Epigenetic research methods and animal models for intervertebral disc degeneration (Review)

XINLIANG CUI[1*], LI ZENG[2*], WEI ZHANG[1], LEIMIN XI[1], RUI WANG[1], DIFF JIA[3], SAFA[1], HAOYU FENG[1] and HUIDONG JIA[2]

[1]Department of Orthopedics, The Third Hospital of Shanxi Medical University, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, Taiyuan, Shanxi 030032, P.R. China; [2]Center for Biomedical Clinical Research, The Third Hospital of Shanxi Medical University, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, Taiyuan, Shanxi 030032, P.R. China; [3]Medical Science Division, University of Oxford, Oxford OX1 4AR, UK

Received January 22, 2026; Accepted April 30, 2026

DOI: 10.3892/mmr.2026.13913

Abstract. Intervertebral disc degeneration (IVDD) is increasingly recognized as a systemic collapse of the epigenetic regulatory network, driven by cellular senescence and environmental stressors. The present review provides an overview of the epigenetic regulatory mechanisms governing IVDD, focusing on the dynamic interplay between DNA methylation, histone modifications, N6-methyladenosine RNA methylation and the non-coding RNA regulatory triad (microRNAs, long non-coding RNAs and circular RNAs). The present study evaluates advanced research methodologies (ranging from site-specific methylation typing and transposase accessible chromatin sequencing to single-cell multi-omics and artificial intelligence-driven predictive modeling) that resolve the spatial and cellular heterogeneities of the degenerating disc niche. Furthermore, the translational constraints of current animal models were critically assessed, advocating for a strategic shift from acute needle-puncture insults to physiologically relevant aging and genetically engineered progeroid models to better recapitulate human 'epigenetic drift'. Finally, the therapeutic potential of targeted epigenetic editing via CRISPR/dCas9 systems and the development of stimuli-responsive nanocarriers for precision delivery are highlighted. By bridging methodological innovation with robust model selection, the present review offers a roadmap for transitioning molecular insights into clinical regenerative therapies for spinal health.

Contents

Correspondence to: Professor Huidong Jia, Center for Biomedical Clinical Research, The Third Hospital of Shanxi Medical University, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, 99 Longcheng Street, Xiaodian, Taiyuan, Shanxi 030032, P.R. China
E-mail: huidongjia@hotmail.com

Professor Haoyu Feng, Department of Orthopedics, The Third Hospital of Shanxi Medical University, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, 99 Longcheng Street, Xiaodian, Taiyuan, Shanxi 030032, P.R. China
E-mail: fenghaoyu@sxbqeh.com.cn

*Contributed equally

Abbreviations: ACAN, aggrecan; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; AGEs, advanced glycation end products; AF, annulus fibrosus; ATAC-seq, transposase accessible chromatin sequencing; CEP, cartilage endplates; ceRNAs, competitive endogenous RNAs; ChIP-Seq, chromatin immunoprecipitation sequencing; circRNAs, circular RNAs; COL2A1, type II collagen; DNMTs, DNA methyltransferases; ECM, extracellular matrix; ERN, epigenetic regulatory network; GAGs, glycosaminoglycans; HATs, histone acetyltransferases; HDACs, histone deacetylases; IVD, intervertebral disc; IVDD, intervertebral disc degeneration; LBP, low back pain; lncRNAs, long non-coding RNAs; miRNAs, microRNAs; MMPs, matrix metalloproteinases; MNase-seq, micrococcal nuclease sequencing; MSP, methylation-specific PCR; mtDNA, mitochondrial DNA; m^6A, N6-methyladenosine; ncRNA, non-coding RNA; NP, nucleus pulposus; NPCs, nucleus pulposus cells; PPARγ, peroxisome proliferator-activated receptor γ; PTM, post-translational modification; ROS, reactive oxygen species; TETs, ten-eleven translocation enzymes; WGBS, whole-genome bisulfite sequencing; 3'UTR, 3' untranslated region; 5-Aza, 5-azacytidine; 5-hmC, 5-hydroxymethylcytosine; 5-mC, 5-methylcytosine

Key words: epigenetic regulations, intervertebral disc degeneration, DNA methylation, histone modifications, non-coding RNAs, animal models in epigenetics

1. Introduction

Humans are predisposed to low back pain (LBP) as an evolutionary trade-off for a mechanically stressed spine that has adapted from quadrupedal to bipedal locomotion. LBP is a prevalent health problem. Statistical reports indicate that the global incidence rate of LBP ranges between 13.1 and 28.5% (1). Intervertebral disc degeneration (IVDD), a complex and progressive process, is the leading cause of chronic LBP and imposes a substantial global health and economic burden (2). The intervertebral disc (IVD) is the largest avascular structure in the human body, serving as the spine's primary mechanical and biological unit. Its highly specialized architecture, comprising a proteoglycan-rich nucleus pulposus (NP), a collagen-dense annulus fibrosus (AF) and superior and inferior cartilage endplates (CEP), enables efficient load distribution while preserving spinal flexibility, stability and mobility (3-6).

Once regarded largely as a consequence of mechanical wear and tear, IVDD is now recognized as a complex, age-associated degenerative process shaped by the interplay between biomechanical forces, systemic metabolic stressors and tightly regulated local biological signals (7). At the cellular level, progressive loss and dysfunction of NP cells (NPCs), including chondrocyte-like, notochordal and progenitor populations, occur through senescence and apoptosis, fundamentally undermining disc homeostasis (8). Increasing evidence suggests that metabolic dysregulation exerts a stronger influence on IVDD progression than biomechanical alterations alone (9). Mendelian randomization analyses have identified type 2 diabetes, hypertriglyceridemia, elevated fasting glucose and increased HbA1c as notable causal risk factors for IVDD, whereas the contribution of obesity-related traits remains less certain (10).

Pathologically, IVDD unfolds through a cascade initiated by disruption of the balance between anabolic and catabolic activities within the disc (2,7,8,11). Hallmark features include extracellular matrix (ECM) degradation, chronic inflammation, oxidative stress, cellular senescence and dysregulated cell death modalities, such as apoptosis, pyroptosis and ferroptosis, accompanied by aberrant neoinnervation and neovascularization. Anabolic synthesis of type I/II collagens, glycosaminoglycans (GAGs) and proteoglycans such as aggrecan (ACAN) and versican progressively declines, while catabolic mediators, notably matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family members, are upregulated, accelerating ECM breakdown and loss of disc hydration and structural integrity (11,12). These molecular and structural derangements culminate in disc bulging, reduced NP cellularity and water content, disc height loss and impaired biomechanical competence. In combination with disc protrusion and local inflammatory responses, such changes may compress adjacent nerve roots and manifest clinically as lumbar disc herniation (13). Compounding these challenges, the avascularity and hostile microenvironment of mature IVDs, characterized by hypoxia, acidosis and restricted nutrient diffusion, severely limit intrinsic regenerative capacity (5,14-17).

Aging and accelerated cellular senescence are central drivers of IVDD and are tightly linked to epigenetic dysregulation. Epigenetic alterations fuel metabolic imbalance, persistent inflammation, oxidative damage, genomic instability and progressive ECM degradation. Key regulatory layers include DNA methylation, histone modifications, diverse classes of non-coding RNAs (ncRNAs) and RNA-specific modifications such as N6-methyladenosine (m^6A) methylation. Specifically, DNA methylation participates in the degenerative process by altering the methylation status of cytosine bases and regulating the expression of genes associated with ECM metabolism, inflammatory responses and ion channel function. Mechanistically, this epigenetic modification involves the addition of methyl groups to cytosine residues, typically silencing gene expression when occurring at promoter regions (18). Histone modifications (such as methylation and acetylation) modulate cellular senescence and inflammatory responses by remodeling chromatin structure and influencing the accessibility of downstream pro-degenerative or protective genes (19). Meanwhile, microRNAs (miRNAs), long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) function as powerful epigenetic regulators by shaping chromatin landscapes, recruiting chromatin-modifying complexes, and controlling mRNA stability and translation (20). Beyond these classical mechanisms, RNA-specific modifications such as m^6A methylation represent a rapidly emerging layer of epigenetic control that influences RNA stability, splicing and translation, and has been implicated in regulating cellular senescence and matrix metabolism in degenerative conditions such as IVDD (21). Through extensive crosstalk with signaling pathways, these mechanisms orchestrate nearly every stage of IVDD pathogenesis.

In the context of aging, epigenetic changes mainly exhibit two patterns: One is a stochastic and cumulative 'epigenetic drift' and the other is a highly programmed 'epigenetic clock'. Epigenetic drift refers to the random and disordered alterations in the epigenome (especially DNA methylation patterns) that occur with advancing age, reflecting the gradual failure of epigenetic maintenance systems, increased genomic instability and exacerbated interindividual heterogeneity (22). By contrast, the epigenetic clock is an algorithmic model constructed by integrating age-dependent DNA methylation profiles at specific CpG sites, which can accurately estimate an individual's biological age (23). Notably, epigenetic drift forms the basis of epigenetic clock signals, yet the two display markedly distinct response patterns to lifespan interventions, developmental processes and cellular dedifferentiation (24). Therefore, deciphering how physiological epigenetic drift transitions into pathological degeneration, how epigenetic signatures evolve across disease stages and populations, and whether targeted epigenetic reprogramming can halt or reverse IVDD represents a critical frontier with notable therapeutic promise (2,13,18,25).

Crucially, the translation of epigenetic discoveries into clinical relevance depends on the choice of appropriate experimental models. Although murine needle-puncture models are widely used, their capacity to mimic the slow, cumulative epigenetic drift characteristic of human disc aging is increasingly questioned due to evolutionary, anatomical and biomechanical disparities. In the present review, current

epigenetic research methodologies applied to IVDD are evaluated, highlighting their strengths and limitations in resolving complex regulatory networks. Commonly used animal models are further critically evaluated, focusing on their translational relevance for epigenetic studies, to provide a framework for more precise and clinically meaningful disc degeneration research.

2. Epigenetic research methods in IVDD

DNA methylation. DNA methylation status reflects a dynamic equilibrium between DNA methyltransferases (DNMTs) and demethylating ten-eleven translocation (TET) enzymes. DNMTs add methyl groups to the fifth carbon of cytosine residues, forming 5-methylcytosine (5-mC) within CpG dinucleotides. The DNMT family orchestrates this landscape: DNMT1 functions as the maintenance methyltransferase during replication, whereas DNMT3A and DNMT3B are responsible for *de novo* methylation. Conversely, these methylation footprints can be removed by TETs, which oxidize 5-mC to 5-hydroxymethylcytosine (5-hmC) and further to formyl and carboxyl-cytosine derivatives (18,26,27). The central role of these enzymes in disease progression and their therapeutic potential has been highlighted.

Mechanistic drivers in IVDD pathogenesis. DNA methylation acts as a pivotal switch in distinct pathological cascades, such as senescence and ferroptosis (DNMT3B axis), and inflammation and matrix catabolism (DNMT3A axis).

Upregulation of DNMT3B can be a dual driver of NPC senescence and ferroptosis. In one pathway, excessive DNMT3B activity, triggered by loss of m^6A modification due to elevated activity of AlkB homolog 5, RNA demethylase (ALKBH5), an m^6A demethylase, induces hypermethylation of the transcription factor E4F1, promoting NPC senescence and IVDD. Silencing ALKBH5 or DNMT3B in IVDD rats confers protection against degeneration *in vivo* (28). In parallel, inside the tert-butyl hydroperoxide-induced degenerative human NPCs, DNMT3B is upregulated and exacerbates ferroptosis and oxidative stress by downregulating the ferroptosis suppressor SLC40A1. Therapeutic intervention with the pan-DNMT inhibitor 5-azacytidine (5-Aza) restores SLC40A1 and alleviates ferroptosis in rat models (29).

DNMT3A abundance and activity increase with disease severity, promoting hypermethylation and suppression of the peroxisome proliferator-activated receptor γ (PPARγ) promoter. This triggers a chain reaction leading to NF-κB activation, exacerbating apoptosis and matrix imbalance. Suppression of DNMT activity effectively preserves NPC viability and matrix homeostasis. Specifically, silencing DNMT3A reduces IL-1β-induced apoptosis and ECM degradation, while 5-Aza treatment counters endoplasmic reticulum stress by inhibiting DNMT1/3A, thereby demethylating the PPARγ promoter to restore its expression *in vitro*. Intradiscal delivery of short hairpin RNA-DNMT3A in rat IVDD models restored ECM anabolism and reduced NPC apoptosis (30,31).

The epigenetic landscape may also differ between symptomatic and asymptomatic IVDD. A study using DNA methylation arrays comparing painful vs. non-painful IVDD discovered differential promoter CpG methylation in equally degenerated discs. Genes related to matrix catabolism, such as MMP2 and MMP28, were less methylated and upregulated in the painful discs, whereas genes associated with matrix anabolism, including CHSY1 and chondroadherin, show an opposite pattern (32). In addition, the global DNA methylation status in rat AF following puncture-induced IVDD has been assessed by measuring epigenetic markers, including 5-mC, 5-hmC, DNMTs and TETs. Elevated global hypermethylation and increased DNMT1 expression are notably associated with abnormal expression of the pain-related ion channel transient receptor potential cation channel subfamily V member 1, suggesting a key role for these epigenetic changes in ectopic nerve innervation of the IVD and pain development (33). However, few studies have directly or systematically stratified epigenetic signatures in IVDD by etiology, sex or other demographic characteristics (34).

Evolution of detection techniques. A plethora of high-throughput detection techniques have been applied to DNA methylation analysis in IVDD, ranging from single-locus (site-specific DNA methylation typing) to global methylome profiling, as well as cutting-edge epigenome editing tools such as CRISPR-dCas9-Tet1-mediated DNA methylation editing. Locus-specific approaches focus on individual CpG sites or regions and employ techniques such as methylation-specific PCR (MSP) and bisulfite sequencing PCR (BSP), sometimes combined with restriction analysis or matrix-assisted laser desorption ionization time-of-flight mass spectrometry for quantitative assessment. Using MSP and BSP, hypermethylation of the secreted protein acidic and rich in cysteine (SPARC) promoter was observed in NP tissues from middle-aged (6 months) mice, increasing further in older (15 months) mice, whereas young (3 months) mice showed minimal methylation. This age-related SPARC silencing was associated with anatomical signs of intervertebral disc degeneration (e.g., disc height loss in mice and imaging evidence of degeneration in humans) and behavioral signs of chronic low back pain (e.g., movement-evoked axial discomfort and cold hypersensitivity in mice, and self-reported chronic pain in humans) in both species (35).

