

Research progress of methylation modification in intervertebral disc degeneration

HAN Tong, TIAN Jiwei

BenQ Medical Center, The Affiliated BenQ Hospital of Nanjing Medical University, Nanjing, Jiangsu 210000, China

Corresponding author: TIAN Jiwei, E-mail: tjw609@163.com

Abstract: Methylation modification is a hot research topic in epigenetics. It plays an important role in cell signal transduction and tissue-specific differentiation by regulating gene expression. Methylation modification also plays a significant role in intervertebral disc degeneration and repair. This article reviews the role and characteristics of methylation modification in the pathogenesis of intervertebral disc degeneration and provides an outlook for future development.

Keywords: Intervertebral disc degeneration; DNA methylation; Histone methylation; N6-methyladenosine

The intervertebral disc is located between vertebrae, consisting of the outer annulus fibrosus (AF), the inner nucleus pulposus (NP), and the adjacent cartilaginous endplate (CEP) on both sides [1]. Long-term overload, injury, aging, smoking and other factors can cause intervertebral disc degeneration (IVDD), manifesting as the loss of disc cells and degradation of the extracellular matrix (ECM) [2]. IVDD is closely related to the occurrence of discogenic low back pain and neurological symptoms, so it is very important to elucidate the regulatory mechanism of IVDD.

Methylation is one of the important research contents of epigenetics, mainly including three modification methods: DNA methylation, histone methylation, and RNA methylation. By regulating gene expression and the activity of functional molecules, it plays an important role in cell signaling and tissue-specific differentiation. This article summarizes the role and characteristics of methylation modification in the pathogenesis of IVDD, aiming to provide new ideas for the prevention and treatment of IVDD.

1. DNA methylation regulates intervertebral disc degeneration

DNA methylation refers to the process of covalently binding a methyl group to a specific base on the DNA sequence under the action of DNA methyltransferase (DNMT) [3]. DNA methylation has various forms such as 5-methylcytosine (5-mC), N6-methyladenine (N6-mA), and 7-methylguanine (7-mG). In humans and mammals, it mainly exists in the form of 5-mC in CpG islands, which is the most widespread form of DNA methylation modification [4]. DNMT mainly consists of DNMT1, DNMT3a, DNMT3b, and DNMT3L. While DNA demethylase, namely TET dioxygenases (TET), mainly includes TET1, TET2, and TET3. These two types of enzymes jointly regulate DNA methylation and demethylation [5]. DNA methylation regulates gene expression levels without changing the DNA sequence and plays an important role in processes such as embryonic development, cell proliferation and differentiation, and

maintaining genome stability [6].

Multiple studies have found a close relationship between DNA methylation levels and IVDD, and have further explored the possible mechanisms of DNA methylation regulating IVDD. Research suggests that when DNA methylation is located in gene promoters and enhancer regions, it usually reduces gene expression levels [7]. Ikuno *et al.* [8] used whole-genome association analysis of human NP, and found that 220 differentially methylated sites were associated with NP degeneration. Kawaguchi *et al.* [9] studied the *growth arrest DNA damage (GADD)45G* gene and *cell cycle-associated protein 1 (CAPRIN1)* gene, which regulate cell cycle and genome stability, and found that *GADD45G* and *CAPRIN1* are representative genes with high methylation levels in the core promoter regions of degenerated NP tissues. Kawai *et al.* [10] examined the genome-wide DNA methylation levels of both coding and non-coding sequences in mice and found that long-term running reduced the global DNA methylation level of the intervertebral disc genome, improving sensory symptoms related to low back pain.

For a long time, inflammatory cytokines have been a research focus in the molecular biology of IVDD. A large number of studies have shown that DNA methylation promotes the expression of inflammatory cytokines related to IVDD. Hou *et al.* [11] constructed a mouse model of lumbar intervertebral disc degeneration and injected adenoviruses carrying short-hairpin RNA (shRNA) targeting DNMT1 into the discs. The experimental results showed that the decrease in methylation levels caused by shDNMT1 significantly reduced the expression of pro-inflammatory cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6, and significantly increased the levels of anti-inflammatory cytokines IL-4 and IL-10 at the same time. This also reduced cell apoptosis and alleviated pain symptoms. Silencing DNMT1 can inhibit the expression of IL-6 and TNF- α , and reduce the expression of NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3), apoptotic speck-like protein containing a CARD (ASC), and caspase-1, thereby relieving intervertebral disc degeneration [12]. Cheng *et al.* [13] found that DNMT3a activated the NF- κ B pathway,

