m6A甲基化调控骨肉瘤的研究进展

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摘要 骨肉瘤是常见的骨恶性肿瘤, 其高复发、高转移率及化疗耐药等问题增加了骨肉瘤的治疗难度。m6A甲基化是哺乳动物中最常见的RNA修饰, 在不改变碱基序列的情况下调控基因转录后的表达。它主要通过甲基化转移酶、甲基化阅读蛋白、去甲基化酶这三类蛋白参与许多生物学过程和疾病的发生。目前已证实, 多种基因的m6A甲基化与骨肉瘤的发生、发展密切相关。该文就m6A甲基化与骨肉瘤关系的研究进行综述, 旨在为骨肉瘤的治疗提供新方向。

关键词 m6A甲基化; 骨肉瘤; 调控

Research Progress of m6A Methylation Regulating Osteosarcoma

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Abstract Osteosarcoma is a common malignant bone tumor. The problems of high recurrence, high metastasis rate and chemotherapy resistance make the treatment of osteosarcoma more difficult. m6A methylation is the most common RNA modification in mammals, regulating the post-transcriptional expression of genes without changing the base sequence. It mainly participates in many biological processes and diseases through the three types of proteins: methyltransferase, methylated reading protein, and demethylase. It has been confirmed that m6A methylation of multiple genes is closely related to the occurrence and development of osteosarcoma. Therefore, this article reviews the researches on the relationship between m6A methylation and osteosarcoma, and aims to provide a new direction for the potential targeted therapy of osteosarcoma.

Keywords m6A methylation; osteosarcoma; regulation

骨肉瘤(osteosarcoma, OS)是常见的骨恶性肿瘤, 多发于儿童、青少年期[1-2]。其在临床上侵袭性强且易发生转移, 是导致儿童和年轻人癌症相关死亡的第二大原因[3]。外科手术联合化疗是OS患者的主要治疗方法。经过标准化治疗后, 非转移性OS患者通常具有相对较好的预后[4]。而肿瘤的复发、高转移率及化疗耐药等问题都会降低患者生存率[5]。

近年来, 众多研究发现表观遗传修饰在调控基因的表达, 影响细胞的生长分化中具有重要作用[6]。表观遗传异常会发生在许多层面, 主要包括DNA[7]、RNA[8]和组蛋白的修饰[9-10]。RNA修饰是指核糖核酸上的化学成分或结构发生变化, 广泛分布于mRNA和非编码RNA中, 目前已经鉴定了150多种RNA修饰[11]。其中, N6-甲基腺苷(N6-methyl-

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adenosine, m6A)修饰是在mRNA的氮-6位置腺苷碱基的甲基化, 它是哺乳动物细胞中最普遍的基因内部修饰[12-13]。m6A的修饰是动态可逆的, 并且在调节前体mRNA成熟、翻译和降解中发挥关键作用[14-16]。

而骨肉瘤的发生、发展及其转移都是非常复杂的过程, 结合近年来发表的与骨肉瘤相关的m6A甲基化的研究, 本文对m6A甲基化的相关概念及其在骨肉瘤进展的作用等进行阐述, 探寻m6A甲基化与骨肉瘤之间的关联, 更好地了解促进骨肉瘤进展的潜在机制并希望能够为骨肉瘤的病理诊断和靶向治疗寻找新的标志物和潜在靶点。

1 m6A甲基化概述

m6A位点主要富集于mRNA的3′非翻译区(3′UTR)、终止密码子附近, 其核心序列为RRACH(R=A/G, H=A/U/C)[17-18]。m6A甲基转移酶、甲基识别蛋白和m6A去甲基化酶这三类蛋白在各种基本生物过程中充当着m6A的“编码器”、“读码器”和“消码器”的功能角色(图1)。