By comparison, global DNA methylome profiling provides genome-wide quantitative information on DNA methylation patterns. These approaches include whole-genome bisulfite sequencing (WGBS), methylation-sensitive restriction enzyme-based sequencing and methylated DNA immunoprecipitation sequencing (36). Human NP studies using WGBS have identified 220 differentially methylated loci in NF-κB, MAPK and Wnt signaling pathways between early and advanced stages, with late-stage samples showing predominant hypermethylation (216 loci). This hypermethylation is associated with silencing of regulatory genes and dysregulation of cell adhesion, contributing to the progression of disc degeneration (37).

Histone modification

Histone acetylation. Post-translational modifications (PTMs) of histones, including methylation, acetylation, phosphorylation, ubiquitination and ADP-ribosylation, modulate chromatin, thereby controlling genome accessibility architecture. Central to this regulation is the dynamic equilibrium of histone acetylation. The dysregulation of histone acetylation at the

N-terminal tail and core domains is catalyzed by histone acetyltransferases (HATs) and histone deacetylases (HDACs) (38). HATs neutralize the positive charge of lysine residues, relaxing chromatin to promote transcription, whereas HDACs restore this charge, tightening histone-DNA interaction and inducing chromatin condensation and transcriptional repression (39). In IVDD, the dysregulation of the HAT/HDAC balance reflects broader epigenetic regulatory network (ERN) instability, with specific HDACs serving opposing roles.

Elevated expression of HDAC7 and HDAC4, which act as detrimental regulators, is associated with disease progression (40-42). Conversely, broad inhibition using HDAC inhibitors (such as SAHA) can shift cytokine-stimulated chondrocytes from a catabolic (MMP-2/3/9, ADAMTS-5) to an anabolic (COL2A1, aggrecan) phenotype (43). However, not all deacetylases are deleterious; HDAC9 and SIRT6 appear protective in both aging and injury models: HDAC9 preserves nucleus pulposus cell viability and suppresses apoptosis by deacetylating RUNX3 and promoting its ubiquitin-proteasomal degradation, while SIRT6 maintains disc health by reducing DNA damage, enhancing autophagy, and inhibiting cellular senescence and the SASP (44,45). Modulating this landscape offers tangible therapeutic avenues, exemplified by the plasmid-mediated delivery of pluripotency factors (Oct4, Krüppel-like factor 4 and Sox2), which reduces repressive H4K20me3 levels to alleviate low back pain and degeneration in rat models (46).

Canonical histone methylation and inflammation. While acetylation acts as a rapid switch, histone methylation provides stable, long-term regulation of gene expression. Previous high-impact evidence highlights the enhancer of zeste homolog 2 (EZH2)-H3K27me3 axis as a critical gatekeeper of inflammation in IVDD. EZH2 is the methyltransferase responsible for placing the repressive H3K27me3 mark. In degenerative NPCs, EZH2 expression is markedly downregulated. This loss leads to the erasure of H3K27me3 at the promoters of key pro-inflammatory and pro-pyroptotic genes, such as Dickkopf Wnt signaling pathway inhibitor 1 (DKK1) and MAPK1. The consequent de-repression of these genes activates the NLRP3 inflammasome and drives pyroptosis (inflammatory cell death). Mechanistically, restoring EZH2 function or inhibiting its downstream targets (such as DKK1) has been demonstrated to re-establish the repressive chromatin state, thereby attenuating the inflammatory cascade and preserving the disc matrix (47,48).

Histone lactylation. Lactate-derived histone lysine lactylation (Kla) has emerged as a novel mechanism linking cellular metabolism directly to gene transcription. Catalyzed by lactyltransferases (such as p300) and removed by delactylases, this modification involves attaching lactyl groups from glycolysis-derived lactate to histone lysines (49).

In IVDD, a metabolic shift toward hyperglycolysis leads to intracellular lactate accumulation. Single-cell RNA sequencing (scRNA-seq) confirms that this excess lactate drives H3K18la (lactylation of H3 lysine 18) specifically at the promoter of acyl-CoA synthetase long-chain family member 4 (ACSL4). The transcriptional activation of ACSL4 primes NPCs for lipid peroxidation and ferroptosis. Consequently, targeting this axis, either by silencing lactate dehydrogenase A [LDHA; via AAV9-small interfering (si)RNA-Ldha] or inhibiting glycolysis with 2-deoxy-D-glucose, suppresses H3K18la levels and ameliorates degeneration. Clinical relevance is further supported by integrated bulk and single-cell analyses showing that histone lactylation levels, and lactylation-related genes such as chromobox 3, are strongly associated with IVDD severity in human tissues (50,51).

High-resolution profiling. Deciphering these complex modifications requires a transition from bulk assays to high-resolution genomic mapping. By combining chromatin immunoprecipitation-sequencing (ChIP-seq) with RNA-seq, researchers have mapped genomic interactions, identifying nuclear receptor 4A3 as a direct downstream target of the regulator early growth response factor 1, whose binding promotes NPC apoptosis and ECM breakdown (52). To understand chromatin accessibility, transposase accessible chromatin sequencing has been integrated with transcriptional profiling, revealing that activating protein-1 transcription factors, particularly c-Fos, increase chromatin accessibility to upregulate cell migration-inducing and hyaluronan-binding protein, a hyaluronidase that disrupts matrix homeostasis. Emerging techniques such as Cleavage Under Targets and Tagmentation (CUT&Tag) now offer higher signal-to-noise ratios than traditional ChIP, revealing that H3K18la is specifically enriched at the promoters of pro-inflammatory genes such as thrombospondin 1 in degenerative NPCs. Furthermore, advanced multi-omics integration has uncovered non-canonical mechanisms, such as the methyltransferase EZH2 monomethylating the RNA-binding protein DDX1. Validated by RNA-seq and RNA immunoprecipitation (RIP)-seq, this modification triggers exon skipping in matrin 3 (MATR3), generating a short isoform (MATR3-S) that disrupts nuclear architecture and activates Wnt signaling (53-56).

m^6A methylation. RNA methylation constitutes the most abundant internal RNA modification. Particularly, m^6A methylation has drawn great attention for its role in fine-tuning gene expression in IVDD. This reversible modification involves adding a methyl group to the nitrogen-6 position of adenosine in RNA, regulating RNA stability, splicing, decay and translation. Imbalances in m^6A writers [methyltransferase 3, N6-adenosine-methyltransferase complex catalytic subunit (METTL3), methyltransferase 14, N6-adenosine-methyltransferase non-catalytic subunit and WT1-associating protein], erasers [α-ketoglutarate-dependent dioxygenase FTO (FTO) and ALKBH5] and reader proteins have been implicated in disease progression.

Advances in profiling the m^6A epitranscriptome are flourishing, driven by a wide spectrum of high-throughput techniques. The current toolkit encompasses three major categories based on detection principles: Antibody-based methods use anti-m^6A antibodies to immunoprecipitate methylated RNA fragments followed by sequencing. Owing to their relative maturity and ease of use, such approaches are the preferred choice for early exploratory research and large-scale sample screening. m^6A-seq/m^6A RNA immunoprecipitation sequencing (MeRIP-seq) represents the foundational approach, enriching m^6A-containing RNA fragments through immunoprecipitation and identifying methylated regions at 100-200 nucleotide resolution; this method has been widely employed for mapping m^6A distribution transcriptome-wide in diverse

biological systems. m^{6}A-seq2 improves upon this with optimized fragmentation and library preparation protocols. m^{6}A individual-nucleotide resolution crosslinking and immunoprecipitation sequencing employs UV crosslinking to covalently link antibodies to m^{6}A sites, enabling single-nucleotide resolution mapping through characteristic mutation signatures at crosslink sites. m^{6}A-level and isoform-characterization sequencing combines immunoprecipitation with full-length transcript sequencing to quantify m^{6}A levels on individual transcript isoforms (57-59).

While antibody-based methods are widely adopted for transcriptome-wide profiling, they require substantial RNA input (typically 300 μg for standard protocols, although optimized versions can work with as little as 500 ng) and may exhibit cross-reactivity with other modifications such as N6,2'-O-di methyladenosine (57,60). Refined protocols using alternative antibodies (such as the highly specific, commercially available monoclonal anti-m6A antibody produced by Cell Signaling Technology, Inc.) have markedly reduced input requirements and costs while maintaining comparable sensitivity (61).

Chemical-based approaches achieve antibody-free detection through selective chemical reactions that distinguish methylated from unmethylated adenosines. By directly labeling or differentiating modified bases via chemical reactions, these approaches enable more accurate quantification and absolute detection. m^{6}A-selective electrophilic affinity labeling sequencing (m^{6}A-SEAL-seq, a chemical strategy that covalently captures oxidized m^{6}A electrophilic intermediates using specific probes) uses FTO-assisted chemical labeling to tag m^{6}A sites, enabling enrichment, imaging and sequencing applications with good sensitivity and specificity (62). m^{6}A-selective allyl chemical labeling-seq employs chemical derivatization to enable base-resolution mapping with quantification capabilities. m^{6}A-label-seq utilizes metabolic labeling combined with chemical reactions.

m^{6}A-glyoxal and nitrite-mediated deamination of unmethylated adenosines-seq represents a breakthrough method that deaminates unmethylated adenosines to inosines while leaving m^{6}A residues intact, enabling absolute quantification of m^{6}A stoichiometry at single-base resolution, conceptually analogous to bisulfite sequencing for DNA methylation (63,64). This unbiased approach reveals clustered m^{6}A modifications with differential distribution and stoichiometry, and characterizes m^{6}A dynamics under stress conditions (64). Updated versions (GLORI 2.0 and 3.0) have dramatically reduced RNA input requirements to as low as 500-1,000 cells while accelerating reaction times and minimizing RNA degradation, greatly expanding applicability for low-input samples (65). Chemical methods offer unbiased, quantitative m^{6}A detection without antibody-related artifacts.

Enzyme-based methods exploit enzymatic activities that discriminate between m^{6}A and unmodified adenosine. By utilizing the specific cleavage or editing activities of enzymes, these approaches offer an antibody-independent alternative, which is particularly suitable for studies requiring high sensitivity or aiming to avoid antibody-related biases. m^{6}A-m^{6}A-sensitive RNA-endoribonuclease-facilitated (MATZER)-seq uses the bacterial endoribonuclease MazF, which cleaves unmethylated ACA motifs but not m^{6}A-containing ACA sequences, generating characteristic cleavage patterns that reveal m^{6}A positions at single-nucleotide resolution. This method permits systematic quantitative profiling of m^{6}A at 16-25% of expressed sites and enables *de novo* discovery of m^{6}A sites, calibration of antibody-based approaches and quantitative tracking of m^{6}A dynamics during differentiation. m^{6}A-sensitive RNA-endoribonuclease-facilitated sequencing with refinement improves upon MAZTER-seq with enhanced computational analysis (66).

m^{6}A-deamination adjacent to RNA modification targets (DART)-seq employs a fusion protein combining the cytidine deaminase apolipoprotein B mRNA editing enzyme catalytic subunit 1 with the m^{6}A-binding YTH domain, which induces C-to-U deamination at sites adjacent to m^{6}A that are then detected by standard RNA-seq (60,67,68). DART-seq requires minimal RNA input (as little as 10-50 ng of total RNA) and can track m^{6}A accumulation in cells over time; long-read DART-seq provides insights into m^{6}A distribution along individual transcripts. However, DART-seq depends on the presence of cytidines near m^{6}A sites and may have variable efficiency across different sequence contexts (68,69).

Each methodological category offers distinct advantages: Antibody-based methods provide established workflows for transcriptome-wide profiling with extensive bioinformatics support; chemical-based approaches enable absolute quantification without antibody bias; and enzyme-based methods offer low-input, antibody-free alternatives with unique detection mechanisms. The choice of method depends on research goals, available sample quantity, desired resolution (region-level vs. single-nucleotide) and whether absolute or relative quantification is needed. For IVDD research involving limited clinical specimens, low-input methods such as GLORI 3.0 or DART-seq may be particularly advantageous.

3. Mapping the degenerative epitranscriptome: Inflammation and autophagy

Global and focal m^{6}A epitranscriptomic changes in IVDD have been mapped both in human and rodent tissues. MeRIP-seq analysis [validated by reverse transcription-quantitative PCR (RT-qPCR)] identified distinct signatures: Annexin A2 transcripts show increased m^{6}A levels in degenerative discs, whereas SLC3A2 and pre-B-cell leukemia transcription factor 3 show decreased methylation. These differentially methylated transcripts were enriched in pathways highly relevant to disc pathology, such as NF-κB signaling and ECM-associated processes, suggesting that m^{6}A modifications are central to influencing inflammation and maintaining matrix homeostasis to IVDD progression (70). Dynamic m^{6}A profiling of aging rat NP tissue using MeRIP-seq combined with RNA-seq further revealed a progressive, age-dependent increase in global m^{6}A levels. This accumulation is associated with gene expression shifts in pathways linked to inflammation, cellular stress responses and ECM metabolism (71).

Beyond these foundational findings, recent evidence highlights the pivotal role of m^{6}A in regulating autophagy and pyroptosis, two critical fate determinants in IVDD. New studies indicate that METTL3-mediated m^{6}A modification stabilizes autophagy related 7 mRNA, a core autophagy gene. In degenerative NPCs, elevated METTL3 promotes senescence by disrupting the autophagic flux (72,73). Conversely,

the eraser FTO has been shown to demethylate NLRP3 mRNA. Downregulation of FTO during degeneration leads to m^6A-dependent stability of NLRP3, thereby activating the inflammasome and driving pyroptosis in NPCs (74). These findings suggest that the m^6A machinery acts as a 'molecular switch' between cell survival (autophagy) and inflammatory death (pyroptosis).

Diagnostic stratification via regulator variability. Beyond pathogenesis, m^6A regulator expression may serve as a sensitive indicator of inter-individual epigenetic variability. A comprehensive analysis using Gene Expression Omnibus datasets, assisted by machine learning and LASSO regression, screened for differentially expressed m^6A regulators and analyzed associated immune infiltration characteristics in IVDD. The study generated a predictive model based on five key regulators (RBM15, YTHDC1, YTHDF3, HNRNPA2B1 and ALKBH5). Validated in rat models, this signature stratifies patients into eight distinct clusters, each associated with unique immune infiltration characteristics. These clusters may underlie subpopulation-specific IVDD trajectories and immune responses, offering a roadmap for personalized epigenetic diagnostics (75).

ncRNAs

Regulatory triad: miRNAs, lncRNAs and circRNAs. ncRNAs orchestrate gene expression at transcriptional or post-transcriptional levels without encoding proteins. In IVDD, three major classes interact synergistically to modulate ECM metabolism, apoptosis, senescence, inflammation and autophagy (20,76-78). miRNAs, which are 18-22 nucleotides in length, act as endogenous silencers by binding to the 3' untranslated regions of target mRNAs, leading to mRNA degradation or translational inhibition. Their effects frequently converge on the NF-κB signaling hub, thereby driving matrix degradation and cell death. These miRNAs are themselves regulated by longer transcripts, including lncRNAs and circRNAs. lncRNAs, typically exceeding 200 nucleotides, modulate gene expression via complex secondary structures that influence chromatin remodeling and mRNA stability. Meanwhile, circRNAs are characterized by a covalent closed-loop structure that renders them resistant to exonuclease degradation, and also serve as protein decoys or scaffolds, influencing diverse cellular processes. A dominant regulatory mechanism in disc homeostasis is the competing endogenous RNA (ceRNA) hypothesis, wherein lncRNAs and circRNAs contain miRNA response elements that sponge specific miRNAs, thereby lifting the repression of downstream target genes. IVDD-associated ncRNAs can be protective or detrimental; what matters most is the perturbation of the regulatory network (20,76-78).