promoted cell apoptosis and ECM degradation by methylating the peroxisome proliferator-activated receptor γ (PPAR γ) promoter. The DNMT3a-mediated PPAR γ /NF- κ B axis may be a new target for the treatment of IVDD. The transient receptor potential vanilloid subfamily 1 (TRPV1) channel is a ligand-gated non-selective cation channel widely distributed in various tissues and organs [14]. During IVDD, TRPV1 is widely activated, causing inflammatory pain [15]. Hong *et al.* [16] found that along with the high expression of DNMT3b, the DNA methylation level of TRPV1 in the NP and AF regions increased after IVDD occurred, and the increase was more pronounced in the AF region than in the NP region. It is worth noting that individual studies have also pointed out the protective role of DNA methylation in the IVDD. Luo *et al.* [17] found that overexpression of DNMT3b could promote NP cell proliferation, upregulate the expression of type II collagen and aggrecan, downregulate the expression of matrix metalloproteinases (MMP)-3 and MMP-9, and inhibit inflammatory reactions. The mechanism is that DNMT3b inhibits the expression of cyclooxygenase-2 (COX-2) by targeting the transient receptor potential ankyrin 1 (*TRPA1*) promoter. COX-2 can promote NP cell apoptosis by inhibiting the expression of Yes-associated protein (YAP). Therefore, DNMT3b reduces NP cell apoptosis and ECM degradation through the *TRPA1*/COX-2/YAP axis, thereby alleviating IVDD in rats.

In human intervertebral discs, abnormal methylation in the DNA promoter region can lead to abnormal expression of miRNAs. Liu *et al.* [18] found that low methylation of the DNA promoter induced up-regulation of miR-132 expression, leading to down-regulation of growth differentiation factor 5 (GDF-5) expression, promoting NP cell degeneration and ECM degradation through the MAPK/ERK signaling pathway. Kang *et al.* [19] found that low methylation of the DNA promoter leads to up-regulation of miR-494 expression, resulting in down-regulation of SOX-9 expression, promoting NP cell apoptosis and ECM degradation. A study conducted by Zhao *et al.* [20] showed that DNA methylation inhibits the expression of miR-129-5p, promoting the synthesis of Beclin-1 and inducing NP cell autophagy. DNA demethylation increases the expression level of miR-143, inhibits the expression of B cell lymphoma-2 protein (Bcl-2), and thereby induces NP cell apoptosis [21]. Chen *et al.* [22] found that the expression level of miR-217 was reduced in degenerate NP cells due to high methylation levels in their promoter region, and overexpression of miR-217 could inhibit IVDD by regulating the FBXO21/ERK pathway.

Hyperhomocysteinemia (HHcy) is a disease caused by a lack of folate and vitamins B6 and B12, leading to elevated serum homocysteine concentrations and resulting in a range of symptoms such as pale skin and fatigue [23]. Zhang *et al.* [24] conducted a clinical epidemiological study showing that HHcy is an independent risk factor for human IVDD. HHcy can promote the expression of DNMT1, DNMT3a, and DNMT3b, and target glutathione peroxidase 4 (GPX4), leading to DNA hypermethylation

and inhibition of GPX4 expression, thereby promoting NP cell degeneration through oxidative stress and ferroptosis. This discovery establishes a new link between HHcy and IVDD.

2. Histone methylation regulates intervertebral disc degeneration

In eukaryotic cells, the genetic information stored in DNA is usually in the form of chromatin structures. Nucleosomes are the core structural elements of chromatin, consisting of four core histones H2A, H2B, H3, and H4, with two molecules of each to form a spherical protein octamer [25]. Various post-translational modifications, such as methylation, acetylation, phosphorylation, and ubiquitination, can be found in histone tails. Among them, histone methylation plays an important role in gene expression, controlling chromatin accessibility, cell cycle regulation, and other activities [26]. Histone methylation mostly occurs on the N-terminal lysine residues extending outward from H3 and H4, and is strictly regulated by lysine methyltransferases (KMTs) and lysine demethylases (KDMs). There are three methylation states: monomethylation (me1), dimethylation (me2), and trimethylation (me3). The methylation of histone lysine determines transcriptional activation or repression based on its position and methylation state. The methylation of H3K4, H3K36, and H3K79 can promote transcription, while the methylation of H3K9, H3K27, and H4K20 inhibit transcription [27].

Recent studies have shown a link between histone methylation and IVDD and discogenic pain. Stover *et al.* [28] clarified that the IL-6/A-kinase anchoring protein 150 (AKAP150) pathway is a potential mechanism for the sensitization of dorsal root ganglion (DRG) neurons in rat IVDD, and targeted H3K9 me3 modification of AKAP150, inhibiting its expression, reducing DRG neuron activity, and relieving inflammatory-driven discogenic pain. Stover *et al.* [29] further found that IVDD could produce IL-6, which increased the activity of nociceptive neurons through *TRPA1*, acid-sensing ion channel 3 (*ASIC3*), and mechanically gated Piezo2 ion channels, leading to discogenic pain. Targeting histone H3K9 methylation at the promoters of *TRPA1*, *ASIC3*, and *Piezo2* genes can inhibit endogenous gene expression and alleviate discogenic pain. Jiang *et al.* [30] found that the histone methyltransferase enhancer of zeste homolog 2 (EZH2) is highly expressed in degenerate CEP tissue. EZH2 inhibits SOX-9 expression through H3K27me3 modification, which promotes intervertebral disc degeneration. Inhibition of EZH2 can upregulate SOX-9 expression, making it a potential therapeutic target for IVDD. Xu *et al.* [31] found that the histone methyltransferase KMT2D was upregulated in human degenerate NP tissue. KMT2D-mediated H3K4me1 and H3K4me2 modifications promote the expression of MMP-3, MMP-9, and MMP-13, leading to ECM degradation and NP degeneration. Silencing KMT2D or using the histone H3K4 methylation inhibitor OICR can reverse this process. In summary, there has been an initial exploration of histone methylation regulating