m6A修饰主要由m6A甲基转移酶复合体催化。经典的复合体由甲基转移酶样3(methyltransferase-like 3, METTL3)、甲基转移酶样14(methyltransferase-like 14, METTL14)和1型Wilms肿瘤相关蛋白(Wilms tumour 1-associated protein, WTAP)组成[19]。METTL3起催化作用, METTL14则辅助与底物结合[20]。WTAP能通过与METTL3-METTL14核心复合物结合, 促进METTL3-MELLT14复合物向核斑点的易位, 这种活性是mRNA有效甲基化所必需的[19]。近年来, 越来越多甲基转移酶复合体中其他组成蛋白为人们所认识, 如病毒样m6A甲基转移酶相关蛋白(Vir like m6A methyltransferase associated protein, VIRMA)[21]、甲基转移酶样蛋白16(methyltransferase-like protein 16, METTL16)[22]、RNA结合基序蛋白15(RNA binding motif protein 15, RBM15)、RBM15B[23]、13锌指CCCH型(zinc finger CCCH-type containing 13, ZC3H13)等[24]。这些蛋白通过相互作用, 进而调节复合体的稳定性、mRNA的m6A甲基化以及细胞定位[25]。

甲基识别蛋白能识别RNA甲基化修饰的信息, 影响含有m6A的mRNA的稳定性、代谢以及翻译[26-27]。第一类甲基识别蛋白主要由YTH家族组成, YT521-B同源(YT521-B homology, YTH)结构域蛋白质家族成员(YTHDF1、YTHDF2、YTHDF3、YTHDC1和YTHDC2)具有特殊的结构域YTH能够直接结合m6A, 识别其介导的生理效应[28-30]。其中YTHDF3与YTHDF1协同促进蛋白质合成, 并影响YTHDF2介导的甲基化mRNA降解[31]。第二类异质核核糖核蛋白(heterogeneous nuclear ribonucleoprotein, HNRNP)家族(HNRNPA2B1、HNRNPC、HNRNP G)能调控RNA底物在核内的成熟过程[32-33]。此外, 随着m6A甲基化检测技术的不断发展, 人们发现了更多通过典型结构域与m6A修饰的RNA结合的蛋白, 如胰岛素样生长因子2 mRNA结合蛋白(insulin-like growth factor 2 mRNA-binding proteins, IGF2BPs)[34]、脆性X智力低下蛋白(Fragile X mental retardation protein, FMRP)[35]。

m6A去甲基化酶能去除RNA中的m6A甲基化

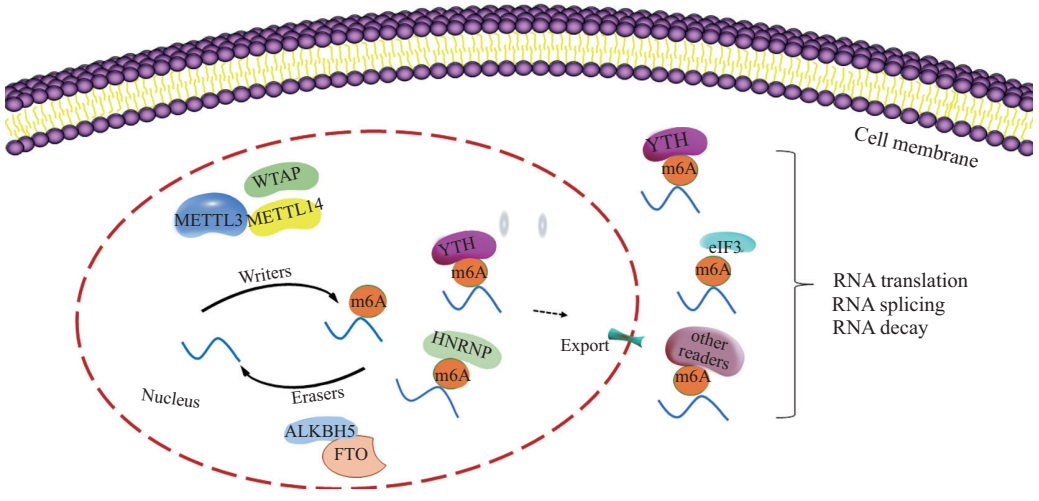

图1 m6A甲基化修饰的基本机制

Fig.1 The basic mechanism of m6A methylation modification

基团, 表明m6A甲基化是一个动态、可逆的过程。常见的有肥胖蛋白(fat mass and obesity-associated protein, FTO)和alkB同源物5(alkB homologue 5, ALKBH5)两种, FTO与ALKBH5均能够定位于核斑点, 催化核RNA中m6A氧化去甲基化, 影响mRNA的出核与代谢[36-37]。随着甲基化测序技术的发展, 越来越多的甲基化相关蛋白被发现。这些蛋白在生物体中发挥着复杂的调节作用, 展示了m6A甲基化修饰的多面性。