High-resolution research methodologies. Research on ncRNAs employs an integrated workflow combining high-throughput discovery with mechanistic validation. The process typically begins with omics-based profiling, using RNA-seq, microarray hybridization or single-cell transcriptomics to map dysregulated profiles (79-81). For instance, microarray analysis has identified the lncRNA RP11-296A18.3 and its neighboring gene Fas associated factor 1 as pro-apoptotic drivers in degeneration (82). Once candidates are identified, their spatial expression is defined using RT-qPCR, FISH or *in situ* hybridization. To confirm the physical interaction between ncRNAs, researchers utilize luciferase reporter assays to verify binding sites and RIP to identify protein partners. Specifically, Argonaute 2 (AGO2)-RIP is crucial for validating the sponging mechanism; since AGO2 is the core component of the RNA-induced silencing complex, enriching for AGO2-bound transcripts confirms the regulatory roles of key molecules such as circVMA21, circFOXO3, circEYA3 and circATXN1 (83-86). Finally, functional roles are assessed via gain- and loss-of-function strategies (using mimics, inhibitors or CRISPR) paired with phenotypic readouts such as TUNEL staining for apoptosis, EdU incorporation for proliferation and Alcian blue staining for matrix synthesis (87-90).

Beyond cytoplasmic sponging, advanced techniques are uncovering novel ncRNA functions in the nucleus and extracellular space. Emerging methods such as CUT&Tag and CUT & Release Using Nuclease bridge RNA-protein binding with genomic localization, revealing that some ncRNAs recruit transcriptional regulators directly to chromatin (91). For example, the nuclear circFUNDC1 was found to recruit CDK9 to the FUNDC1 promoter, boosting transcription and promoting mitophagy to protect against oxidative stress (92). Furthermore, previous evidence highlights that exosomes serve as critical vehicles for intercellular rejuvenation. Urine-derived stem cell exosomes have been shown to deliver the matrilin-3 protein to NPCs, where it activates the TGF-β signaling pathway to promote matrix synthesis and suppress senescence (93). These findings establish exosomal ncRNA tracking and delivery as a promising non-viral strategy for disc repair.

Notably, the plasticity and complexity of ncRNA networks are best illustrated by the conflicting roles of the lncRNA HOTAIR, which suggests a biphasic function driven by disease stage or stress type. Although some studies report that HOTAIR is downregulated in patients, where its restoration exerts a protective effect by suppressing MMP-13 via the sponging of miR-642a-5p (94) or preventing apoptosis by sequestering miR-34a (95,96), more evidence suggests that HOTAIR upregulation is associated with IVDD severity by activating the Wnt/β-catenin pathway and AMPK/mTOR/ULK1 pathway to drive the NPC degenerative changes. Moreover, targeted inhibition via siHOTAIR effectively improved ECM composition and attenuated IVDD symptoms in rat models (97,98). This discrepancy likely stems from variations in sample source, as 'control' samples often differ (such as idiopathic scoliosis vs. trauma) and 'disease' samples represent different pathological stages (acute inflammation vs. chronic oxidative stress). It remains an intriguing research avenue to discern how regulators such as HOTAIR may transition from an anti-apoptotic factor under inflammatory cues to a pro-degenerative factor under chronic stress conditions.

Advances in mitochondrial epigenetics (mitoepigenetics) in IVDD. Mitoepigenetics represents an emerging regulatory layer distinct from nuclear epigenetics, with critical implications for IVDD. Epigenetic mechanisms regulate mitochondrial quality control (MQC) through interconnected pathways involving DNA methylation, histone modifications

and m^{6}A RNA methylation, directly influencing mitochondrial biogenesis, mitophagy, dynamics and oxidative stress responses in NPCs (14,99,100).

Distinguishing mitochondrial from nuclear epigenetics. Mitoepigenetics differs fundamentally from nuclear epigenetics in DNA packaging, modification patterns and functional consequences. Nuclear DNA is packaged with histone proteins into nucleosomes, allowing extensive histone modifications (acetylation, methylation and phosphorylation) that regulate gene expression (99,101). By contrast, mitochondrial DNA (mtDNA) exists in nucleoids, DNA-protein complexes lacking histones that instead contain mitochondrial transcription factor A and other nucleoid-associated proteins (99,102). Epigenetic regulation in mitochondria therefore relies on PTMs of these nucleoid proteins rather than histone modifications (103).

Although DNA methylation occurs in both genomes, critical differences exist. Nuclear DNA methylation primarily occurs at CpG dinucleotides and typically represses gene transcription when present at promoters (104). mtDNA methylation involves multiple patterns including 5-mC, 5-hmC, and N6-methyladenine (105). Notably, experimental evidence suggests that GpC methylation, not CpG methylation, may be the primary functional regulator of mitochondrial gene expression, and mtDNA methylation at promoters appears to activate rather than repress transcription; diametrically opposed to nuclear DNA methylation effects (105,106).

Mitoepigenetic modifications utilize mitochondrial isoforms of nuclear enzymes. DNMTs (mtDNMT1 and DNMT3B) localize to mitochondria and methylate mtDNA, while TET-like hydroxymethylase activity has been detected in mitochondria for demethylation (106). A unique aspect of mitochondrial epigenetics is its bidirectional relationship with nuclear epigenetics: Mitochondria produce metabolites (acetyl-CoA, α-ketoglutarate and S-adenosylmethionine) that serve as substrates for nuclear epigenetic enzymes, while nuclear-encoded factors regulate mitochondrial epigenetic machinery through anterograde signaling (101,104,107).

mtDNA D-loop hypermethylation in IVDD. Recent studies indicate that hypermethylation of the mtDNA displacement loop (D-loop), the control region for mitochondrial replication and transcription, is markedly elevated in degenerative human NP tissues. This D-loop hypermethylation, mediated by DNMT1 translocation to mitochondria, represses mitochondrial gene expression (such as ND1 and COX1), driving metabolic reprogramming and oxidative stress (108-110). In other disease models, D-loop methylation levels are inversely associated with mtDNA copy number, suggesting compensatory mechanisms in response to mitochondrial dysfunction (109,111).

Single-cell approaches to mitochondrial dysfunction. scRNA-seq has proven particularly valuable for dissecting cellular heterogeneity and identifying mitochondrial dysfunction signatures in degenerative disc tissues (112,113). Recent scRNA-seq-guided studies revealed critical roles for mtDNA/SPARC-STING signaling pathways in fibrotic phenotype polarization of NP cells during IVDD progression. These findings have informed novel therapeutic strategies, including engineered mitochondrial transplantation that improves MQC in NP cells under pathological conditions (114-116).

Nuclear epigenetic regulation of MQC. DNA methylation and mitochondrial oxidative damage. DNMT3B-mediated DNA hypermethylation represents a key epigenetic driver of mitochondrial dysfunction in IVDD. DNMT3B is highly expressed in degenerated NP tissue and promotes hypermethylation of genes enriched in oxidative stress and ferroptosis pathways. Specifically, DNMT3B-mediated hypermethylation silences the ferroptosis suppressor gene SLC40A1 (ferroportin), leading to iron accumulation, lipid peroxidation and mitochondrial oxidative damage. Treatment with the DNA methylation inhibitor 5-azacytidine (5-Aza) alleviates IVDD in rat models by restoring SLC40A1 expression and reducing ferroptosis-associated mitochondrial dysfunction (29,100).

Histone modifications and metabolic homeostasis. H3K4me3 catalyzed by SET domain containing protein 1A (SETD1A) serves a protective role in maintaining mitochondrial metabolic function. H3K4me3 levels are markedly decreased in degenerated NP tissues (117). SETD1A regulates glycolytic metabolism, the primary energy pathway in the avascular intervertebral disc., through the H3K4me3-HELZ2/PPARα-hypoxia inducible factor 1α (HIF1α) axis. Loss of SETD1A reduces H3K4me3 enrichment at the HELZ2 promoter, suppressing the HELZ2/PPARα complex and downregulating HIF1α, which impairs glycolytic capacity and induces cellular senescence (117). Since mitochondria participate in metabolic regulation beyond glycolysis in disc cells, this histone modification pathway indirectly affects mitochondrial quality by altering cellular metabolic homeostasis.

m^{6}A methylation and mitophagy regulation. m^{6}A methylation represents a critical epitranscriptomic mechanism linking metabolic intermediates to MQC. METTL3-mediated m^{6}A modification regulates α-ketoglutarate (α-KG)-dependent mitophagy through the MALAT1/miR-23c/isocitrate dehydrogenase 1 (IDH1) axis. α-KG, a tricarboxylic acid cycle intermediate which is markedly decreased in degenerated NP tissues, serves dual functions: As a metabolic substrate and as a cofactor for TET DNA demethylases and Jumonji-C histone demethylases. Mechanistically, METTL3-mediated m^{6}A modification destabilizes the lncRNA MALAT1, releasing miR-23c to suppress IDH1, the enzyme responsible for α-KG production. Reduced α-KG levels impair mitophagy, leading to accumulation of dysfunctional mitochondria, increased reactive oxygen species (ROS) production and enhanced apoptosis. Supplementation with α-KG restores NP cell proliferation, reduces apoptosis and reestablishes ECM homeostasis (100,118).

m^{6}A modifications also regulate autophagy-related genes under stress conditions, providing additional control over mitochondrial quality (21). Multiple m^{6}A regulators including RBM15, YTHDC1, YTHDF3, HNRNPA2B1 and ALKBH5 show differential expression in IVDD, with METTL3/YTHDC1 co-regulation mediating m^{6}A hypermethylation of RNF41 mRNA to impair autophagy and ECM integrity (119,120).

Bidirectional metabolic-epigenetic crosstalk. A bidirectional relationship exists between mitochondrial metabolism and epigenetic regulation in IVDD. Mitochondria-derived metabolites serve as essential cofactors for epigenetic enzymes: NAD^+ for sirtuin deacetylases, α-KG for TET demethylases

and Jumonji-C demethylases, and S-adenosylmethionine for methyltransferases (100,107). Conversely, epigenetic modifications regulate expression of genes encoding MQC machinery, including those governing mitochondrial dynamics (fusion/fission), mitophagy pathways (PINK1-PRKN) and mitochondrial biogenesis (PPARGC1A/PGC-1α) (121,122).

Protein-level integration with epigenetic control. The NOD-like receptor X1 (NLRX1)-SLC39A7 complex exemplifies how protein-level regulation integrates with epigenetic control to maintain mitochondrial quality. This complex orchestrates mitochondrial dynamics and mitophagy by modulating mitochondrial zinc trafficking, coupling mitochondrial fission factors (dynamin-1-like protein), fusion regulators (OPA1 mitochondrial dynamin like GTPase) and mitophagy activity. Loss of NLRX1 triggers compensatory activation of the PINK1-PRKN pathway, leading to excessive mitophagy and accelerated NP cell senescence (123,124). NLRX1 is the only known mitochondria-localized NLR family member and functions as a negative regulator of inflammation while modulating mitochondrial metabolism and autophagy (124,125).

Targeted epigenetic editing. To establish a definitive causal link between epigenetic modulation and IVDD, and to facilitate the transition from observation to therapy, the field is increasingly adopting targeted epigenetic editing. Unlike traditional gene therapy that alters the genomic sequence, platforms such as CRISPR/dCas9, transcription activator-like effector nucleases or zinc-finger proteins fused to chromatin-modifying enzymes (such as DNMT3A, TET1, KRAB and p300) allow for the precise, reversible regulation of gene expression. These approaches are currently in various stages of *in vivo* investigation across a spectrum of metabolic, ophthalmic, neurobiological and musculoskeletal disorders (126,127). The translational feasibility of this technology in complex tissue environments has been demonstrated in non-spinal models; for instance, mice receiving lipid nanoparticle-derived mRNA encoding engineered zinc-finger repressors for PCSK9 achieved stable transcriptional repression through repressive chromatin remodeling, offering a potential treatment for hypercholesterolemia (128). Similarly, AAV-mediated delivery of dSaCas9 fused to transcriptional repression domains derived from KRAB and MeCP2 successfully induced localized chromatin repression of ApoE in the mouse brain, mitigating the most prominent genetic risk factor for Alzheimer's disease (129).

In the specific context of IVDD and discogenic pain, research has progressed from germline-edited models [such as zygote microinjection with dCas9-DNMT3A (130) or scaffold-based recruitment systems (131)] to somatic, therapeutically relevant interventions. A primary focus has been the modulation of the 'gut-disc-nerve' or inflammatory axes. Utilizing *in vitro* and *ex vivo* models, researchers have employed lentiviral dCas9-KRAB constructs to target the promoters of inflammatory receptors IL6st, TNFR1 and IL1R1 in rat dorsal root ganglion neurons. This multiplex epigenome editing effectively silenced cytokine signaling cascades, thereby desensitizing nociceptive neurons to the inflammatory milieu of the degenerative disc (132,133). Building on this, the same group demonstrated the *in vivo* therapeutic efficacy of CRISPR epigenome editing of TNFR1 in a needle puncture-induced rat model, demonstrated that targeted repression of inflammation can alleviate behavioral signs of pain (134).

However, halting inflammation addresses only one side of the degenerative coin; restoring the matrix requires reactivating silenced anabolic genes. Complementing repressive strategies, recent 'CRISPR activation' (CRISPRa) approaches, utilizing dCas9 fused to transcriptional activators such as VPR or p300, are now being explored to rejuvenate the disc matrix. Emerging evidence suggests that targeting the promoters of core anabolic genes, such as ACAN and COL2A1, can reverse the age-related epigenetic silencing of these loci. By depositing active histone marks (such as H3K27ac) at these promoters, CRISPRa systems can restore physiological gene expression levels and promote functional matrix repair in degenerative NPCs, offering a dual-pronged strategy when combined with inflammatory repression (Table I) (28-30,33,35,37,50,52,54-56,70,71,79-86, 92,133-135).

