管发育的关系和潜在机制任重道远。

4 H3K36甲基化与心血管疾病

心血管疾病(cardiovascular disease, CVD)是世界上导致死亡的主要原因之一。年龄、吸烟、肥胖、糖尿病和高血压等因素都会增加心血管疾病的风险。然而,心血管疾病高死亡率并不能完全归因于这些传统危险因素,包括表观遗传模式改变在内的非传统因素在近年来也成为心血管疾病高发的重要原因。表观遗传变化包括DNA甲基化、组蛋白修饰、非编码RNA以及差异RNA剪接等,已证明其在心血管生物学中的作用^[85],但组蛋白甲基化,尤其是H3K36甲基化及其相关HMTs和HDMs在心血管疾病中的功能仍有待总结。因此,本部分内容将综述与H3K36甲基化相关的HMTs和HDMs在心血管疾病中的作用。

4.1 HMTs与心血管疾病

*NSD1*基因编码HMTs,通过调节染色质重塑参与转录调控。该基因的点突变和缺失是导致Sotos综合征的主要原因之一^[86]。Sotos综合征的心脏表现出结构性心脏缺损,其中房室隔缺损最常见,其次为肥厚型心肌病和心律失常^[87]。Sotos综合征心脏相关疾病的发生率在不同的研究中有所不同,从8%到23.5%不等^[88-90]。一些文献报道,其会导致室间隔完整型肺动脉闭锁、肥厚性心肌病和主动脉缩窄的发生^[90]。另外一些报道描述了Sotos综合征的心律失常,但心律失常的发生似乎比相关的结构缺陷或心肌病少。同时,Sotos综合征也与神经发育和先天性甲状腺功能减退有关^[91,92]。有报道显示,在Sotos综合征中,位于PHD位置的突变能够减少*NSD1*与特定甲基化位点(H3K4和H3K9)的结合,并减少辅因子(如锌指蛋白NIZP1)的募集,二者共同作用导致转录调控的异常^[93]。

有文献报道,*NSD2*的表达水平在心室重构过程中升高。在心肌中特异性敲除*NSD2*基因后,H3K36me2而非H3K27me2的表达下调^[94]。此外,*NSD2*基因敲除可减轻心室重构症状,提示*NSD2*可能成为心室重构个体化治疗的新靶点^[70]。

在病理性心肌肥大的细胞模型中,异丙肾上腺素(isoprenaline, ISO)刺激能够诱导*NSD3*表达水平下降。心肌*NSD3*是一种重要的心脏功能、结构重塑以及肥厚标志物心房利钠因子(atrial natriuretic factor, ANF)转录的重要调控因子。*NSD3*及其在*ANF*启动子上相关的H3K27me2/me3修饰在ISO刺激下减少,但*ANF*启动子的H3K27ac修饰增加。然而,*NSD3*的过表达可通过与BRD4相互作用抑制*ANF*启动子的H3K27ac修饰,从而减轻ISO诱导的心肌细胞肥大^[29]。

4.2 HDMs与心血管疾病

JMJD2A又称为KDM4A[Lysine (K)-specific demethylase 4A],是JmjC结构域家族JMJD成员之一,可催化H3K9me3和H3K36me3的去甲基化。Zhang等^[95]的研究结果表明,在TAC手术模型中,心肌细胞中JMJD2A对含四个半LIM结构域蛋白1(four and a half lim domains protein 1, *FHL1*)启动子处H3K9me3的去甲基化促进了基因转录,从而重新编程参与心肌肥大的基因表达。JMJD2A响应TAC并与*FHL1*启动子结合,上调*FHL1*表达,下调H3K9me3。JMJD2A通过介导SRF和心肌素上调*FHL1*,并且需要其去甲基化酶活性。同时,作者发现JMJD2A在人类肥厚型心肌病患者中上调,因此推断JMJD2A在心肌肥大相关基因重编程过程中发挥重要作用。

目前对与H3K36甲基化相关的HDMs研究相对较少,但是HDMs对其他位点甲基化的研究表明H3K36甲基化对心血管疾病的影响可能具有广泛的研究空间。

5 H3K36甲基化结合因子与心血管疾病

除了上述与心血管发育和疾病相关的HMTs及HDMs外,一些“reader”蛋白也与心血管发育和疾病密切相关。在血管紧张素Ⅱ(angiotensin Ⅱ, Ang Ⅱ)诱导的心肌肥大模型中,*PHF19*敲低能够降低左心室缩短分数(fractional shortening, FS)和左心室射血分数(ejection fraction, EF)。同时,*PHF19*敲低还抑制了Ang Ⅱ介导的心脏增重和心肌肥大,并降低了心房钠尿肽和脑钠肽等心肌肥大标记基因的表达。相比之下,*PHF19*在体外的过表达促进了Ang Ⅱ诱导的心肌细胞肥大。进一步的机