IVDD, but the specific mechanisms of histone methylation at different positions and methylation states regulating IVDD remain to be further studied.

3. RNA methylation regulates intervertebral disc degeneration

RNA methylation includes N6-methyladenosine (m6A), N1-methyladenosine (m1A), 5-methylcytidine (m5C), etc. Among them, m6A is the most studied form of RNA methylation modification, which participates in the regulation of RNA cycling stages such as transcription, maturation, translation, and degradation [32]. m6A modification is dynamically reversible and is co-regulated by methyltransferase "Writers", demethylase "Erasers", and methylation reader proteins "Readers". The currently known that m6A methyltransferases mainly include methyltransferase-like protein (METTL) 3, METTL14, METTL16, Wilms tumor 1-associated protein (WTAP), virus-like m6A methyltransferase-related protein (VIRMA), Zinc finger CCCH domain-containing protein 13 (ZC3H13), and RNA-binding motif protein 15 (RBM15). m6A demethylases include fat mass and obesity-associated protein (FTO), AlkB homolog 5 (ALKBH5), and ALKBH3. The m6A methylation reading protein is mainly the YTH domain protein family, including YTHDF1, YTHDF2, and YTHDF3 [32]. A large number of studies have shown that m6A modification plays an important regulatory role in cell senescence and senescence-related diseases [33], and is closely related to bone-related diseases such as osteoporosis and osteosarcoma [34].

In recent years, the role of m6A modification in the process of IVDD has become a research hotspot. Xiao *et al.* [35] found that the expression level of METTL3 and the level of m6A modification were significantly elevated in human degenerated CEP tissue. Tension stimulation destroys the stability of SOX-9 mRNA and downregulates the expression of SOX-9 through METTL3-mediated m6A modification, thereby inhibiting the synthesis of ECM in the cartilaginous endplate. Xiao *et al.* [36] also found that METTL3 promotes the expression of miR-126-5p through m6A modification, thereby inhibiting the expression of the target gene phosphoinositide-3-kinase regulatory subunit 2 (PIK3R2) and inhibiting the protective effect of the PI3K/AKT pathway on chondrocytes, leading to metabolic disorders and endplate degeneration. Zhu *et al.* [37] used the water escape behavior of mice to construct an IVDD model and detected m6A modification in the NP tissue of mice after IVDD through methylated RNA immunoprecipitation (MeRIP) technology. The results showed that the expression of METTL3 and METTL14 was significantly upregulated in degenerated NP cells, while the expression of FTO was significantly downregulated. Their further research [38] found that METTL14 upregulates the expression of miR-34a-5p through m6A modification, increases the recognition and binding level of miR-34a-5p with DGCR8, thereby reducing the expression level of silent information regulator 1 (SIRT1) mRNA and promoting NP cell

senescence. Chen *et al.* [39] found that oxidative stress increased the degradation of methionine adenosyltransferase 2A (MAT2A) precursor mRNA by upregulating METTL16-mediated m6A modification, and downregulating the expression of MAT2A will cause a decrease in S-adenosylmethionine (SAM) and apoptosis of NP cells. Wang *et al.* [40] constructed a static compression model of mouse tails and compared the m6A modification levels of NP tissue before and after degeneration. The results showed that most differentially expressed mRNAs and long non-coding RNAs (lncRNAs) exhibited significant demethylation after degeneration. The m6A regulatory factor zinc finger protein 217 (ZFP217) promotes the demethylation of these differentially expressed RNAs by activating the transcription of FTO. Circular RNA (circRNA), as an important component of non-coding RNA, has been shown to participate in the progression of various diseases including IVDD [41]. The role of circRNA methylation in IVDD deserves further exploration. Chen *et al.* [42] detected the m6A modification level of differentially expressed circRNA in the NP tissue of IVDD patients and found that circGPATCH2L was upregulated with the decrease of m6A level. circGPATCH2L, as a decoy for tripartite motif-containing protein 28 (TRIM28), causes DNA damage accumulation and apoptosis by inhibiting the phosphorylation of TRIM28 and the degradation of P53, leading to NP degeneration. After m6A modification, the circGPATCH2L can be recognized, cut and degraded by the ribonuclease endonuclease complex YTHDF2-RPL10-RNaseP/MRP to maintain the physiological state of NP cells. This study expounds the role of circRNA methylation in maintaining the physiological state of NP cells, and provides a new target for the treatment of IVDD.