4. Animal models used in IVDD epigenetics research

Common models and translational challenges. Animal models remain indispensable for IVDD epigenetics research because chromatin remodeling is dynamically regulated by systemic cues (such as vascularization, immune surveillance and neural interaction) that are notably absent in static *in vitro* or organ-culture systems (136). The experimental repertoire is extensive, utilizing both small animals (murine, rabbit or canine) for mechanistic screening and large animals (ovine, porcine or non-human primates) for pre-clinical validation (137). Recently, weight-bearing species such as camelids and kangaroos have been introduced to improve approximation of human spinal loading. These models are generally classified into three distinct categories: Spontaneous, induced and genetically engineered. Spontaneous models, such as the sand rat (*Psammomys obesus*) or chondrodystrophoid (ChD) dogs, naturally replicate age-related degeneration, with porcine models offering anatomical dimensions that closely mirror human disc aging (138,139). Conversely, induced models utilize surgical needle puncture, enzymatic injection or controlled mechanical vibration to trigger rapid, reproducible degeneration, making them ideal for isolating specific mechanobiological pathways. Bridging these approaches, genetically engineered models introduce targeted mutations to probe specific signaling or metabolic mediators; notably, the lamin A/C G609G/G609G knock-in mouse, which models Hutchinson-Gilford progeria, develops spontaneous IVDD that closely mimics the accelerated epigenetic aging seen in humans (140).

Despite this diversity, species-specific variations impose notable constraints on translational relevance. The primary limitation is biomechanical; the quadrupedal posture of most models fails to replicate the axial loading forces of the human spine. While primates and surgically bipedal rodents offer partial solutions, their use is limited by ethical concerns and high costs (135,141,142). To address this, a novel non-surgical bipedal rat model has been developed by encouraging upright posture via specially designed cages; this approach induces progressive degeneration over months, improving the mimicking of chronic mechanical stress compared with acute

Table I. Core epigenetic techniques in IVDD research.

Epigenetic category	Methodological approach	Specific techniques/tools	Key applications& insights in IVDD	(Refs.)
DNA methylation	Locus-specific methylation typing	MSP/BSP, COBRA/MALDI-TOF MS	Observed age-related hypermethylation of the SPARC promoter associated with chronic low back pain.	(35)
		BSP + dot blot	Revealed global hypermethylation and DNMT1 expression associated with the upregulation of the pain-related channel TRPV1 in rat AF tissue.	(33)
		MSP/western blot	5-Azacytidine inhibits ER stress and apoptosis in NPCs by preser ving PPARγexpression via promoter demethylation.	(30)
		BSP/ChIP/western blot	Oxidative stress upregulates DNMT3B, causing hypermethylation of SLC40A1 (ferroptosis inhibitor), leading to ferroptosis; reversed by 5-Azacytidine.	(29)
	Global methylome profiling	WGBS	Identified 220 differentially methylated loci distinguishing early vs. late-stage IVDD.	(37)
m^6A methylation	High-throughput profiling	MeRIP-seq	Identified altered m^6A methylation in ANXA2, SLC3A2 and PBX3, which are enriched in NF-κB and ECM degradation pathways.	(70)
		RNA-seq/LC-MS/MS	Characterized age-dependent increases in global m^6A levels in rat NP tissue that regulate ECM metabolism and inflammation.	(71)
	Mechanistic validation	MeRIP-qPCR/RIP	Demonstrated that ALKBH5-mediated m^6A hypomethylation of DNMT3B transcripts promotes IVDD via E4F1 deficiency.	(28)
Histone modifications	Chromatin profiling	ChIP-seq + RNA-seq	Identified NR4A3 as a direct downstream target of the transcription regulator EGR1, promoting oxidative stress-induced apoptosis.	(52)
		CUT&Tag	Revealed that mitophagy reduces H3K18la enrichment at the THBS1 promoter, linking mitophagy and lactate metabolism.	(56)
	Chromatin accessibility	ATAC-seq	Demonstrated that upregulated CEMIP promotes IVDD by altering chromatin accessibility via the AP-1 transcription factor.	(54)
	Proteomics and chromatin	LC-MS/MS	Discovered that EZH2-mediated methylation of DDX1 alters MATR3 splicing, initiating chromatin reprogramming and degeneration.	(55)
	Metabolic-epigenetic link	scRNA-seq + ChIP-seq	Detected metabolic shifts (glycolysis) driving H3K18la enrichment at the ACSL4 promoter, which activates ferroptosis in NPCs.	(50)
Non-coding RNA	Expression profiling	RNA-seq/microarray	Systematic identification of dysregulated miRNA, lncRNA and circRNA expression profiles in degenerative human disc tissues.	(79-82)
	Functional interaction	Luciferase/RIP/RNA pull-down	Validated circRNA-miRNA-mRNA axes (such as circVMA21/ miR-200c/XIAP, circEYA3/miR-196a-5p/EBF1) that regulate apoptosis and ECM balance.	(83-86)
		CUT&Tag/ChIP	Demonstrated that circFUNDC1 recruits CDK9 to the FUNDC1 promoter to enhance mitophagy under oxidative stress.	(92)

Table I. Continued.

Epigenetic category	Methodological approach	Specific techniques/tools	Key applications& insights in IVDD	(Refs.)
Targeted epigenetic editing	Multiplex repression	Lentiviral dCas9-KRAB	Simultaneous repression of IL6st, TNFR1 and IL1R1 in DRG neurons completely abolished IVDD-induced mechanical sensitivity.	(133)
	In vivo epigenome editing	AAV-CRISPR-dCas9	Successful therapeutic epigenome editing of TNFR1 in rat IVDD models significantly reduced inflammation and pain behavior.	(134)

MSP, methylation-specific PCR; BSP, Bisulfite Sequencing PCR; AF, annulus fibrosus; ER, endoplasmic reticulum; NP, nucleus pulposus; NPCs, nucleus pulposus cells; COBRA, combined bisulfite restriction analysis; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; ChIP, chromatin immunoprecipitation; ChIP-seq, chromatin immunoprecipitation sequencing; WGBS, whole genome bisulfite sequencing; MeRIP-seq, methylated RNA immunoprecipitation sequencing; MeRIP-qPCR, methylated RNA immunoprecipitation quantitative PCR; RIP, RNA immunoprecipitation; LC-MS/MS, liquid chromatography with tandem mass spectrometry; ATAC-seq, assay for transposase-accessible chromatin with high-throughput sequencing; CUT&Tag, Cleavage Under Targets and Tagmentation; scRNA-seq, single-cell RNA sequencing; CRISPR, clustered regularly interspaced short palindromic repeats; dCas9, Dead Cas9 (Catalytically inactive Cas9); AAV, adeno-associated virus; IVDD, intervertebral disc degeneration; circRNA, circular RNA, miR/miRNA, microRNA, lncRNA, long non-coding RNA; ECM, extracellular matrix.

injury models, although it still incompletely replicates human kinematics (143). Furthermore, the persistence of notochordal cells into adulthood in rodents contrasts with their disappearance in humans, obscuring the study of the 'chondrocyte-like' cells that populate the adult human NP.

From an epigenetic perspective, the 'time gap' presents a formidable barrier. The short lifespan of rodents prevents the modeling of gradual, accumulative 'epigenetic drift', the loss of stable methylation patterns over decades, that characterizes human disease. Moreover, previous comparative epigenomics data from the Encyclopedia of DNA Elements (ENCODE) project highlights that while DNA sequences may be conserved, the functional activity of regulatory elements (enhancers/promoters) often diverges significantly between mice and humans, meaning an epigenetic therapy valid in mice may lack a functional target in humans (144). Consequently, the field is increasingly integrating human pluripotent stem cell-derived organoids alongside animal models. These 'disc-on-a-chip' systems provide a human genetic background to validate epigenetic mechanisms found in animals, offering a bridge to overcome species-specific regulatory divergence while adhering to the 3R principles of animal research (145).

Needle-puncture models used in IVDD epigenetics

Model utility and biological relevance. For epigenetic studies, murine needle puncture remains the overwhelmingly dominant method to induce controlled AF or NP disruption. The protocol allows for precise modulation through variables such as the insertion site (lumbar vs. coccygeal disc), needle gauge (21G to 32G), needle manipulation (retained or rotated) and intradiscal reagent injections (135,146). Despite the biomechanical differences inherent to quadrupeds, the murine caudal disc serves as a surprisingly robust surrogate for the human lumbar spine; it mirrors the human phenotype in disc height, anteroposterior width, torsional resistance, axial compressive loading and biochemical composition, particularly in glycosaminoglycan content. Consequently, the model rapidly recapitulates a spectrum of pathological features strikingly similar to human IVDD, including radial/concentric annular tears, endplate trabecular bone deposition, profound NP matrix degradation, cell apoptosis and inflammatory responses. These extensive biological parallels, combined with low cost, short life cycles and amenability to genetic manipulation, establish the murine tail disc as a clinically relevant proxy for studying structural degeneration (147).

Epigenetic limitations: Acute injury and evolutionary divergence. However, the application of this model to epigenetics requires rigorous scrutiny due to two fundamental disconnects. First, the temporal nature of the epigenetic modifications differs; because the alterations detected in puncture models are triggered by acute mechanical injury, they generate an 'acute inflammatory epigenetic spike' that may obscure or completely miss the gradual, wear-and-tear-related epigenetic drift that cumulatively drives IVDD onset in humans (148). Second, epigenetic findings from murine models must be interpreted with caution due to evolutionary divergence in regulatory elements (REs). Data from the latest phase of the ENCODE project reveals a notable discordance between sequence conservation and functional activity. While a substantial proportion of epigenome REs appear orthologous

at the sequence level (~56% of human REs and 72% of mouse REs), only a minority retain conserved regulatory activity. This functional drop-off is particularly severe when translating from humans to mice: Only 18% of human REs display analogous activity in mice, whereas 46% of mouse REs have functional counterparts in humans (149,150). This disparity, attributed to species-specific regulatory evolution, context-dependent enhancer usage and heterogeneity in bulk-tissue profiling, implies that an epigenetic target validated in a murine puncture model has a high probability of lacking a functional equivalent in the human genome.

Non-puncture models used in IVDD epigenetics

Genetic engineering: Dissecting molecular homeostasis. In contrast to acute injury models, only a few non-puncture IVDD animal models have been used in IVDD epigenetic research, including natural aging, gene editing (alone or combined with aging) and models based on advanced glycation end products (AGEs) or more systemic conditions. These models capture distinct facets of epigenetic modifications. For example, age-related SPARC downregulation due to hypermethylation is linked to disc degeneration, as aged and SPARC-null mice exhibit highly similar pain phenotypes (35). HDAC9 knockout mice at different ages exhibit accelerated IVDD due to impaired NPC vitality and increased apoptosis. HDAC9 enhances acetylation and ubiquitin-proteasomal degradation of RUNX3, and its overexpression restores NPC viability *in vivo* (44). miR-141 knockout prevents both spontaneous IVDD in aged mice and IVDD progression in a puncture-induced mouse model, acting through the SIRT1/NF-κB pathway. Local injection of nanoparticle-coupled miR-141 inhibitors demonstrated therapeutic benefit (87). The epigenetic regulator SIRT6, a nuclear NAD^+-dependent HDAC, maintains disc homeostasis by supporting anabolic and proliferative responses via IGF-1 and MYC. Disc-specific Sirt6-knockout causes global H3K9 hyperacetylation, increased H3K27 and H3K36 methylation, altered chromatin accessibility and dysregulation of DNA-repair pathways. Together these changes result in accumulated DNA damage (γH2AX foci), reduced autophagy, increased cellular senescence with a heightened senescence-associated secretory phenotype burden in NP and AF cells, and accelerated structural disc degeneration in an age-dependent manner (45). Disc cell-specific Foxo3 knockout induces IVDD with pronounced type II collagen and ECM loss, revealing a protective mechanism whereby FOXO3 activates the lncRNA HOTTIP, which sequesters miR-615-3p to preserve COL2A1 expression (151).

Chemical and metabolic induction: The 'slow-burn' of aging. Using a rat IVDD model induced by AGEs, researchers show that lentiviral overexpression of lncRNA FAM83H-AS1 competitively binds miR-22-3p via a ceRNA mechanism, reducing the inflammatory mediators IL-1β and TNF-α (152). Unlike mechanical injury models, AGE administration-based models reproduce a gradual accumulation of crosslinked matrix proteins, oxidative stress, inflammation and activation of the receptor for AGE, the 'slow-burn' aging chemistry that contributes to IVDD. A similar AGE-based rat model has also been used to evaluate the therapeutic effects of palladium nanoparticles in IVDD, which facilitates autophagic degradation of AGEs (153). However, exogenous AGE delivery may not fully reflect the temporal or tissue-specific distribution of AGE accumulation seen in natural aging, and it underrepresents other contributors such as mechanical loading and vascular or immune responses.

Systemic regulators: Exercise and circadian rhythms. Animal studies have also explored how systemic conditions may have a strong impact on epigenetic changes and disease development. Although lumbar IVDs from SPARC-null and age-matched wild-type mice show comparable global DNA methylation, as assessed via 5-mC ELISA, long-term exercise lowers global methylation in both groups and attenuates LBP-related symptoms manifested in a sex-specific manner. In males, exercise reduces Dnmt3a, Mecp2 and Tet1 mRNA expression, while in females it reduces Dnmt3b and Mecp2 but increases Mbd2a/b, with Mecp2a reduction shared across sexes, especially in SPARC-null mice. Because the exercise intervention began at 8 months (middle-aged) and ended at 14 months, this comparison reflects the function of SPARC in age-associated methylation (154). In a streptozotocin (STZ)-induced diabetic rat model, hyperglycemia markedly suppresses SIRT1 expression and activity in NPCs. As a class III HDAC, SIRT1 deacetylates multiple substrates, including the tumor suppressor protein p53. Its reduction increases p53 acetylation, enhancing its transcriptional activity that favors apoptosis and cellular senescence. Pharmacological or gene-based reactivation of SIRT1 reverses these effects and protects NPCs from diabetes-associated IVDD (155).

Expanding on systemic regulation, recent work has established circadian rhythm disruption models (such as environmental light-cycle shifting or Bmal1 knockout) as a novel non-puncture paradigm. These models reveal that the core circadian clock protein BMAL1 directly binds to the promoters of anabolic genes (such as aggrecan and Col2a1) to maintain their rhythmic expression, a mechanism critical for cartilaginous tissue integrity. Furthermore, in the specific context of the intervertebral disc, disrupting this rhythm leads to degeneration through an autophagy-dependent axis; the loss of BMAL1 impairs the rhythmic expression of nuclear receptors that govern autophagic flux, thereby compromising NPC survival (156). Collectively, these models underscore that the IVDD epigenome is not a static entity but a dynamic record of genetic, metabolic and lifestyle inputs (156,157).

Common pitfalls and misinterpretations in IVDD epigenetics research. Progress in IVDD epigenetics research into clinical trials is limited by several obstacles, including over-reliance on single epigenetic marks, neglect of disc zonal heterogeneity, conflation of correlation with causation, and mismatches between models and methods.