2 m6A甲基化在骨肉瘤中的作用

m6A是表观遗传学研究的新生领域, 目前发现m6A可调节的生理过程包括昼夜节律、热休克反应、T细胞稳态、果蝇性别、癌症发展等[38-39]。m6A水平的任何波动都可能导致功能障碍或疾病发生[40]。越来越多的证据表明, m6A甲基化在肿瘤细胞的生长、分化、凋亡过程中起着重要的调节作用[41-45]。目前研究发现, m6A甲基化修饰在调控骨肉瘤的发生、发展中具有重要作用, 进一步深入研究发现, m6A甲基化修饰调控发生的位点主要有: GTP发育调控结合蛋白1(developmentally regulated GTP-binding protein 1, DRG1)、三磷酸腺苷酶家族蛋白2(ATPase family, AAA domain containing 2, ATAD2)、浆细胞瘤多样异位基因1(plasmacytoma variant translocation gene 1, PVT1)、淋巴增强因子-1(lymphoid enhancer factor-1, LEF-1)、丝氨酸/苏氨酸激酶Akt等。

2.1 DRG1

DRG1也被称为NEDD3, 属于GTP结合蛋白超家族。DRG1有G1~G5的结构域, 参与GTP的结合[46-47]。*DRG1*是一个高度保守的基因, 在成年组织和脊椎动物胚胎中都有着广泛的表达, 在胚胎发育中有着重要作用[46,48]。尽管DRG1在出生后被下调, 但在快速生长的组织和器官中DRG1仍是高表达的[49]。越来越多的研究表明, DRG1的异常表达在肿瘤的发生、发展中有着重要的影响。DRG1能促进肺腺癌细胞和黑色素瘤细胞的增殖, 表现出一定的促癌作用[50-51]。而在乳腺癌患者中, DRG1的低表达与患者的极低生存率相关[52]。因此, DRG1在不同类型的癌症中扮演着不同的角色。LING等[53]研究发现与癌旁组织相比, 骨肉瘤患者组织中DRG1的mRNA和蛋白水平较高, 且DRG1的高表达与骨肉瘤的肿瘤大小和临床分期相关。沉默DRG1会抑制骨肉瘤细胞迁移和集落形成能力, 阻滞细胞周期, 导致细胞凋亡。在骨肉瘤组织中METTL3表达增加, DRG1的m6A甲基化水平增高。而沉默METTL3能降低DRG1的mRNA和蛋白质水平。同时, 该研究还发现, m6A甲基识别蛋白ELAVL1能与DRG1的m6A序列相结合, 且DRG1的表达与ELAVL1的表达呈正相关。这一研究表明, METTL3和ELAVL1介导的m6A修饰共同促进了*DRG1* mRNA的稳定性, 进而增加了DRG1的蛋白表达水平, 促进骨肉瘤的进展。

2.2 ATAD2

*ATAD2*是一种新出现的癌基因, 与多种人类恶性肿瘤的发生发展、疾病复发以及患者生存期密切相关[54-55]。ZHOU等[56]研究发现, 沉默METTL3能通过抑制ATAD2的表达显著抑制骨肉瘤细胞的增殖、迁移和侵袭能力, 同时通过调节Bcl-2/Bax轴和Caspase 3的激活促进骨肉瘤细胞凋亡。这表明METTL3通过调节ATAD2在骨肉瘤生长和侵袭中起癌基因的作用, 提示METTL3是骨肉瘤治疗的潜在靶点。