制研究表明, PHF19结合SIRT2的启动子并调节H3K27me3和H3K36me3之间的平衡, 从而在体内和体外抑制SIRT2的表达^[96]。

DNMT3B作为一种从头开始介导甲基化的DNA甲基转移酶(DNA methyltransferase, DNMT), 在心血管发育和疾病中充当重要角色。在Wnt信号通路被抑制后, 心肌细胞高表达的miR-133a重新定位到细胞核中并直接调节DNMT3B的转录^[97]。在哺乳动物中, DNMT3B介导的非CpG甲基化和抑制因子1[repressor element 1 (RE1), REST]结合的相互作用共同调控心脏发育过程中的基因沉默^[98]。抑癌基因(gene of phosphate and tension homology deleted on chromosome ten, *P TEN*)缺失可以通过DNMT3B调节非CpG DNA甲基化来抑制心肌细胞分化^[99]。同型半胱氨酸可以通过DNMT3B/SP1通路抑制清道夫受体B类成员1并加速动脉粥样硬化^[100]。MiR-125b靶向DNMT3B并介导p53 DNA甲基化参与同型半胱氨酸诱导的血管平滑肌细胞增殖^[101]。但是具体机制是否依赖其H3K36甲基化“reader”蛋白的功能还有待探究。

*DNMT3A*缺乏会导致多种免疫细胞群出现几种潜在的促进动脉粥样硬化表型^[102,103]。同样, DNMT3A抑制了HADC11在miR-195-3p启动子处的结合并促进其转录来缓解同型半胱氨酸介导的动脉粥样硬化^[104]。相反, *DNMT3A*敲低限制了巨噬细胞中I型干扰素的产生^[105], 可能潜在防止动脉粥样硬化的发展^[106,107]。当然, DNMT3A对动脉粥样硬化的作用机制是否通过其H3K36甲基化“reader”蛋白发挥作用也有待探究。

6 结论与展望

组蛋白PTM是表观遗传学的重要内容, 不仅表现在组蛋白PTM可以直接调节基因表达, 还可以通过DNA修饰影响基因活性。单一组蛋白PTM通常不能单独发挥作用, 通常需要多个组蛋白修饰的协同作用共同调控转录组基因表达。本文主要关注了H3K36甲基化相关HMTs和HDMs在心血管发育和疾病中的重要作用。目前, 我们对心血管发育和疾病中组蛋白甲基化尤其是H3K36甲基化的理解还不够深入。虽然H3K36甲基化修饰与心血管发育和疾病相关, 但仍需进一步深入研究以阐

明H3K36甲基化修饰调控心血管发育和疾病的具体分子机制。

HMTs和HDMs对组蛋白PTM在基因表达和转录调控中发挥重要作用, 调节HMTs和HDMs酶活性的小分子化合物可能是一种潜在的心脏病治疗方法。目前, KDM1A抑制剂在治疗急性髓细胞白血病方面的前景良好^[108]。这项研究也提供了一些潜在的数据, 提示可以通过药物抑制剂调节HDMs治疗心血管疾病。KDM6B过表达导致的心肌肥厚可以通过其抑制剂GSK-J4的治疗得到改善^[109]。在细胞中, 通过KDM3C已知的抑制剂JIB-04可以防止血管紧张素II介导的心肌肥厚^[110]。未来的研究可以考虑测试其他HDMs抑制剂对人类心血管疾病的治疗效果。但是, 由于HDMs一般不具有组织器官表达特异性, 目前尚不清楚针对心血管疾病的HDMs抑制剂是否会对其他组织器官造成影响, 仍需深入研究。

本文旨在为后续关于H3K36甲基化与心血管发育和疾病的研究提供了研究思路。未来还需要继续深入研究H3K36特异性HMTs和HDMs以及H3K36不同水平甲基化在心血管发育和疾病中的作用机制, 并探索通过特异性干预H3K36甲基化水平预防或治疗心血管疾病的新方法。同时, 除了导致心血管发育和疾病的遗传因素外, 外部环境因素也十分重要。目前研究主要关注外部环境因素通过怎样的途径引起表观遗传学的改变、不同表观遗传修饰是否对特定的环境因素易感、针对不同环境因素引起的表观遗传改变是否存在靶向干预手段等科学问题。在这些问题中, 全面细致地研究H3K36甲基化修饰的功能和作用可能为探索心血管发育及相关疾病的发病机制和治疗方法提供新的认识和理解。

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