A persistent limitation in IVDD epigenetic studies is the tendency to focus on isolated epigenetic features, such as DNA methylation or individual histone marks, rather than the coordinated behavior of the ERN. Age-associated epigenetic alterations accumulate in a non-random manner, preferentially affecting genes involved in development and transcriptional regulation, indicating that degeneration reflects network-level erosion rather than discrete molecular events. Studies that fail to account for this functional redundancy risk oversimplifying disc aging as a sum of independent alterations rather than a systemic disruption of regulatory control.

The IVD exhibits pronounced spatial heterogeneity, with distinct epigenetic and transcriptional programs across the NP, AF and CEP. Failure to resolve these zonal differences, particularly in bulk epigenomic assays, can obscure focal chromatin architectures that drive localized degeneration. Advanced spatial and single-cell epigenomic approaches are therefore critical for accurately interpreting disc-specific regulatory states and avoiding the conflation of global aging signatures with region-specific pathology.

Early machine learning studies successfully identified senescence-associated hub genes, such as TATA-box binding protein associated factor 13 (158), and predictive mRNA triads (BTG anti-proliferation factor 2, MDM4 regulator of p53, acyl-CoA oxidase 1) using approaches including weighted gene co-expression network analysis, random forest and support vector machine-recursive feature elimination (159). While these correlation-based frameworks are valuable for hypothesis generation, they cannot establish causality. Without functional perturbation or longitudinal validation, there remains a risk of misinterpreting downstream epigenetic consequences as primary drivers of degeneration.

A critical and often overlooked pitfall lies in mismatching experimental models with the biological timescale of human IVDD. Selecting an appropriate animal model requires consideration of its age-related characteristics relative to human IVDD, as different species have distinct life spans and aging rates that shift the timing of degeneration (160). Across mammals, shorter-lived species have a higher rate of epigenetic drift than longer-lived species, and this rate scales with maximum lifespan. Age-associated epigenetic drift accumulates faster in genome regions with low CpG density, which are more common in shorter-lived species, whereas longer-lived species tend to possess CpG-dense regulatory elements in shared genes and appear to maintain more robust protective mechanisms, such as sirtuin efficiency and DNA repair capacity. This discrepancy is detectable even within murine rodents. When measured per unit chronological time, epigenetic disorder occurs more rapidly in rats than in mice (22). Such mismatches undermine translational relevance when acute injury models are used to infer mechanisms of chronic degeneration.

5. Future directions

Methodological innovations needed. The avascular nature of disc tissue and limited cell yields necessitate low-input and single-cell epigenomic technologies. Integrating single-cell multi-omics, spatial transcriptomics and long-read epigenomics will enable high-resolution mapping of chromatin states while preserving zonal and cellular context. Human IVDD is defined by slow, progressive epigenetic drift rather than abrupt molecular change. Longitudinal sampling strategies, particularly in aging animal models, are essential to distinguish stochastic drift from pathological transitions and to identify early epigenetic states that predispose discs to failure. Establishing standardized, disc-specific epigenetic reference maps across age, degeneration stage and disc region will provide a foundational framework for interpreting disease-associated changes. Such atlases are critical for cross-study comparability and for anchoring artificial intelligence (AI)-driven analyses in biologically meaningful ground truth.

Challenges in therapeutic delivery remain stubborn. The NP tissue presents formidable barriers, creating a dilemma between achieving tissue penetration and maintaining therapeutic retention. The avascular nature and dense ECM make it exceptionally challenging for systemically administered drugs to reach target cells, necessitating local intradiscal injection as the clinically relevant delivery approach (161). However, direct injection suffers from rapid diffusion outside the injection site, resulting in short-lived benefits while potentially causing systemic toxicity (162). The high negative fixed charge density of the NP, driven by aggrecan glycosaminoglycans, can be exploited to enhance intra-NP residence time through positively charged delivery vehicles that utilize long-range electrostatic interactions (163).

Additionally, degenerative disc tissues develop fibrotic barriers that further impede drug penetration, further complicating therapeutic delivery (164). Advanced nanocarrier systems have emerged as promising solutions to overcome these dual challenges. Injectable hydrogel gene delivery systems incorporating functionalized nanoparticles enable sustained, on-demand release of therapeutic agents directly into the NP, often utilizing MMP-responsive mechanisms (165). Tannic acid-based nanoparticles provide both antioxidant and anti-inflammatory effects while serving as effective gene delivery vectors (166). Multiple engineering strategies can enhance nanocarrier performance. Stimuli-responsive nanoparticles offer precise targeting and controlled therapeutic release, improving drug localization and enabling sustained delivery (167). Inflammation-responsive drug release systems can exploit the pathological microenvironment, with nanoscaffolds demonstrating disc-mimetic stiffness, excellent biodegradability and robust scavenging of ROS and cell-free nucleic acids (168). Phenylboronic acid-functionalized microspheres create microenvironment-responsive systems enabling sustained gene release while modulating inflammation and alleviating apoptosis (169). Charge-reversal and self-propelling nanocarriers represent cutting-edge approaches to the penetration-retention paradox. Avidin-grafted dextran nanostructures utilize electrostatic interactions to achieve month-long intra-discal retention, with binding strong enough to prevent rapid clearance yet reversible enough to allow movement throughout the tissue (163). Self-enriching nanocarriers catalyze hydrogen peroxide to generate asymmetric bubble propulsion, enabling selective penetration into degenerated tissues without accumulation in healthy tissues, overcoming fibrotic barriers that impede conventional passive diffusion (164). Surface modification further enhances functionality. NP-targeting nanocarriers facilitate miRNA-based therapeutics by enhancing transportation to target cells, which enables effective *in vivo* inhibition of pathological miRNAs (166). Multifunctional nanocarriers can simultaneously deliver multiple therapeutic agents, such as combining gene therapy with mitochondrial-targeted peptides, to address both ECM metabolism and mitochondrial dysfunction (170).

Despite these challenges, nanocarrier-based delivery systems have demonstrated the capacity to enhance targeted delivery, improve local drug concentration and sustain drug retention. This provides disease-modifying therapeutic strategies that address fundamental pathophysiological mechanisms of IVDD (Fig. 1) (162,171,172).

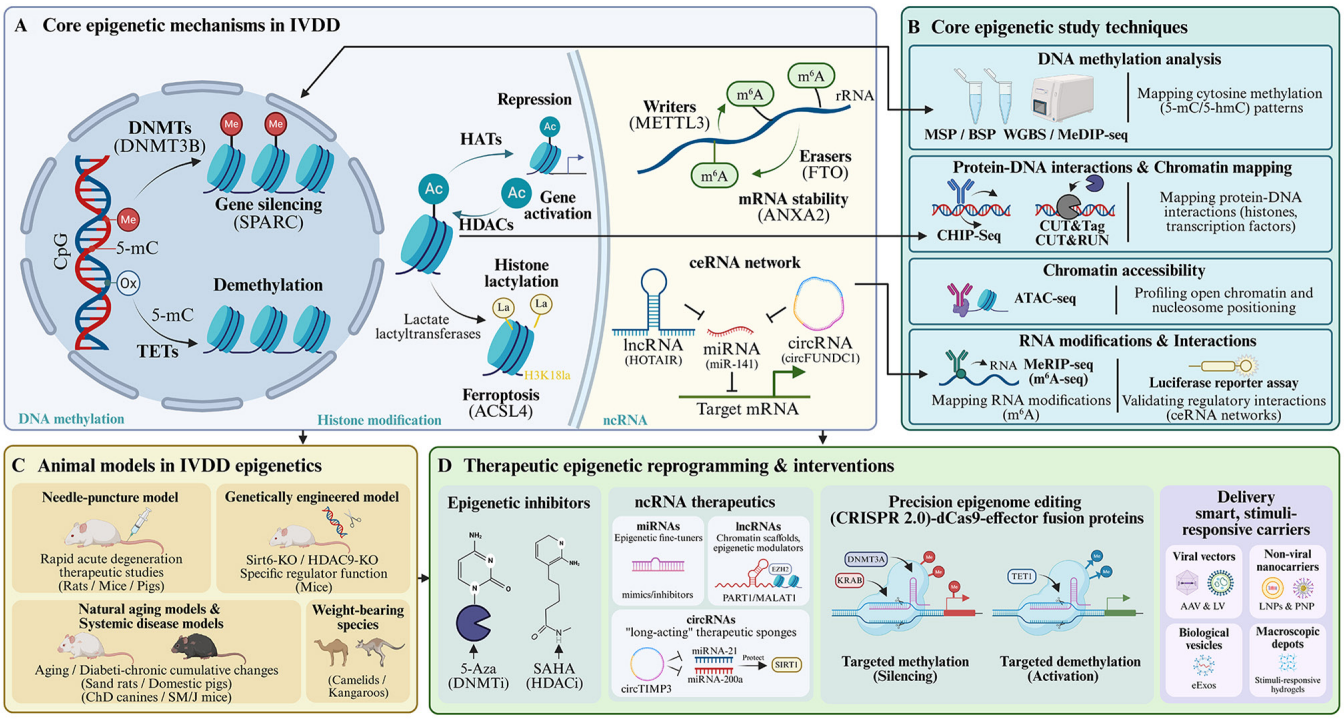

Figure 1. Epigenetic toolkit in IVDD: Mechanisms, techniques, models and translational applications. (A) Core epigenetic mechanisms in IVDD. (B) Core epigenetic study techniques (C) Animal models in IVDD epigenetics. (D) Therapeutic epigenetic reprogramming and interventions. 5-mc, 5-methylcytosine; 5-hmc, 5-hydroxymethylcytosine; DNMTs, DNA methyltransferases; TETs, Ten-eleven translocation enzymes; HATs, histone acetyltransferases; MSP, methylation-specific PCR; BSP, bisulfite sequencing PCR; WGBS, whole-genome bisulfite sequencing; MeDIP-seq, methylated DNA immunoprecipitation sequencing; CHIP-Seq, chromatin immunoprecipitation sequencing; CUT&Tag, Cleavage Under Targets and Tagmentation; CUT&RUN, Cleavage Under Targets and Release Using Nuclease; ATAC-seq, assay for transposase-accessible chromatin using sequencing; MeRIP-seq, methylated RNA immunoprecipitation sequencing; m^{6}A-seq, N6-methyladenosine sequencing; AAV, adeno-associated virus; LV, lentivirus; LNPs, lipid nanoparticles; PNP, polymeric nanoparticles; eExos, engineered exosomes; miRNA, microRNA, HDAC, histone deacetylase.

Model innovation. Non-human primates such as *Macaca mulatta* live to about 27 years and age at about three times faster than humans. This degeneration, similar to that of middle-aged and elderly humans (40-60 years old), appears at 8-12 years in monkeys (173). ChD dogs exhibit clinical IVDD between 3 and 7 years of age (174), whereas gerbils develop early degeneration at 4 months, progressing extensively by 9 months, which helps model the continuum from youth to old age in humans (175). Future research must prioritize such longer-lived models to capture the slow, cumulative epigenetic drift that characterizes human pathology, while permitting controlled mechanical perturbation. Humanized and genetically engineered progeroid models represent a promising direction for aligning epigenetic timescales with human pathology. These systems allow interrogation of pre-existing epigenetic vulnerabilities that are invisible in acute injury paradigms yet decisive for clinical translation.

From descriptive epigenetics to causal biology. Recent deep learning models published between 2024 and 2025 have pushed analysis far beyond correlation-based methods, enabling the end-to-end prediction of complex regulatory interactions directly from DNA sequence. Key advances include DeepEPI (176) and EPI-Trans (177), which combine convolutional neural networks to capture local motifs with transformers to model long-range 3D chromatin dependencies. Building on this, DeepMethyGene integrates multi-scale methylation features (CpG islands, shores and shelves) to predict gene expression with high interpretability (178). These models enable hypothesis-driven perturbation experiments that test causality rather than correlation. Advanced architectures incorporating transformers and Kolmogorov-Arnold Networks (such as KansformerEPI) facilitate interpretable modeling of long-range chromatin dependencies. By learning the non-linear regulatory grammar of the disc, these tools support experimental validation of cooperative network behaviors underlying senescence. More sophisticated architectures add biological flexibility; EPI-DynFusion introduces dynamic fusion gates to adaptively weigh sequence representations based on cell context (179), while KansformerEPI replaces traditional perceptrons with Kolmogorov-Arnold Networks, combining functional expressiveness with interpretability (180). Together, these tools are reshaping the paradigm from studying isolated epigenetic marks towards learning the non-linear, context-dependent regulatory grammar governing disc senescence.

6. Conclusions

IVDD represents a systemic collapse of the ERN, rather than isolated molecular failures. Future research must integrate single-cell multi-omics, spatial transcriptomics and AI to decode the cumulative erosion of cooperative network behaviors and the complex regulatory mechanisms of disc senescence, prioritizing physiologically relevant aging models to identify epigenetic states predisposing to degeneration. Successful

clinical translation depends on coupling these insights with advanced delivery strategies that navigate the disc's avascular environment, shifting the paradigm from symptom preservation toward active epigenetic reprogramming and rejuvenation of the aging spine.

Acknowledgements

Fig. 1. was created in BioRender. c, C. (2026; https://BioRender.com/hzv1ofn) with a publication license.

Funding

This work was supported by the Research and Innovation Team Project for Scientific Breakthroughs at Shanxi Bethune Hospital (grant no. 2024ZHANCHI10).

Availability of data and materials

Not applicable.