4. Methylation jointly regulates intervertebral disc degeneration

Recent studies have found that there was a close relationship among the three methylation modifications, which could jointly regulate gene expression through specific interactions [43-45]. In the research process of the mechanism of intervertebral disc degeneration, some studies have reported the role of methylation co-regulation. Tu *et al.* [46] found that smoking increased the release of mast cell-restricted tetramer-forming tryptases (MC-TTs) in the intervertebral disc, removed the inhibitory H3K9me3 modification on the METTL14 promoter, caused increased METTL14 activity, upregulated the m6A modification level of the DIX domain containing 1 (DIXDC1) mRNA, increased the mRNA stability of DIXDC1 and promoted its interaction with Disrupted in Schizophrenia-1 (DISC1), activating the canonical Wnt signaling pathway to accelerate the senescence of NP cells. Li *et al.* [47] found that decreased expression of KDM5a can increase the H3K4me3 level in the WTAP promoter region and promote the expression of WTAP in senescent NP cells. WTAP promotes the m6A modification of non-coding RNA activated by DNA damage (NORAD), and induces the degradation of NORAD through the

recognition of YTHDF2, leading to more PUMILIO (PUM) 1/2 binding to E2F transcription factor 3 (E2F3) mRNA, promoting the degradation of E2F3 and accelerating the senescence of NP cells. Li *et al.* [48] found that after the decrease of H3K9me3 modification mediated by KDM4a upregulation, the expression of ALKBH5 increases, leading to the demethylation of DNMT3b mRNA. After demethylation, the expression of DNMT3b increases, inhibiting its expression through methylation modification of the E4F1 promoter, promoting the occurrence of IVDD.

5. Conclusion and prospect

Various studies have shown that methylation modification plays an important role in the degeneration and repair of intervertebral discs. Methylation modification has broad therapeutic prospects in various diseases. For example, DNMT inhibitors azacitidine and decitabine were approved for the treatment of hematologic malignancies, such as myelodysplastic syndromes, at the beginning of this century. And EZH2 inhibitor--tazemetostat has been demonstrated to have strong antitumor effects in clinical trials [49]. Currently, the use of methylation in the treatment of intervertebral disc degeneration and its corresponding complications is still in the exploratory stage. Studies have confirmed the feasibility of treating intervertebral disc degeneration and discogenic pain using clustered regularly interspaced short palindromic repeats (CRISPR) epigenome editing technology [50]. It is a feasible research direction to combine epigenetic drugs with biological therapies based on growth factors, stem cell-mediated cell therapies, gene therapies, and tissue engineering to form different combined treatment strategies. In addition, age-related diseases are often polygenic diseases, and different genes involve methylation changes in different directions. The current gene knockout and overexpression models can cause global changes in the methylome and cannot achieve methylation at specific sites or genes. Precise methylation editing technology is the future direction and challenge. Finally, the main research objects of intervertebral disc degeneration are currently mice and rabbits, and the effectiveness, specificity, and safety of methylation editing therapy in humans need to be further verified. Future research is needed to clarify the above issues, further reveal the relevant mechanisms of methylation modification, and provide new ideas for the diagnosis and treatment of intervertebral disc degeneration.

Conflict of Interest: None

References

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甲基化修饰调控椎间盘退变的研究进展

韩通, 田纪伟

南京医科大学附属明基医院, 江苏 南京 210000

摘要: 甲基化修饰作为表观遗传学的研究热点, 通过调节基因的表达, 在细胞信号转导和组织特异性分化等方面发挥着重要作用。在椎间盘的退变与修复过程中, 甲基化修饰扮演着重要角色。本文综述了甲基化修饰在椎间盘退变发病机制中的作用及特点, 并对其未来发展前景进行展望。

关键词: 椎间盘退变; DNA 甲基化; 组蛋白甲基化; N6-甲基腺嘌呤

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HAN Tong, TIAN Jiwei

BenQ Medical Center, The Affiliated BenQ Hospital of Nanjing Medical University, Nanjing, Jiangsu 210000, China

Corresponding author: TIAN Jiwei, E-mail: tjw609@163.com

Abstract: Methylation modification is a hot research topic in epigenetics. It plays an important role in cell signal transduction and tissue-specific differentiation by regulating gene expression. Methylation modification also plays a significant role in intervertebral disc degeneration and repair. This article reviews the role and characteristics of methylation modification in the pathogenesis of intervertebral disc degeneration and provides an outlook for future development.

