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REVIEW

The epigenetic landscape of rheumatoid arthritis: Pathogenesis and drug therapeutic potentials

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Abstract Rheumatoid arthritis (RA) represents a persistent autoimmune condition distinguished by a multifaceted etiology that encompasses both genetic and environmental factors. Recent progress in understanding the mechanisms behind RA pathogenesis has delved into the critical role of epigenetic regulatory processes, including DNA methylation, histone modifications, and the regulation by microRNAs (miRNAs). These findings provide new insights into the intricate nature of RA and pave the way for innovative therapeutic strategies. This review consolidates the latest developments in the epigenetic regulation of RA, concentrating on how these mechanisms affect the dysregulated signaling pathways associated with the disease. We analyze the roles of specific proteins that function as 'writers', 'erasers', and 'readers' in epigenetic modifications, highlighting their potential as targets for therapeutic intervention. Additionally, in view of the significance of miRNAs in the pathogenesis of RA, we deliberate on their involvement in disease progression and explore miRNA-based treatment strategies. By integrating these diverse epigenetic dimensions, this review offers a comprehensive epigenetic perspective on RA pathogenesis and identifies promising avenues for future research and therapeutic interventions.

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1. Introduction

Rheumatoid arthritis (RA) is an enduring inflammatory autoimmune disorder marked by synovial inflammation and the gradual deterioration of cartilage and bone, ultimately leading to joint damage, deformities, disability, and, in some cases, can be fatal[1]. It is estimated that this condition impacts roughly 0.5% to 1.0% of the global population, with a higher prevalence among smokers, females, and individuals with a familial predisposition to the disease. Females are two to three times more prone to develop RA compared to males[2,3]. The heterogeneity of RA complicates the elucidation of its precise pathogenesis; however, it is widely accepted that the pathological features of joints affected by RA involve multiple dysfunctional cell types and inflammatory mediators[4]. Immune cells, fibroblast-like synoviocytes (FLSs), and bone-related cells respond to changes in the microenvironment, disrupting cytokine secretion, signal transduction, and homeostasis. Under pathological conditions, aberrant leukocytes extensively infiltrate synovial tissue and accumulate within inflamed joints. Activated T cells release pro-inflammatory cytokines, such as interleukins (ILs) and tumor necrosis factor (TNF), while B cells generate autoantibodies, including rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA). Within this inflammatory milieu, FLSs adopt an invasive phenotype, secreting inflammatory mediators, RANK ligand (RANKL), and matrix metalloproteinases (MMPs). MMPs degrade the extracellular matrix, giving rise to damage of joint cartilage and bone, whereas RANKL promotes osteoclastogenesis, thereby contributing to bone erosion. Additionally, synoviocytes secrete pro-angiogenic factors that facilitate angiogenesis[5,6]. The interplay among these cellular components culminates in joint inflammation, swelling, and persistent damage, ultimately resulting in functional disability (Fig. 1). In advanced stages, RA can manifest with extra-articular symptoms, affecting the eyes, lungs, heart, and other organs[7,8]. Current therapies for RA mainly focus on pain relief and inflammation reduction, utilizing nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids (GCs), disease-modifying antirheumatic drugs (DMARDs), and biologics[9]. While a majority of patients have experienced clinical benefits from various pharmacological treatments, a substantial proportion continues to demonstrate inadequate or resistant responses to conventional regimens[10]. Moreover, the long-term administration of existing therapies is often accompanied by significant adverse effects, creating an urgent need for new strategies with higher target specificity to improve treatment outcomes.

The etiology of RA is complex, arising from the interaction between environmental influences and genetic susceptibility. Environmental risk factors associated with RA include smoking, infection, obesity, and alterations in the microbiota[1]. Individuals who are first-degree relatives of RA patients have a significantly higher likelihood of developing the disease than the general population[11], with heritability estimates suggesting that genetics account for approximately 65% of the risk. A major contributor to genetic susceptibility is linked to HLA haplotypes, especially the HLA-DRB1 alleles residing in the major histocompatibility complex (MHC) region. Additionally, over 100 susceptibility loci for RA beyond the MHC region have been identified through genome-wide association studies[12−14]. However, genetic factors alone do not fully explain RA risk. The concordance rate for RA among monozygotic twins is only 12%−15%[15], indicating that non-genetic mechanisms play a pivotal role in disease pathogenesis. This gap in understanding has prompted researchers to explore epigenetic mechanisms as a potential bridge between environmental and genetic factors in RA development. Through pre-transcriptional mechanisms (DNA methylation and histone modifications) and post-transcriptional mechanisms (non-coding RNAs), epigenetic regulatory processes can convey environmental information to cells, thereby influencing disease progression (Fig. 1). Changes in epigenetics can also modify how disease-related genes are expressed and contribute to the aberrant activation of multiple signaling pathways. The activation of these pathways contributes to an increase in pro-inflammatory cytokines, chemokines, and other mediators of inflammation, ultimately resulting in disease manifestation[16,17]. Thus, developing novel pharmacological therapies that target epigenetic mechanisms may open promising avenues for RA treatment.