Authors' contributions

XLC contributed to the conceptualization, writing and figures and tables creation. LZ contributed to the conceptualization and writing. HJ contributed to the conceptualization and reviewing and editing the manuscript. WZ contributed to the writing. LX and DJ contributed to the review, editing and language checking. RW and S contributed to the conceptualization. HF contributed to the review and editing. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Maher C, Underwood M and Buchbinder R: Non-specific low back pain. Lancet 389: 736-747, 2017.
2. Samanta A, Lufkin T and Kraus P: Intervertebral disc degeneration-current therapeutic options and challenges. Front Public Health 11: 1156749, 2023.
3. Taylor W and Erwin WM: Intervertebral disc degeneration and regeneration: New molecular mechanisms and therapeutics: Obstacles and potential breakthrough technologies. Cells 13: 2103, 2024.
4. Mohd Isa IL, Teoh SL, Mohd Nor NH and Mokhtar SA: Discogenic low back pain: Anatomy, pathophysiology and treatments of intervertebral disc degeneration. Int J Mol Sci 24: 208, 2022.
5. Kirnaz S, Capadona C, Wong T, Goldberg JL, Medary B, Sommer F, McGrath LB Jr and Härtl R: Fundamentals of intervertebral disc degeneration. World Neurosurg 157: 264-273, 2022.
6. Du J: Global, regional, and national burden of low back pain: Findings from the global burden of disease study 2021 and projections to 2050. Spine (Phila Pa 1976) 50: E389, 2025.
7. Oichi T, Taniguchi Y, Oshima Y, Tanaka S and Saito T: Pathomechanism of intervertebral disc degeneration. JOR spine 3: e1076, 2020.
8. Ferreira JR, Moura ML, Pilão S, Castro AL, Fiordalisi M, Leite Pereira C, Caldeira J and Gonçalves RM: Decoding intervertebral disc cell populations: Challenges in isolation and phenotype definition. Tissue Eng Part B Rev: tenteb20240350, 2025 (Epub ahead of print).
9. Urban JPG and Fairbank JCT: Current perspectives on the role of biomechanical loading and genetics in development of disc degeneration and low back pain; a narrative review. J Biomech 102: 109573, 2020.
10. Guo W, Li BL, Zhao JY, Li XM and Wang LF: Causal associations between modifiable risk factors and intervertebral disc degeneration. Spine J 24: 195-209, 2024.
11. Vo NV, Hartman RA, Yurube T, Jacobs LJ, Sowa GA and Kang JD: Expression and regulation of metalloproteinases and their inhibitors in intervertebral disc aging and degeneration. Spine J 13: 331-341, 2013.
12. Tseranidou S, Segarra-Queralt M, Chemorion FK, Le Maitre CL, Piñero J and Noailly J: Nucleus pulposus cell network modelling in the intervertebral disc. NPJ Syst Biol Appl 11: 13, 2025.
13. Kaneda G, Zila L, Wechsler JT, Shafi K, Cheema K, Bae H, Kim SD, Tuchman A, Li D and Sheyn D: What a pain in the back: Etiology, diagnosis and future treatment directions for discogenic low back pain. Bone Res 13: 89, 2025.
14. Kang L, Zhang H, Jia C, Zhang R and Shen C: Epigenetic modifications of inflammation in intervertebral disc degeneration. Ageing Res Rev 87: 101902, 2023.
15. Ząbek Z, Wyczałkowska-Tomasik A, Poboży K, Adamus JP, Turek G, Ząbek M and Pączek L: Understanding the microenvironment of intervertebral disc degeneration: A comprehensive review of pathophysiological insights and therapeutic implications. Int J Mol Sci 26: 9938, 2025.
16. Xu J, Shao T, Lou J, Zhang J and Xia C: Aging, cell senescence, the pathogenesis and targeted therapies of intervertebral disc degeneration. Front Pharmacol 14: 1172920, 2023.
17. Hickman TT, Rathan-Kumar S and Peck SH: Development, pathogenesis, and regeneration of the intervertebral disc: Current and future insights spanning traditional to omics methods. Front Cell Dev Biol 10: 841831, 2022.
18. Li G, Zhang W, Liang H and Yang C: Epigenetic regulation in intervertebral disc degeneration. Trends Mol Med 28: 803-805, 2022.
19. Zhang Y, Wang J, Hua D, Fan C, He W, Deng Y, Tang M, Geng D, Wu X and Mao H: Histone modifications: Unveiling the epigenetic enigma of degenerative skeletal diseases. J Orthop Translat 55: 245-266, 2025.
20. Jiang C, Chen Z, Wang X, Zhang Y, Guo X, Xu Z, Yang H and Hao D: The potential mechanisms and application prospects of non-coding RNAs in intervertebral disc degeneration. Front Endocrinol (Lausanne) 13: 1081185, 2022.
21. Tao Y, Yu X, Li X, Xu Y, Wang H, Zhang L, Lin R, Wang Y and Fan P: M6A methylation-regulated autophagy may be a new therapeutic target for intervertebral disc degeneration. Cell Biol Int 48: 389-403, 2024.
22. Bertucci-Richter EM and Parrott BB: The rate of epigenetic drift scales with maximum lifespan across mammals. Nat Commun 14: 7731, 2023.
23. Moqri M, Poganik JR, Horvath S and Gladyshev VN: What makes biological age epigenetic clocks tick. Nat Aging 5: 335-336, 2025.
24. Bertucci-Richter EM, Shealy EP and Parrott BB: Epigenetic drift underlies epigenetic clock signals, but displays distinct responses to lifespan interventions, development, and cellular dedifferentiation. Aging (Albany NY) 16: 1002-1020, 2024.
25. Diwan AD and Melrose J: Intervertebral disc degeneration and how it leads to low back pain. JOR Spine 6: e1231, 2022.
26. Younesian S, Mohammadi MH, Younesian O, Momeny M, Ghaffari SH and Bashash D: DNA methylation in human diseases. Heliyon 10: e32366, 2024.
27. Wu X and Zhang Y: TET-mediated active DNA demethylation: Mechanism, function and beyond. Nat Rev Genet 18: 517-534, 2017.
28. Li G, Luo R, Zhang W, He S, Wang B, Liang H, Song Y, Ke W, Shi Y, Feng X, *et al*: m6A hypomethylation of DNMT3B regulated by ALKBH5 promotes intervertebral disc degeneration via E4F1 deficiency. Clin Transl Med 12: e765, 2022.

29. Chen J, Yang X, Li Q, Ma J, Li H, Wang L, Chen Z and Quan Z: Inhibiting DNA methyltransferase DNMT3B confers protection against ferroptosis in nucleus pulposus and ameliorates intervertebral disc degeneration via upregulating SLC40A1. Free Radic Biol Med 220: 139-153, 2024.
30. Cheng P, Li H, Chen HW, Wang ZQ, Li PW and Zhang HH: 5-Azacytidine inhibits endoplasmic reticulum stress and apoptosis of nucleus pulposus cells by preserving PPARγ via promoter demethylation. In Vitro Cell Dev Biol Anim 61: 288-297, 2025.
31. Cheng P, Wei HZ, Chen HW, Wang ZQ, Mao P and Zhang HH: DNMT3a-mediated methylation of PPARγ promote intervertebral disc degeneration by regulating the NF-κB pathway. J Cell Mol Med 28: e18048, 2024.
32. Yeater TD, Kawarai Y, Lee S, Belani KG, Beebe DS, Sheyn D, Pinto MR and Stone LS: Investigating the epigenetic landscape of symptomatic disk degeneration: A case study. Pain Rep 10: e1237, 2025.
33. Hong JY, Kim H, Jeon WJ, Lee J, Yeo C, Lee YJ and Ha IH: Epigenetic changes within the annulus fibrosus by DNA methylation in rat intervertebral disc degeneration model. Cells 11: 3547, 2022.
34. Karchevskaya AE, Poluektov YM and Korolishin VA: Understanding intervertebral disc degeneration: Background factors and the role of initial injury. Biomedicines 11: 2714, 2023.
35. Tajerian M, Alvarado S, Millecamps M, Dashwood T, Anderson KM, Haglund L, Ouellet J, Szyf M and Stone LS: DNA methylation of SPARC and chronic low back pain. Mol Pain 7: 65, 2011.
36. Li S and Tollefsbol TO: DNA methylation methods: Global DNA methylation and methylomic analyses. Methods 187: 28-43, 2021.
37. Ikuno A, Akeda K, Takebayashi SI, Shimaoka M, Okumura K and Sudo A: Genome-wide analysis of DNA methylation profile identifies differentially methylated loci associated with human intervertebral disc degeneration. PLoS One 14: e0222188, 2019.
38. Sun P, Huang T, Huang C, Wang Y and Tang D: Role of histone modification in the occurrence and development of osteoporosis. Front Endocrinol (Lausanne) 13: 964103, 2022.
39. Dueva R, Akopyan K, Pederiva C, Trevisan D, Dhanjal S, Lindqvist A and Farnebo M: Neutralization of the positive charges on histone tails by RNA promotes an open chromatin structure. Cell Chem Biol 26: 1436-1449.e5, 2019.
40. Higashiyama R, Miyaki S, Yamashita S, Yoshitaka T, Lindman G, Ito Y, Sasho T, Takahashi K, Lotz M and Asahara H: Correlation between MMP-13 and HDAC7 expression in human knee osteoarthritis. Mod Rheumatol 20: 11-17, 2010.
41. Zheng Q, Li XX, Xiao L, Shao S, Jiang H, Zhang XL, Sun LY and Xu HG: MicroRNA-365 functions as a mechanosensitive microRNA to inhibit end plate chondrocyte degeneration by targeting histone deacetylase 4. Bone 128: 115052, 2019.
42. Xiao L, Gong D, Liang L, Liang A, Liang H, Xu X and Teng H: Inhibition of HDAC4 by GSK3β leads to downregulation of KLF5 and ASK1 and prevents the progression of intravertebral disc degeneration. Clin Epigenetics 13: 53, 2021.
43. Makki MS and Haqqi TM: Histone deacetylase inhibitor vorinostat (SAHA) suppresses IL-1β-induced matrix metallopeptidase-13 expression by inhibiting IL-6 in osteoarthritis chondrocyte. Am J Pathol 186: 2701-2708, 2016.
44. Lei M, Lin H, Shi D, Hong P, Song H, Herman B, Liao Z and Yang C: Molecular mechanism and therapeutic potential of HDAC9 in intervertebral disc degeneration. Cell Mol Biol Lett 28: 104, 2023.
45. Ramteke P, Watson B, Toci M, Tran VA, Johnston S, Tsingas M, Barve RA, Mitra R, Loeser RF, Collins JA and Risbud MV: SIRT6 loss causes intervertebral disc degeneration in mice by promoting senescence and SASP status. bioRxiv [Preprint]: 2024.09.09.612072, 2024.
46. Ma W, Wang W, Zhao L, Fan J, Liu L, Huang L, Peng B, Wang J, Xu B, Liu H, *et al*: Reprogramming to restore youthful epigenetics of senescent nucleus pulposus cells for mitigating intervertebral disc degeneration and alleviating low back pain. Bone Res 13: 35, 2025.
47. Yao Q, Lei Y, Zhang Y, Chen H, Dong X, Ye Z and Liang H: EZH2-H3K27me3-mediated epigenetic silencing of DKK1 induces nucleus pulposus cell pyroptosis in intervertebral disc degeneration by activating NLRP3 and NAIP/NLRC4. Inflammation 48: 902-918, 2025.
48. Zhou M, He SJ, Liu W, Yang MJ, Hou ZY, Meng Q and Qian ZL: EZH2 upregulates the expression of MAPK1 to promote intervertebral disc degeneration via suppression of miR-129-5p. J Gene Med 24: e3395, 2022.
49. Zhang D, Tang Z, Huang H, Zhou G, Cui C, Weng Y, Liu W, Kim S, Lee S, Perez-Neut M, *et al*: Metabolic regulation of gene expression by histone lactylation. Nature 574: 575-580, 2019.
50. Sun K, Shi Y, Yan C, Wang S, Han L, Li F, Xu X, Wang Y, Sun J, Kang Z and Shi J: Glycolysis-derived lactate induces ACSL4 expression and lactylation to activate ferroptosis during intervertebral disc degeneration. Adv Sci (Weinh) 12: e2416149, 2025.
51. Shi Y, Li F, Lin W, Han L, Wang J, Yan C, Sun J, Ji C, Shi J and Sun K: Integrating bulk RNA and single-cell RNA sequencing identifies and validates lactylation-related signatures for intervertebral disc degeneration. J Cell Mol Med 28: e70262, 2024.
52. Zheng SK, Zhao XK, Wu H, He DW, Xiong L and Cheng XG: Oxidative stress-induced EGR1 upregulation promotes NR4A3-mediated nucleus pulposus cells apoptosis in intervertebral disc degeneration. Aging (Albany NY) 16: 10216-10238, 2024.
53. Saotome M, Goodman J and Takaku M: Capturing chromatin organization by MNase-seq and ATAC-seq. Methods Mol Biol 2889: 167-180, 2025.
54. Shu S, Zhang X, Feng Z, Liu Z, Wang K, Li F, Wu Y, Shi B, Qiu Y, Zhu Z and Bao H: Upregulated CEMIP promotes intervertebral disc degeneration via AP-1-mediated change in chromatin accessibility. Clin Transl Med 15: e70322, 2025.
55. Zhu D, Liang H, Tong B, Du Z, Li G, Zhang W, Wu D, Zhou X, Lei J, Zhang X, *et al*: DDX1 methylation mediated MATR3 splicing regulates intervertebral disc degeneration by initiating chromatin reprogramming. Nat Commun 16: 6153, 2025.
56. Wang K, Wu X, Li H, Xu X, Cheng F, Chen J, Zhou H, Xu J, Yu C, Li Y, *et al*: Mitophagy reprograms lactate metabolism to suppress THBS1 via H3K18la reduction, alleviating intervertebral disc degeneration. Research (Wash D C) 8: 0957, 2025.
57. Zeng Y, Wang S, Gao S, Soares F, Ahmed M, Guo H, Wang M, Hua JT, Guan J, Moran MF, *et al*: Refined RIP-seq protocol for epitranscriptome analysis with low input materials. PLoS Biol 16: e2006092, 2018.
58. Dominissini D, Moshitch-Moshkovitz S, Amariglio N and Rechavi G: Transcriptome-wide mapping of N^6-methyladenosine by m^6A-Seq. Methods Enzymol 560: 131-147, 2015.
59. Körtel N, Rücklé C, Zhou Y, Busch A, Hoch-Kraft P, Sutandy FXR, Haase J, Pradhan M, Musheev M, Ostareck D, *et al*: Deep and accurate detection of m6A RNA modifications using miCLIP2 and m6Aboost machine learning. Nucleic Acids Res 49: e92, 2021.
60. Yang Y, Lu Y, Wang Y, Wen X, Qi C, Piao W and Jin H: Current progress in strategies to profile transcriptomic m6A modifications. Front Cell Dev Biol 12: 1392159, 2024.
61. Xia W, Guo L, Su H, Li J, Lu J, Li H and Huang B: A low-cost, low-input method establishment for m6A MeRIP-seq. Biosci Rep 44: BSR20231430, 2024.
62. Wang Y, Xiao Y, Dong S, Yu Q and Jia G: Antibody-free enzyme-assisted chemical approach for detection of N^6-methyladenosine. Nat Chem Biol 16: 896-903, 2020.
63. Zhang LS, Dai Q and He C: Base-resolution sequencing methods for whole-transcriptome quantification of mRNA modifications. Acc Chem Res 57: 47-58, 2024.
64. Liu C, Sun H, Yi Y, Shen W, Li K, Xiao Y, Li F, Li Y, Hou Y, Lu B, *et al*: Absolute quantification of single-base m^6A methylation in the mammalian transcriptome using GLORI. Nat Biotechnol 41: 355-366, 2023.
65. Sun H, Lu B, Zhang Z, Xiao Y, Zhou Z, Xi L, Li Z, Jiang Z, Zhang J, Wang M, *et al*: Mild and ultrafast GLORI enables absolute quantification of m^6A methylome from low-input samples. Nat Methods 22: 1226-1236, 2025.
66. Garcia-Campos MA, Edelheit S, Toth U, Safra M, Shachar R, Viukov S, Winkler R, Nir R, Lasman L, Brandis A, *et al*: Deciphering the 'm^6A Code' via antibody-independent quantitative profiling. Cell 178: 731-747.e16, 2019.
67. Capitanchik C, Toolan-Kerr P, Luscombe NM and Ule J: How do you identify m^6 A methylation in transcriptomes at high resolution? A comparison of recent datasets. Front Genet 11: 398, 2020.
68. Meyer KD: DART-seq: An antibody-free method for global m^6A detection. Nat Methods 16: 1275-1280, 2019.
69. Tegowski M, Zhu H and Meyer KD: Detecting m^6A with in vitro DART-Seq. Methods Mol Biol 2404: 363-374, 2022.
70. Shen J, Zhang Q, Lan Y, Peng Q, Ji Z, Wu Y and Liu H: Identification and characterisation of potential targets for N6-methyladenosine (m6A) modification during intervertebral disc degeneration. Front Biosci (Landmark Ed) 29: 405, 2024.