2.3 PVT1

PVT1是定位于人类染色体8q24区域的长链非编码RNA[57]。研究表明, PVT1能通过调控miRNA的表达促进骨肉瘤的进展。PVT1通过调节miR-497/HK2轴促进骨肉瘤细胞糖酵解和细胞增殖[58]。同时PVT1能通过吸附抑制miR-486表达, 促进骨肉瘤细胞的迁移和侵袭[59]。此外, PVT1还能靶向抑制miR-152并激活c-MET/PI3K/Akt途径增强骨肉瘤对吉西他滨的耐药性[60]。CHEN等[61]研究表明, PVT1表达与骨肉瘤临床分期、肿瘤大小显著相关, 且PVT1表达高的患者预后较差。与正常组织相比, 骨肉瘤组织中去甲基化酶*ALKBH5* mRNA表达明显上调, 且ALKBH5的过表达与OS患者预后差有关。此外, *ALKBH5* mRNA水平与PVT1转录本水平呈正相关。表明ALKBH5和PVT1可能是OS患者潜在的预后标志物。而进一步的细胞和小鼠实验证明, ALKBH5能通过降低PVT1的m6A甲基化水平, 抑制甲基化识别蛋白YTHDF2与PVT1的结合, 进而抑制PVT1的降解, 促进骨肉瘤的肿瘤发生。有趣的是, PVT1的缺失并不能消除ALKBH5对骨肉瘤的促进作用, 这意味着ALKBH5很可能同时通过介导其他转录本的m6A去甲基化促进骨肉瘤进展[61]。

2.4 LEF1

LEF1是Wnt/β-catenin途径的主要成分, 并且

会加重骨肉瘤的进展[62]。MIAO等[63]发现, 在人骨肉瘤组织和骨肉瘤细胞系中, m6A甲基化水平和METTL3的表达水平均上调。沉默METTL3降低了LEF1的m6A甲基化和总mRNA水平, 随后抑制 了Wnt/β-catenin信号通路的活性, 进而抑制了骨肉瘤细胞的增殖、迁移和侵袭能力。当LEF1过表达时, 则消除了METTL3沉默对骨肉瘤细胞增殖、迁移和侵袭能力的抑制作用。WANG等[64]通过阿霉素DXR诱导人的OS细胞系MG63, 建立了多药耐药性细胞株MG63/DXR。从RNA和蛋白水平检测与m6A甲基化相关的主要酶发现, METTL3和ALKBH5在MG63/DXR中显著性上调。通过MeRIP-seq分析发现与MG63相比, 在MG63/DXR中有2 372个明显上调的m6A峰和3 229个明显下调的m6A峰。将含有差异甲基化位点的基因进行GO富集和KEGG通路分析, 发现m6A甲基化修饰可能对骨肉瘤干细胞的细胞全能性有重要影响, 主要通过Wnt和Notch信号通路发挥作用。以上结果表明, m6A甲基转移酶METTL3能通过调节LEF1的m6A水平并激活Wnt/β-catenin信号通路来促进骨肉瘤细胞的进展。而之前已有多项研究表明, Wnt/β-catenin通路能影响骨肉瘤细胞对阿霉素[65]、吉西他滨[66]和甲氨蝶呤[67]的耐药性。这提示, m6A甲基化可能会影响骨肉瘤的化疗耐药性, 但目前还未见相关的动物模型的研究, 仍需要进一步的探索。

2.5 Akt

丝氨酸/苏氨酸激酶Akt也叫做蛋白激酶B(protein kinase B, PKB), 位于信号网络的中枢, 主要通过磷脂酰肌醇3-激酶(phosphatidylinositol 3-kinase, PI3K)/Akt信号通路被激活。该通路在调节细胞周期、增殖、凋亡等方面有着重要作用, 在各种癌症中经常被激活, 并被认为是有希望的治疗靶标[68]。该途径常在骨肉瘤中过度活化并促进疾病的发生和发展, 包括促进肿瘤细胞的增殖、侵袭和转移, 抑制细胞凋亡[69]; 此外还能作用于肿瘤的血管生成和耐药性[70-71]。