Keywords: Intervertebral disc degeneration; DNA methylation; Histone methylation; N6-methyladenosine

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椎间盘位于椎体之间, 由外层纤维环(annulus fibrosus, AF)、内部髓核(nucleus pulposus, NP)组织以及两侧邻近的软骨终板(cartilaginous endplate, CEP)组成^[1]。长期超负荷、损伤、衰老、吸烟等多种因素可引起椎间盘退变(intervertebral disc degeneration, IVDD), 表现为椎间盘细胞丢失和细胞外基质(extracellular matrix, ECM)降解^[2]。IVDD 与椎间盘源性腰痛和神经症状的出现密切相关, 因此全面阐明 IVDD 的调控机制十分重要。

甲基化是表观遗传学的重要研究内容之一, 主要包括 DNA 甲基化、组蛋白甲基化和 RNA 甲基化三种修饰方式, 通过调节基因的表达, 调控功能分子的活性, 对细胞信号转导和组织特异性分化具有重要作用。在此, 本文总结了目前甲基化修饰在 IVDD 发病机制中的作用及特点, 旨在为预防和治疗 IVDD 提供新思路。

1 DNA 甲基化调控椎间盘退变

DNA 甲基化是指 DNA 序列上的特定碱基在 DNA 甲基转移酶(DNA methyltransferase, DNMT)的作用下, 共价结合一个

甲基基团的过程^[3]。DNA 甲基化有 5-甲基胞嘧啶(5-mC)、N6-甲基腺嘌呤(N6-mA)、7-甲基鸟嘌呤(7-mG)等多种形式, 在人类及哺乳动物中主要以 5-mC 的形式存在于 CpG 岛, 是目前研究最多的、最广泛的一种 DNA 甲基化修饰形式^[4]。DNMT 主要包括 DNMT1、DNMT3a、DNMT3b、DNMT3L, 而 DNA 去甲基化酶(DNA demethylase), 即 TET 双加氧酶(TET dioxygenases, TET), 主要包括 TET1、TET2、TET3, 两者共同调控 DNA 甲基化与去甲基化^[5]。DNA 甲基化在不改变 DNA 序列的前提下, 调控基因表达水平, 在胚胎发育、细胞增殖与分化、维持基因组稳定等过程中发挥重要作用^[6]。

多项研究发现 DNA 甲基化水平与 IVDD 密切相关, 并深入探讨了 DNA 甲基化调控 IVDD 的可能机制。研究表明, 当 DNA 甲基化位于基因启动子和增强子区域时, 通常会降低基因表达水平^[7]。Ikuno 等^[8]使用人类 NP 全基因组关联分析, 发现 220 个差异甲基化位点与 NP 退变相关。Kawaguchi 等^[9]研究调节细胞周期及基因组稳定性的生长停滞和 DNA 损伤诱导伽马(growth arrest and DNA damage-inducible gamma, GADD45G)基因, 以及细胞周期相关蛋白 1(cell cycle-

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通信作者: 田纪伟, E-mail: tjw609@163.com

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associated protein 1, CAPRIN1) 基因,发现 GADD45G 和 CAPRIN1 是退变 NP 组织中代表性的核心启动子区域高甲基化水平基因。Kawarai 等^[10]检测了小鼠包括编码序列和非编码序列的全基因组 DNA 甲基化水平,发现长期跑步降低了椎间盘基因组全局 DNA 甲基化水平,改善了腰痛相关的感觉症状。

长期以来炎症因子是 IVDD 分子生物学机制的研究热点。大量研究共同表明,DNA 甲基化促进了 IVDD 相关炎症因子的表达。Hou 等^[11]构建腰椎间盘退变小鼠模型,原位注射携带 DNMT1 的短发夹 RNA(short-hairpin RNA, shRNA)的腺相关病毒(adeno-associated virus, AAV)。实验结果表明,shDNMT1 引起的甲基化水平降低显著减少了促炎细胞因子肿瘤坏死因子(TNF- α)、白介素(IL)-1 β 和 IL-6 的表达,同时显著升高了抗炎细胞因子 IL-4 和 IL-10 的水平,减少了细胞凋亡并缓解了疼痛症状。沉默 DNMT1 可以抑制 IL-6 和 TNF- α 的表达,并降低 NOD 样受体热蛋白结构域相关蛋白 3(NLRP3)、凋亡相关斑点样蛋白(ASC)和半胱氨酸蛋白酶-1(caspase-1)的表达,缓解椎间盘退变^[12]。Cheng 等^[13]的研究表明,DNMT3a 通过对过氧化物酶体增殖物激活受体 γ (peroxisome proliferator-activated receptor γ , PPAR γ)启动子进行甲基化修饰,从而激活 NF- κ B 通路,促进细胞凋亡和 ECM 降解。DNMT3a 介导的 PPAR γ /NF- κ B 轴可能为治疗 IVDD 的新靶点。瞬时感受器电位香草酸亚型 1(transient receptor potential vanilloid subfamily 1, TRPV1)通道是一种配体门控非选择性阳离子通道,在多种组织器官内广泛分布^[14]。在 IVDD 过程中,TRPV1 被广泛激活,引起炎性疼痛^[15]。Hong 等^[16]发现伴随着 DNMT3b 的高表达,IVDD 发生后 NP 和 AF 区域中 TRPV1 的 DNA 甲基化水平升高,且 AF 区域比 NP 区域升高更明显。值得注意的是,个别文献也指出了 DNA 甲基化在 IVDD 过程中的保护作用。Luo 等^[17]发现,DNMT3b 过表达可促进 NP 细胞增殖,上调 II 型胶原和聚集蛋白聚糖的表达,同时下调基质金属蛋白酶(matrix metalloproteinase, MMP)-3 和 MMP-9 的表达,并抑制炎症反应。其机制为 DNMT3b 通过靶向瞬时受体电位 A1(transient receptor potential ankyrin 1, TRPA1)启动子,抑制环氧化酶-2(cyclooxygenase-2, COX-2)的表达,后者可通过抑制 Yes 相关蛋白(Yes-associated protein, YAP)的表达促进 NP 细胞凋亡。因此,DNMT3b 通过 TRPA1/COX-2/YAP 轴减少 NP 细胞凋亡及 ECM 降解,从而缓解大鼠 IVDD。