71. Liu L, Sun H, Zhang Y, Liu C, Zhuang Y, Liu M, Ai X, Long D, Huang B, Li C, *et al*: Dynamics of N6-methyladenosine modification during aging and their potential roles in the degeneration of intervertebral disc. JOR Spine 7: e1316, 2024.
72. Chen X, Gong W, Shao X, Shi T, Zhang L, Dong J, Shi Y, Shen S, Qin J, Jiang Q and Guo B: METTL3-mediated m^6A modification of ATG7 regulates autophagy-GATA4 axis to promote cellular senescence and osteoarthritis progression. Ann Rheum Dis 81: 87-99, 2022.
73. Wu O, Jin Y, Zhang Z, Zhou H, Xu W, Chen L, Jones M, Kwan KYH, Gao J, Zhang K, *et al*: KMT2A regulates the autophagy-GATA4 axis through METTL3-mediated m^6A modification of ATG4a to promote NPCs senescence and IVDD progression. Bone Res 12: 67, 2024.
74. Sun R, Wu XT, Shi H, Wang F, Gao JW, Wang PY, Xu ZY, Gan WW, Wang YT and Zhang C: Mechanism of FTO-mediated m6A demethylation regulation of YAP1 in nucleus pulposus cell senescence. Mech Ageing Dev 227: 112101, 2025.
75. Xu X, Shen L, Qu Y, Li D, Zhao X, Wei H and Yue S: Experimental validation and comprehensive analysis of m6A methylation regulators in intervertebral disc degeneration subpopulation classification. Sci Rep 14: 8417, 2024.
76. Guo C, Chen Y, Wang Y and Hao Y: Regulatory roles of noncoding RNAs in intervertebral disc degeneration as potential therapeutic targets (Review). Exp Ther Med 25: 44, 2022.
77. Jiang J, Sun Y, Xu G, Wang H and Wang L: The role of miRNA, lncRNA and circRNA in the development of intervertebral disk degeneration (Review). Exp Ther Med 21: 555, 2021.
78. Wang C, Cui L, Gu Q, Guo S, Zhu B, Liu X, Li Y, Liu X, Wang D and Li S: The mechanism and function of miRNA in intervertebral disc degeneration. Orthop Surg 14: 463-471, 2022.
79. Li Y, Wang B, Sun W, Kong C, Ding J, Hu F, Li J, Chen X and Lu S: Construction of circ_0071922-miR-15a-5p-mRNA network in intervertebral disc degeneration by RNA-sequencing. JOR Spine 7: e1275, 2023.
80. Zhao B, Lu M, Wang D, Li H and He X: Genome-wide identification of long noncoding RNAs in human intervertebral disc degeneration by RNA sequencing. Biomed Res Int 2016: 3684875, 2016.
81. Guo ZL, Liu YY, Gao Y, Guan XM, Li H and Cheng M: Circ-RNA expression pattern and circ-RNA-miRNA-mRNA network in the pathogenesis of human intervertebral disc degeneration. Cell J 23: 218-224, 2021.
82. Wan ZY, Song F, Sun Z, Chen YF, Zhang WL, Samartzis D, Ma CJ, Che L, Liu X, Ali MA, *et al*: Aberrantly expressed long noncoding RNAs in human intervertebral disc degeneration: A microarray related study. Arthritis Res Ther 16: 465, 2014.
83. Cheng X, Zhang L, Zhang K, Zhang G, Hu Y, Sun X, Zhao C, Li H, Li YM and Zhao J: Circular RNA VMA21 protects against intervertebral disc degeneration through targeting miR-200c and X linked inhibitor-of-apoptosis protein. Ann Rheum Dis 77: 770-779, 2018.
84. Yu C, Zhao J, Cheng F, Chen J, Chen J, Xu H, Shi K, Xia K, Ding S, Wang K, *et al*: Silencing circATXN1 in aging nucleus pulposus cell alleviates intervertebral disc degeneration via correcting progerin mislocalization. Research (Wash D C) 7: 0336, 2024.
85. Wang T, Yan X, Song D, Li Y, Li Z and Feng D: CircEYA3 aggravates intervertebral disc degeneration through the miR-196a-5p/EBF1 axis and NF-κB signaling. Commun Biol 7: 390, 2024.
86. Chen X, Song Y, Chen G, Zhang B, Bai Y, Sun C, Fan D and Chen Z: Circular RNA CircFOXO3 functions as a competitive endogenous RNA for acid-sensing ion channel subunit 1 mediating oxeiptosis in nucleus pulposus. Biomedicines 12: 678, 2024.
87. Ji ML, Jiang H, Zhang XJ, Shi PL, Li C, Wu H, Wu XT, Wang YT, Wang C and Lu J: Preclinical development of a microRNA-based therapy for intervertebral disc degeneration. Nat Commun 9: 5051, 2018.
88. Huang Y, Zhang Z, Wang J, Shen S, Yao T, Xu Y, Chen Z, Fang B and Ma J: circSPG21 protects against intervertebral disc disease by targeting miR-1197/ATP1B3. Exp Mol Med 53: 1547-1558, 2021.
89. Du X, Chen S, Cui H, Huang Y, Wang J, Liu H, Li Z, Liang C, Zheng Z and Wang H: Circular RNA hsa_circ_0083756 promotes intervertebral disc degeneration by sponging miR-558 and regulating TREM1 expression. Cell Prolif 55: e13205, 2022.
90. Xie L, Huang W, Fang Z, Ding F, Zou F, Ma X, Tao J, Guo J, Xia X, Wang H, *et al*: CircERCC2 ameliorated intervertebral disc degeneration by regulating mitophagy and apoptosis through miR-182-5p/SIRT1 axis. Cell Death Dis 10: 751, 2019.
91. Yao W, Hu X and Wang X: Crossing epigenetic frontiers: The intersection of novel histone modifications and diseases. Signal Transduct Target Ther 9: 232, 2024.
92. Gu T, He Y, Zhou J, Qiu X, Yang W, Zhu Q, Liang Y, Zheng Y, Yik JHN, Haudenschild DR, *et al*: CircFUNDC1 interacts with CDK9 to promote mitophagy in nucleus pulposus cells under oxidative stress and ameliorates intervertebral disc degeneration. Cell Death Dis 16: 94, 2025.
93. Guo Z, Su W, Zhou R, Zhang G, Yang S, Wu X, Qiu C, Cong W, Shen N, Guo J, *et al*: Exosomal MATN3 of urine-derived stem cells ameliorates intervertebral disc degeneration by antisenescence effects and promotes NPC proliferation and ECM synthesis by activating TGF-β. Oxid Med Cell Longev 2021: 5542241, 2021.
94. Wang Z, Zhang J, Zheng W and He Y: Long non-coding RNAs H19 and HOTAIR implicated in intervertebral disc degeneration. Front Genet 13: 843599, 2022.
95. Yu Y, Zhang X, Li Z, Kong L and Huang Y: LncRNA HOTAIR suppresses TNF-α induced apoptosis of nucleus pulposus cells by regulating miR-34a/Bcl-2 axis. Biomed Pharmacother 107: 729-737, 2018.
96. Shao T, Hu Y, Tang W, Shen H, Yu Z and Gu J: The long noncoding RNA HOTAIR serves as a microRNA-34a-5p sponge to reduce nucleus pulposus cell apoptosis via a NOTCH1-mediated mechanism. Gene 715: 144029, 2019.
97. Zhang S, Song S, Cui W, Liu X and Sun Z: Mechanism of long noncoding RNA HOTAIR in nucleus pulposus cell autophagy and apoptosis in intervertebral disc degeneration. Evid Based Complement Alternat Med 2022: 8504601, 2022.
98. Zhan S, Wang K, Song Y, Li S, Yin H, Luo R, Liao Z, Wu X, Zhang Y and Yang C: Long non-coding RNA HOTAIR modulates intervertebral disc degenerative changes via Wnt/β-catenin pathway. Arthritis Res Ther 21: 201, 2019.
99. Donato L, Mordà D, Scimone C, Alibrandi S, D'Angelo R and Sidoti A: From powerhouse to regulator: The role of mitoepigenetics in mitochondrion-related cellular functions and human diseases. Free Radic Biol Med 218: 105-119, 2024.
100. Hou Y, Liu L, Guo Y and Shi J: Epigenetic crossroads in intervertebral disc degeneration: Unlocking novel therapeutic avenues (Review). Mol Med Rep 33: 113, 2026.
101. Wiese M and Bannister AJ: Two genomes, one cell: Mitochondrial-nuclear coordination via epigenetic pathways. Mol Metab 38: 100942, 2020.
102. Cao K, Feng Z, Gao F, Zang W and Liu J: Mitoepigenetics: An intriguing regulatory layer in aging and metabolic-related diseases. Free Radic Biol Med 177: 337-346, 2021.
103. Sharma J, Kumari R, Bhargava A, Tiwari R and Mishra PK: Mitochondrial-induced epigenetic modifications: From biology to clinical translation. Curr Pharm Des 27: 159-176, 2021.
104. Matilainen O, Quirós PM and Auwerx J: Mitochondria and epigenetics-crosstalk in homeostasis and stress. Trends Cell Biol 27: 453-463, 2017.
105. Yin MT and Guo L: Mitochondrial DNA methylation: State-of-the-art in molecular mechanisms and disease implications. J Adv Res 83: 455-473, 2026.
106. Shock LS, Thakkar PV, Robinson JM and Taylor SM: Cytosine methylase and hydroxymethylase activity in mammalian mitochondria. Front Cell Dev Biol 13: 1677402, 2025.
107. Santos JH: Mitochondria signaling to the epigenome: A novel role for an old organelle. Free Radic Biol Med 170: 59-69, 2021.
108. Liu YF, Zhu JJ, Yu Tian X, Liu H, Zhang T, Zhang YP, Xie SA, Zheng M, Kong W, Yao WJ, *et al*: Hypermethylation of mitochondrial DNA in vascular smooth muscle cells impairs cell contractility. Cell Death Dis 11: 35, 2020.
109. Coppedè F and Stoccoro A: Mitoepigenetics and neurodegenerative diseases. Front Endocrinol (Lausanne) 10: 86, 2019.
110. Mishra M and Kowluru RA: Epigenetic modification of mitochondrial DNA in the development of diabetic retinopathy. Invest Ophthalmol Vis Sci 56: 5133-5142, 2015.
111. Zhang J, Shang J, Wang F, Huo X, Sun R, Ren Z, Wang W, Yang M, Li G, Gao D, *et al*: Decreased mitochondrial D-loop region methylation mediates an increase in mitochondrial DNA copy number in CADASIL. Clin Epigenetics 14: 2, 2022.
112. Hu X, Wang Z, Zhang H, Cui P, Li Y, Chen X, Kong C, Wang W and Lu S: Single-cell sequencing: New insights for intervertebral disc degeneration. Biomed Pharmacother 165: 115224, 2023.
113. Yu C, Yang W, Wang Y and Zhang Y: Single-cell sequencing reveals key biological insights into intervertebral disc development and degeneration. Curr Mol Med: Jan 13, 2026 (Epub ahead of print).