TIAN等[72]研究发现, 在骨髓间充质干细胞(bone mesenchymal stem cell, BMSC)中, 敲低*METTL3*不仅降低了血管内皮生长因子A(vascular endothelial growth factor, Vegfa)的表达, 而且降低了其剪接变体Vegfa-164和Vegfa-188的水平。同时Akt磷酸化水平显著降低, 表明在成骨分化过程中BMSC中的*METTL3*敲低抑制了PI3K/Akt信号传导。而VU等[73]研究发现, 在人类造血干/祖细胞中敲除*METTL3*降低了10号染色体上磷酸酶与张力蛋白同源缺失基因(phosphatase and tensinhology deleted from chromosome 10, *PTEN*)的m6A甲基化水平, 阻碍其翻译效率, 同时增加Akt磷酸化水平, 进而促进急性髓细胞白血病(acute myelocytic leukemia, AML)细胞的分化和凋亡。以上研究表明, 敲除*METTL3*很可能影响PI3K/Akt通路的信号传导。而在骨肉瘤中m6A甲基化水平改变能否靶向PI3K/Akt途径调节骨肉瘤细胞增殖和凋亡, 还需要进一步的研究。

3 总结与展望

综上所述, m6A甲基化相关酶能通过影响靶基因的m6A甲基化水平调控*DRG1*、*ATAD2*、*PVT1*等癌基因的表达和Wnt/β-catenin、PI3K/Akt/mTOR骨肉瘤相关信号通路的激活, 在骨肉瘤的发生发展及转移中有着重要的作用(图2)。骨肉瘤恶性程度高, 能迅速破坏周围组织进行转移。尽管目前的手术切除结合化疗或放疗等骨肉瘤治疗策略已取得了进步, 但骨肉瘤患者的存活率仍然不太乐观[74]。而m6A甲基化修饰在调控骨肉瘤的增殖、迁移和侵袭能力中具有重要作用, 这成为骨肉瘤的潜在治疗靶点, 为今后的药物开发提供了一定的基础。

目前, 对于m6A甲基化和骨肉瘤的研究主要集中在细胞层面, 还需要更多动物实验进行进一步的验证。m6A甲基化是动态可逆的过程, 而甲基转移酶和去甲基化酶如何协调作用, 以及甲基识别蛋白在识别RNA甲基化后如何发挥作用都还需要进一步挖掘。

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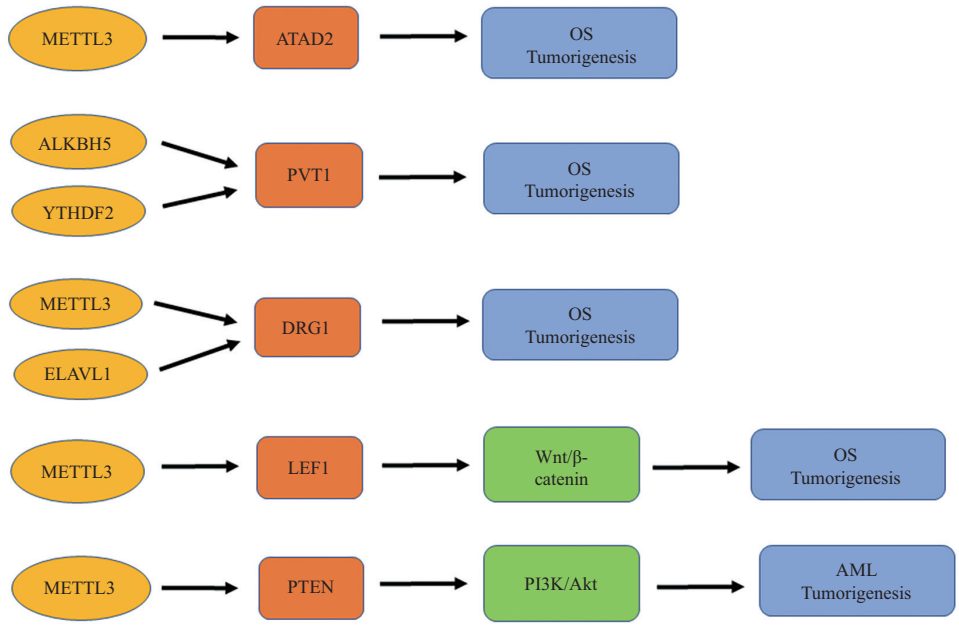

图2 m6A甲基化调控骨肉瘤的相关机制

Fig.2 Mechanism of m6A methylation in osteosarcoma

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