在人类椎间盘中,DNA 启动子区域的异常甲基化可导致 miRNA 的异常表达。Liu 等^[18]的研究发现 DNA 启动子低甲基化诱导 miR-132 表达上调,引起生长分化因子 5(growth differentiation factor 5, GDF-5)表达下调,通过 MAPK/ERK 信号通路促进 NP 细胞退变和 ECM 降解。Kang 等^[19]发现 DNA 启动子低甲基化导致 miR-494 表达上调,引起 Y 染色体性别决定区-盒转录因子 9(SOX-9)表达下调,促进人 NP 细胞凋亡和 ECM 降解。一项由 Zhao 等^[20]进行的研究表明,DNA 甲基化抑制了 miR-129-5p 的表达,促进 Beclin-1 的合成,

诱导 NP 细胞自噬。而 DNA 去甲基化使 miR-143 表达水平升高,抑制 B 细胞淋巴瘤-2 蛋白(B cell lymphoma 2, Bcl-2)表达,进而诱导 NP 细胞凋亡^[21]。Chen 等^[22]发现退变 NP 细胞中 miR-217 的表达水平降低由于其启动子区域的高甲基化水平所致,而过表达 miR-217 可通过调控 FBXO₂₁/ERK 通路抑制 IVDD。

高同型半胱氨酸血症(hyperhomocysteinemia, HHcy)是一种由叶酸和维生素 B6、B12 的缺乏引起的血清同型半胱氨酸浓度升高的疾病,可导致皮肤苍白、乏力等一系列症状^[23]。Zhang 等^[24]的临床流行病学研究表明,HHcy 是人类 IVDD 的独立危险因素。HHcy 可促进 DNMT1、DNMT3a 和 DNMT3b 的表达,并靶向谷胱甘肽过氧化物酶 4(glutathione peroxidase 4, GPX4),导致 GPX4 的 DNA 高甲基化并抑制其表达,从而通过氧化应激和铁死亡促进 NP 细胞退变。这一发现为 HHcy 与 IVDD 之间建立了新的联系。

2 组蛋白甲基化调控椎间盘退变

在真核细胞中,储存在 DNA 中的遗传信息通常以染色质结构的形式存在。核小体是染色质的核心结构元件,由 4 种核心组蛋白 H2A、H2B、H3 和 H4 各两分子组成一个球形蛋白八聚体^[25]。组蛋白尾部可以进行甲基化、乙酰化、磷酸化和泛素化等多种翻译后修饰,其中组蛋白甲基化修饰(histone methylation)在基因表达、控制染色质可及性、细胞周期调控等多种活动中发挥重要作用^[26]。组蛋白甲基化多发生在 H3、H4 中赖氨酸残基向外延伸的 N 端,受赖氨酸甲基转移酶(lysine methyltransferases, KMTs)和赖氨酸去甲基化酶(lysine demethylases, KDMs)的严格调控,有单甲基化(me1)、二甲基化(me2)和三甲基化(me3)三种甲基化状态。组蛋白赖氨酸甲基化依据其位置和甲基化状态决定转录激活或抑制,通常来说,H3K4、H3K36 和 H3K79 甲基化可促进转录,而 H3K9、H3K27 和 H4K20 甲基化则抑制转录^[27]。