114. Yang G, Dong C, Wu Z, Wu P, Yang C, Li L, Zhang J and Wu X: Single-cell RNA sequencing-guided engineering of mitochondrial therapies for intervertebral disc degeneration by regulating mtDNA/SPARC-STING signaling. Bioact Mater 48: 564-582, 2025.
115. Zhao L, Liang K, Cheng W, Zhou X, Yang L, Mao X, Qi W, Jin H, Zhang W, Pan H and Wang D: A multifaceted strategy for intra- and extracellular nucleic acid regulation to alleviate intervertebral disc degeneration. Nat Commun 16: 7936, 2025.
116. Zhou H, Liang C, Cheng F, Wang K, Zhang Y, Chen J, Xu H, Yu C, Xia K, Li Y, *et al*: Mitochondria-targeting polymeric micelles for intervertebral disc degeneration alleviation via coordinated cascade energetic intervention. ACS Nano 19: 41121-41135, 2025.
117. Fu J, Leng X, Long J, Liao Z, An X, Ai X, Long D, Li C, Huang B, Zhou Y, *et al*: SETD1A regulates glycolysis and senescence of nucleus pulposus cells via H3K4me3-HELZ2/PPARα-HIF1α axis to drive intervertebral disc degeneration. Adv Sci (Weinh): e75105, 2026 (Epub ahead of print).
118. Wu O, Jin Y, Weng S, Zhang Z, Tu K, Sun J, Chen L, Chen Q, Chen Z, Jones M, *et al*: METTL3 regulates α-KG-dependent mitophagy and apoptosis in nucleus pulposus cells through the MALAT1/miR-23c/IDH1 axis. Cell Rep 45: 116793, 2026.
119. Yao B, Wan L, Deng J, Chen Z, Zhao L, Wang W and Han Z: m6A-mediated silencing of RNF41 by METTL3/YTHDC1 disrupts autophagy to drive intervertebral disc degeneration. Cell Biol Toxicol 41: 147, 2025.
120. Yang Q, Huang F, Wang C, Liang X, Huang L, Xu H, Liu J, Wei Q and Jiang H: METTL3-mediated m^6A modification promotes intervertebral disc degeneration. Ann Med 57: 2546670, 2025.
121. Song C, Xu Y, Peng Q, Chen R, Zhou D, Cheng K, Cai W, Liu T, Huang C, Fu Z, *et al*: Mitochondrial dysfunction: A new molecular mechanism of intervertebral disc degeneration. Inflamm Res 72: 2249-2260, 2023.
122. Qiu B, Xie X and Xi Y: Mitochondrial quality control: The real dawn of intervertebral disc degeneration? J Transl Med 22: 1126, 2024.
123. Song Y, Liang H, Li G, Ma L, Zhu D, Zhang W, Tong B, Li S, Gao Y, Wu X, *et al*: The NLRX1-SLC39A7 complex orchestrates mitochondrial dynamics and mitophagy to rejuvenate intervertebral disc by modulating mitochondrial Zn^{2+} trafficking. Autophagy 20: 809-829, 2024.
124. Liu M, Liu K, Cheng D, Zheng B, Li S and Mo Z: The regulatory role of NLRX1 in innate immunity and human disease. Cytokine 160: 156055, 2022.
125. Stokman G, Kors L, Bakker PJ, Rampanelli E, Claessen N, Teske GJD, Butter L, van Andel H, van den Bergh Weerman MA, Larsen PWB, *et al*: NLRX1 dampens oxidative stress and apoptosis in tissue injury via control of mitochondrial activity. J Exp Med 214: 2405-2420, 2017.
126. Roth GV, Gengaro IR and Qi LS: Precision epigenetic editing: Technological advances, enduring challenges, and therapeutic applications. Cell Chem Biol: S2451-9456(24)00309-X, 2024 (Epub ahead of print).
127. Yuan L, Xiong Y, Zhang Y, Gu S and Lei Y: Epigenome editing based treatment: Progresses and challenges. Mol Ther 34: 46-67, 2026.
128. Cappelluti MA, Mollica Poeta V, Valsoni S, Quarato P, Merlin S, Merelli I and Lombardo A: Durable and efficient gene silencing in vivo by hit-and-run epigenome editing. Nature 627: 416-423, 2024.
129. Kantor B, O'Donovan B, Rittiner J, Hodgson D, Lindner N, Guerrero S, Dong W, Zhang A and Chiba-Falek O: The therapeutic implications of all-in-one AAV-delivered epigenome-editing platform in neurodegenerative disorders. Nat Commun 15: 7259, 2024.
130. Lei Y, Zhang X, Su J, Jeong M, Gundry MC, Huang YH, Zhou Y, Li W and Goodell MA: Targeted DNA methylation in vivo using an engineered dCas9-MQ1 fusion protein. Nat Commun 8: 16026, 2017.
131. Horii T, Morita S, Hino S, Kimura M, Hino Y, Kogo H, Nakao M and Hatada I: Successful generation of epigenetic disease model mice by targeted demethylation of the epigenome. Genome Biol 21: 77, 2020.
132. Stover JD, Trone MA, Lawrence B and Bowles RD: Multiplex epigenome editing of ion channel expression in nociceptive neurons abolished degenerative IVD-conditioned media-induced mechanical sensitivity. JOR Spine 6: e1253, 2023.
133. Stover JD, Farhang N, Lawrence B and Bowles RD: Multiplex epigenome editing of dorsal root ganglion neuron receptors abolishes redundant interleukin 6, tumor necrosis factor alpha, and interleukin 1β signaling by the degenerative intervertebral disc. Hum Gene Ther 30: 1147-1160, 2019.
134. Stover JD, Trone MAR, Weston J, Lewis C, Levis H, Farhang N, Philippi M, Zeidan M, Lawrence B and Bowles RD: Therapeutic CRISPR epigenome editing of inflammatory receptors in the intervertebral disc. Mol Ther 32: 3955-3973, 2024.
135. Liang T, Gao B, Zhou J, Qiu X, Qiu J, Chen T, Liang Y, Gao W, Qiu X and Lin Y: Constructing intervertebral disc degeneration animal model: A review of current models. Front Surg 9: 1089244, 2023.
136. Tang SN, Bonilla AF, Chahine NO, Colbath AC, Easley JT, Grad S, Haglund L, Le Maitre CL, Leung V, McCoy AM, *et al*: Controversies in spine research: Organ culture versus in vivo models for studies of the intervertebral disc. JOR Spine 5: e1235, 2022.
137. Daly C, Ghosh P, Jenkin G, Oehme D and Goldschlager T: A review of animal models of intervertebral disc degeneration: Pathophysiology, regeneration, and translation to the clinic. Biomed Res Int 2016: 5952165, 2016.
138. Choi H, Tessier S, Silagi ES, Kyada R, Yousefi F, Pleshko N, Shapiro IM and Risbud MV: A novel mouse model of intervertebral disc degeneration shows altered cell fate and matrix homeostasis. Matrix Biol 70: 102-122, 2018.
139. Bergknut N, Rutges JPHJ, Kranenburg HJC, Smolders LA, Hagman R, Smidt HJ, Lagerstedt AS, Penning LC, Voorhout G, Hazewinkel HAW, *et al*: The dog as an animal model for intervertebral disc degeneration? Spine (Phila Pa 1976) 37: 351-358, 2012.
140. Xu X, Wang D, Zheng C, Gao B, Fan J, Cheng P, Liu B, Yang L and Luo Z: Progerin accumulation in nucleus pulposus cells impairs mitochondrial function and induces intervertebral disc degeneration and therapeutic effects of sulforaphane. Theranostics 9: 2252-2267, 2019.
141. Castillo ER and Lieberman DE: Lower back pain. Evol Med Public Health 2015: 2-3, 2015.
142. Alini M, Diwan AD, Erwin WM, Little CB and Melrose J: An update on animal models of intervertebral disc degeneration and low back pain: Exploring the potential of artificial intelligence to improve research analysis and development of prospective therapeutics. JOR Spine 6: e1230, 2023.
143. Kong M, Gao C, Han S, Jin C, Hao M, Zhao J, Luan J, Lin Y, Li Q and Ma X: Establishing an intervertebral disc degeneration model in bipedal rats via a modified feeding approach. Sci Rep 15: 35319, 2025.
144. Cheng Y, Ma Z, Kim BH, Wu W, Cayting P, Boyle AP, Sundaram V, Xing X, Dogan N, Li J, *et al*: Principles of regulatory information conservation between mouse and human. Nature 515: 371-375, 2014.
145. Mainardi A, Cambria E, Occhetta P, Martin I, Barbero A, Schären S, Mehrkens A and Krupkova O: Intervertebral disc-on-a-chip as advanced in vitro model for mechanobiology research and drug testing: A review and perspective. Front Bioeng Biotechnol 9: 826867, 2022.
146. Poletto DL, Crowley JD, Tanglay O, Walsh WR and Pelletier MH: Preclinical in vivo animal models of intervertebral disc degeneration. Part 1: A systematic review. JOR Spine 6: e1234, 2022.
147. Romaniyanto F, Mahyudin F, Utomo DN, Suroto H, Sari WA, Fachreza MS, Sadewa D, Dzikri DN and Nofaldi F: Effectivity of puncture method for intervertebral disc degeneration animal models: Review article. Ann Med Surg (Lond) 85: 3501-3505, 2023.
148. Sakai D and Grad S: Advancing the cellular and molecular therapy for intervertebral disc disease. Adv Drug Deliv Rev 84: 159-171, 2015.
149. ENCODE Project Consortium; Moore JE, Purcaro MJ, Pratt HE, Epstein CB, Shoresh N, Adrian J, Kawli T, Davis CA, Dobin A, *et al*: Expanded encyclopaedias of DNA elements in the human and mouse genomes. Nature 583: 699-710, 2020.
150. Zhou H, Clark E, Guan D, Lagarrigue S, Fang L, Cheng H, Tuggle CK, Kapoor M, Wang Y, Giuffra E and Egidy G: Comparative genomics and epigenomics of transcriptional regulation. Annu Rev Anim Biosci 13: 73-98, 2025.
151. Hao Y, Zhu G, Yu L, Ren Z, Zhou W, Zhang P and Lian X: FOXO3-activated HOTTIP sequesters miR-615-3p away from COL2A1 to mitigate intervertebral disc degeneration. Am J Pathol 194: 280-295, 2024.

152. Jiang X and Chen D: LncRNA FAM83H-AS1 maintains intervertebral disc tissue homeostasis and attenuates inflammation-related pain via promoting nucleus pulposus cell growth through miR-22-3p inhibition. Ann Transl Med 8: 1518, 2020.
153. Yang X, Cao X, Wang X, Guo J, Yang Y, Lu L, Zhang P, Yang H, Rong K, Zhou T, *et al*: Palladium nanoparticles degrade advanced glycation end products via valosin-containing protein mediated autophagy to attenuate high-glucose/high-fat-induced intervertebral disc degeneration. Exploration (Beijing) 5: 20230174, 2025.
154. Kawarai Y, Jang SH, Lee S, Millecamps M, Kang H, Gregoire S, Suzuki-Narita M, Ohtori S and Stone LS: Exercise attenuates low back pain and alters epigenetic regulation in intervertebral discs in a mouse model. Spine J 21: 1938-1949, 2021.
155. Zhang Z, Lin J, Nisar M, Chen T, Xu T, Zheng G, Wang C, Jin H, Chen J, Gao W, *et al*: The Sirt1/P53 axis in diabetic intervertebral disc degeneration pathogenesis and therapeutics. Oxid Med Cell Longev 2019: 7959573, 2019.
156. Zhang TW, Li ZF, Dong J and Jiang LB: The circadian rhythm in intervertebral disc degeneration: An autophagy connection. Exp Mol Med 52: 31-40, 2020.
157. Dudek M, Gossan N, Yang N, Im HJ, Ruckshanthi JP, Yoshitane H, Li X, Jin D, Wang P, Boudiffa M, *et al*: The chondrocyte clock gene Bmal1 controls cartilage homeostasis and integrity. J Clin Invest 126: 365-376, 2016.
158. Xiang M, Peng Q, Dai F, Wu Y, Liu L and Liu H: Machine learning-enabled identification of nucleus pulposus senescence-associated genes as potential biomarkers for intervertebral disc degeneration. Eur J Med Res 30: 887, 2025.
159. Huang K, Shen L, Guan H, Dai L, Huang X, Zhang X, Xu X and Liu C: Construction of a lncRNA-miRNA-mRNA network for biomarker identification in intervertebral disc degeneration. J Musculoskelet Neuronal Interact 25: 316-327, 2025.
160. Murphy K, Lufkin T and Kraus P: Development and degeneration of the intervertebral disc-insights from across species. Vet Sci 10: 540, 2023.
161. Hu Y, Yang R, Liu S, Song Z and Wang H: The emerging roles of nanocarrier drug delivery system in treatment of intervertebral disc degeneration-current knowledge, hot spots, challenges and future perspectives. Drug Des Devel Ther 18: 1007-1022, 2024.
162. Ding Y, Li F, Wang Y, Pan W, Fu X and Tan S: Nanomedicine approaches for intervertebral disc regeneration: From bench to bedside. Pharmaceutics 17: 313, 2025.
163. Wagner EK, Vedadghavami A, Jacobsen TD, Goel SA, Chahine NO and Bajpayee AG: Avidin grafted dextran nanostructure enables a month-long intra-discal retention. Sci Rep 10: 12017, 2020.
164. Zhou H, Ning H, Liu Q, Tan Q, Zeng D, Feng X, Wang D, Zhao Q, Wu X, He H, *et al*: A single-cell-inspired self-enrichment therapeutic strategy delays intervertebral disc degeneration by inhibiting pyroptosis. Adv Mater 38: e16405, 2026.
165. Wang Y, Wu Y, Zhang B, Zheng C, Hu C, Guo C, Kong Q and Wang Y: Repair of degenerative nucleus pulposus by polyphenol nanosphere-encapsulated hydrogel gene delivery system. Biomaterials 298: 122132, 2023.
166. Wang Y, Deng M, Wu Y, Zheng C, Zhang F, Guo C, Zhang B, Hu C, Kong Q and Wang Y: A multifunctional mitochondria-protective gene delivery platform promote intervertebral disc regeneration. Biomaterials 317: 123067, 2025.
167. Shi S, Ou X, Liu C, Li R, Zheng Q and Hu L: Nanotechnology-enhanced pharmacotherapy for intervertebral disc degeneration treatment. Int J Nanomedicine 19: 14043-14058, 2024.
168. Yang L, Bhujel B, Hou Y, Luo J, An SB, Han I and Lee KB: Effective modulation of inflammation and oxidative stress for enhanced regeneration of intervertebral discs using 3D porous hybrid protein nanoscaffold. Adv Mater 35: e2303021, 2023.
169. Guo C, Liu Y, Zhao Z, Wu Y, Kong Q and Wang Y: Regulating inflammation and apoptosis: A smart microgel gene delivery system for repairing degenerative nucleus pulposus. J Control Release 365: 1004-1018, 2024.
170. Chen Z, Liao Z, Liu M, Lin F, Chen S, Wang G, Zheng Z, Liu B, Li C, Wang Z, *et al*: Nucleus pulposus-targeting nanocarriers facilitate mirna-based therapeutics for intervertebral disc degeneration. Adv Healthc Mater 12: e2301337, 2023.
171. Song T, Chen H, Huang J, Tian Y, Zhang X, Chen H, Xie Y and Chen L: Engineering nanomaterials for the treatment of intervertebral disc degeneration: From application to therapeutic mechanisms. Small 21: e09806, 2025.
172. Liu W, Ma Z, Wang Y and Yang J: Multiple nano-drug delivery systems for intervertebral disc degeneration: Current status and future perspectives. Bioact Mater 23: 274-299, 2022.
173. Bailey JF, Fields AJ, Liebenberg E, Mattison JA, Lotz JC and Kramer PA: Comparison of vertebral and intervertebral disc lesions in aging humans and rhesus monkeys. Osteoarthritis Cartilage 22: 980-985, 2014.
174. Thompson K, Moore S, Tang S, Wiet M and Purmessur D: The chondrodystrophic dog: A clinically relevant intermediate-sized animal model for the study of intervertebral disc-associated spinal pain. JOR Spine 1: e1011, 2018.
175. Gruber HE and Hanley EN Jr: Morphologic features of spontaneous annular tears and disc degeneration in the aging sand rat (*Psammomys obesus* obesus). Biotech Histochem 92: 402-410, 2017.
176. Tabatabaei SF, Akbari Roknabadi S and Koohi S: DeepEPI: CNN-transformer-based model for extracting TF interactions through predicting enhancer-promoter interactions. Bioinform Adv 5: vbaf221, 2025.
177. Ahmed FS, Aly S and Liu X: EPI-Trans: An effective transformer-based deep learning model for enhancer promoter interaction prediction. BMC Bioinformatics 25: 216, 2024.
178. Yan Y, Chai X, Liu J, Wang S, Li W and Huang T: DeepMethyGene: A deep-learning model to predict gene expression using DNA methylations. BMC Bioinformatics 26: 99, 2025.
179. Zhang A, Jia J, Sun M and Wei X: EPI-DynFusion: Enhancer-promoter interaction prediction model based on sequence features and dynamic fusion mechanisms. Front Genet 16: 1614222, 2025.
180. Zhang T, Shao S, Zhang H, Zhao Z, Zhao X, Zhang X, Wang Z and Wang G: KansformerEPI: A deep learning framework integrating KAN and transformer for predicting enhancer-promoter interactions. Brief Bioinform 26: bbaf272, 2025.