近年来相关研究表明组蛋白甲基化与 IVDD 及椎间盘源性疼痛存在联系。Stover 等^[28]阐明了 IL-6/A 型激酶锚定蛋白 150(A-kinase anchoring protein 150, AKAP150)通路是大鼠 IVDD 的背根神经节(dorsal root ganglion, DRG)神经元敏化的潜在机制,并针对 AKAP150 进行 H3K9 me3 修饰,抑制了 AKAP150 的表达,降低了 DRG 神经元活性,减轻了炎症驱动的椎间盘源性疼痛。Stover 等^[29]的进一步研究发现 IVDD 产生 IL-6,通过 TRPA1、酸敏感离子通道 3(acid-sensing ion channel 3, ASIC3)和机械门控 Piezo2 离子通道引起了伤害性神经元活性增加,产生椎间盘源性疼痛。靶向 TRPA1、ASIC3 和 Piezo2 基因启动子组蛋白 H3K9 甲基化,可以抑制内源性基因表达,减轻椎间盘源性疼痛。Jiang 等^[30]发现组蛋白甲基转移酶 zeste 增强子同系物 2(enhancer of zeste homolog 2, EZH2)在退变 CEP 组织中高表达。EZH2 通过 H3K27me3 修饰,抑制 SOX-9 的表达,促进椎间盘退变。抑制 EZH2 可以上调 SOX-9 的表达,是 IVDD 的潜在治疗靶点。Xu 等^[31]发现组蛋白甲基转移酶 KMT2D 在人类退变 NP 组织中表达上调。

KMT2D 介导的 H3K4me1 和 H3K4me2 修饰促进 MMP-3、MMP-9 和 MMP-13 的表达,进而引起 ECM 分解和 NP 退变。沉默 KMT2D 或使用组蛋白 H3K4 甲基化抑制剂 OICR 可以逆转这一进程。总之,组蛋白甲基化调控 IVDD 已有了初步探索,但不同位置和甲基化状态的组蛋白甲基化调控 IVDD 的具体机制有待进一步研究。

3 RNA 甲基化调控椎间盘退变

RNA 甲基化包括 N6-甲基腺嘌呤 (N6-methyladenosine, m6A)、N1-腺苷酸甲基化 (m1A)、胞嘧啶甲基化 (m5C) 等形式,其中 m6A 是目前研究最多的 RNA 甲基化修饰形式,参与了转录、成熟、翻译、降解等 RNA 循环阶段的调控^[32]。m6A 修饰是动态可逆的,由甲基化转移酶“Writers”、去甲基化酶“Erasers”和甲基化阅读蛋白“Readers”共同调控。目前已知的 m6A 甲基化转移酶主要包括甲基化转移酶样蛋白 (methyltransferase like, METTL) 3、METTL14、METTL16、Wilms 肿瘤 1 关联蛋白 (Wilms tumor 1 associated protein, WTAP)、类病毒 m6A 甲基化转移酶相关蛋白 (VIRMA)、CCH 锌指蛋白 13 (ZC3H13)、RNA 结合基序蛋白 15 (RBM15)。m6A 去甲基化酶包括脂肪堆积和肥胖相关蛋白 (fat mass and obesity associated, FTO)、AlkB 同源物 5 (AlkB homolog 5, ALKBH5) 和 ALKBH3。m6A 甲基化阅读蛋白主要为 YTH 结构域蛋白家族,包括 YTHDF1、YTHDF2 和 YTHDF3^[32]。大量研究表明,m6A 修饰在细胞衰老及衰老相关疾病中发挥重要调控作用^[33],并与骨质疏松、骨肉瘤等骨相关疾病密切相关^[34]。

近年来,IVDD 过程中 m6A 修饰的作用成为了研究热点。Xiao 等^[35]在人退变的 CEP 组织中发现,METTL3 表达水平和 m6A 修饰水平显著升高。张力刺激通过 METTL3 介导的 m6A 修饰,破坏 SOX-9 mRNA 稳定性并下调 SOX-9 表达,从而抑制软骨终板 ECM 的合成。Xiao 等^[36]还发现 METTL3 通过 m6A 修饰促进 miR-126-5p 的表达,进而抑制靶基因磷酸肌醇-3 激酶调节亚基 2 (phosphoinositide-3-kinase regulatory subunit 2, PIK3R2) 的表达,抑制 PI3K/AKT 通路对软骨终板细胞的保护作用,导致代谢失调和终板退变。Zhu 等^[37]利用小鼠的水逃逸行为构建 IVDD 模型,通过甲基化 RNA 免疫共沉淀 (MeRIP) 技术检测 IVDD 后小鼠 NP 组织的 m6A 修饰,结果表明,退变 NP 细胞内 METTL3 和 METTL14 的表达显著上调,而 FTO 的表达显著下调;进一步研究发现 METTL14 通过 m6A 修饰上调了 miR-34a-5p 的表达,提高了 miR-34a-5p 与 DGCR8 的识别和结合水平,进而降低沉默信息调节因子 (silent information regulator 1, SIRT1) mRNA 的表达水平,促进 NP 细胞衰老^[38]。Chen 等^[39]的研究表明,氧化应激通过上调 METTL16 介导的 m6A 修饰,增加甲硫氨酸腺苷转移酶 2A (methionine adenosyltransferase 2A, MAT2A) 前体 mRNA 的降解,而下调 MAT2A 的表达,则会引起 S-腺苷甲硫氨酸 (S-adenosylmethionine, SAM) 的减少和 NP 细胞的凋亡。Wang 等^[40]构建了鼠尾静态压迫模型,对比退变前后 NP 组织的 m6A 修饰水平。结果表明,退变后大部分差异表达的 mRNA 和长链

非编码 RNA (lncRNA) 表现出明显的去甲基化。m6A 调控因子锌指蛋白 217 (Zinc finger protein 217, ZFP217) 通过激活 FTO 的转录,促进这些差异表达的 RNA 的去甲基化。环状 RNA (circRNA) 作为非编码 RNA 的重要组成部分,已被证实参与到 IVDD 在内的多种疾病的进展^[41]。circRNA 甲基化在 IVDD 中的作用值得进一步探索。Chen 等^[42]检测了 IVDD 患者 NP 组织中差异表达 circRNA 的 m6A 修饰水平,发现 circGPATCH2L 随着 m6A 水平的降低而上调。circGPATCH2L 作为三结构域蛋白 28 (tripartite motif containing 28, TRIM28) 的诱饵,通过抑制 TRIM28 的磷酸化和 P53 的降解,引起 DNA 损伤积累和细胞凋亡,从而导致 NP 退变。m6A 修饰后的 circGPATCH2L 可以被核糖核酸内切酶复合物 YTHDF2-RPL10-RNaseP/MRP 识别并切割降解,以维持 NP 细胞的生理状态。该研究阐述了 circRNA 甲基化在维持 NP 细胞生理状态中的作用机制,为 IVDD 治疗提供新的靶点。

4 甲基化共同调控椎间盘退变

最新的研究发现,三种甲基化修饰之间存在密切联系,可以通过特异性识别相互作用,共同调控基因表达^[43-45]。在椎间盘退变机制的研究过程中,已有部分文献报道了甲基化共调控作用。Tu 等^[46]发现吸烟增加椎间盘内肥大细胞限制性四聚体类胰蛋白酶 (mast cell-restricted tetramer-forming tryptases, MC-TTs) 的释放,去除了 METTL14 启动子上的抑制性 H3K9me3 修饰,引起了 METTL14 活性增加,上调了 DIX 结构域包含蛋白 1 (DIX domain containing 1, DIXDC1) mRNA 的 m6A 修饰水平,增加了 DIXDC1 的 mRNA 稳定性并促进其与 Disrupted in Schizophrenia-1 (DISC1) 的相互作用,激活经典 Wnt 信号通路加速 NP 细胞的衰老。Li 等^[47]在衰老的 NP 细胞中发现,KDM5a 表达减少可提高 WTAP 启动子区域的 H3K4me3 水平并促进 WTAP 的表达。WTAP 促进 DNA 损伤激活的非编码 RNA (non-coding RNA activated by DNA damage, NORAD) 的 m6A 修饰,并通过 YTHDF2 的识别诱导 NORAD 的降解,导致更多的 PUMILIO (PUM) 1/2 与 E2F 转录因子 3 (E2F transcription factor 3, E2F3) mRNA 结合,促进 E2F3 的降解,加速 NP 细胞的衰老。Li 等^[48]发现 KDM4a 上调介导的 H3K9me3 修饰减少后,ALKBH5 的表达增加,导致 DNMT3b 的 mRNA 去甲基化。去甲基化后 DNMT3b 的表达增加,通过对 E4F1 启动子进行甲基化修饰从而抑制其表达,促进 IVDD 的发生。

5 总结与展望

各项研究表明甲基化修饰在椎间盘退变与修复中发挥重要作用。甲基化修饰在各类疾病中具有广阔的治疗前景,如 DNMT 抑制剂阿扎胞苷和地西他滨,在本世纪初被批准用于治疗骨髓增生异常综合征等血液系统疾病,以及 EZH2 抑制剂他泽司他,在现有的临床试验中被证实具有较强的抗肿瘤作用^[49]。目前甲基化在治疗椎间盘退变及相应并发症上还处于探索阶段,已有研究证实了基于规律成簇的间隔短回文

重复 (clustered regularly interspaced short palindromic repeats, CRISPR) 表观基因组编辑技术治疗椎间盘退变及椎源性疼痛的可行性^[50]。将表观遗传药物与生长因子为主的生物疗法、干细胞介导的细胞疗法、基因疗法、组织工程结合形成不同的联合治疗策略,是可行的研究方向。此外,衰老相关疾病常为多基因疾病,不同基因涉及不同方向的甲基化改变。目前的基因缺失及过表达模型会造成甲基化组学的全局性变化,而不能实现特定位点或特定基因的甲基化。精确甲基化编辑技术是未来发展的方向和挑战。最后,目前椎间盘退变主要的研究对象为小鼠和家兔,人类体内甲基化编辑治疗的有效性、特异性和安全性有待进一步验证。未来需要通过更深入的研究阐明上述问题,进一步揭示甲基化修饰的相关作用机制,为椎间盘退变诊疗提供新思路。

利益冲突 无

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