At present, the exploration of clinical trials involving epigenetic therapies for the treatment of RA remains relatively limited. Nevertheless, significant advancements have been made with the selective histone deacetylase 6 inhibitor CKD-506, which has successfully completed phase II clinical trials (NCT04204603) designed to assess its effects on the signs and symptoms of moderate to severe RA in patients who inadequately responded to methotrexate treatment. Additionally, ITF-2357, an orally active pan-HDAC inhibitor, has entered phase II clinical trials to evaluate its safety and efficacy for active systemic juvenile idiopathic arthritis (SJIA), a condition related to RA. This compound has been shown to reduce disease activity, alongside favorable tolerability and manageable adverse effects (NCT00570661)[18]. Alterations in microRNA (miRNA) expression levels may function as biomarkers indicative of both the advancement of RA and the response to therapeutic interventions[19,20]. Other epigenetic agents targeting DNA methyltransferases (DNMTs, as 'writers'), histone deacetylases (HDACs, as 'erasers'), bromodomain and extra-terminal (BET, as 'readers') proteins, and miRNAs, also demonstrate considerable potential for the treatment of RA based on findings from various preclinical studies conducted *in vitro* and *in vivo*. In summary, it is expected that epigenetic drugs will assume a significant role in RA therapy moving forward.

This review aims to elucidate several signal transduction pathways related to RA, the diverse epigenetic modifications implicated in its pathology, and the epigenetic targets currently being explored in clinical or preclinical phases of drug development. Additionally, it will also cover the main epigenetic compounds being studied for potential treatment of RA, as well as miRNA-based therapeutic strategies. By offering an overview of these emerging therapeutic approaches, this review intends to underscore the potential of epigenetic interventions in addressing the intricate and multifaceted nature of RA.

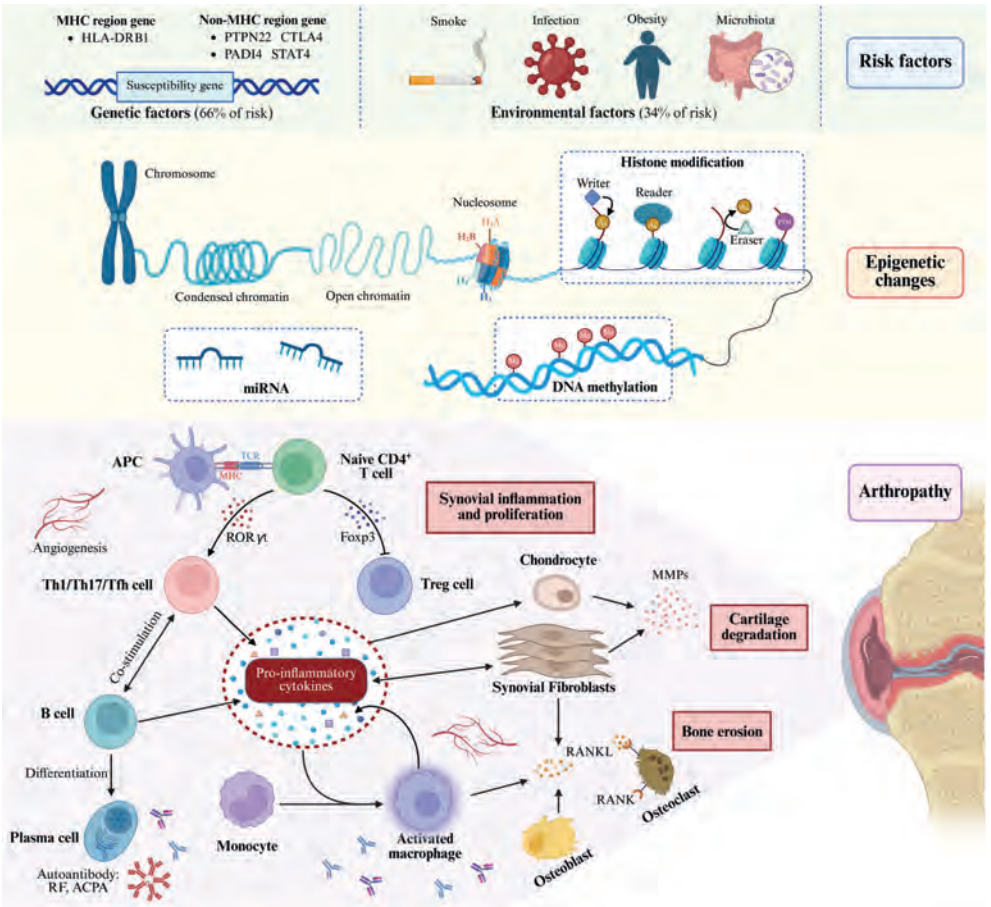


Figure 1 Overview of the pathological process underlying RA. Genetic and environmental risk factors initiate the onset of RA, while gene-environment interactions involving epigenetic mechanisms further modulate gene expression associated with RA development. This activates inflammatory responses, creating an initial inflammatory microenvironment. Within the joint lesions, intercellular crosstalk prompts activated immune cells, FLSs, and bone-related cells to secrete pro-inflammatory and osteoclastogenic factors, exacerbating the inflammatory microenvironment and resulting in synovitis, cartilage degradation, and bone erosion.

2. Signaling pathways and epigenetic mechanism in RA

2.1. Signaling pathways in RA

The intricate cross-talk between cytokines and dysregulated signaling pathways worsens the inflammatory microenvironment within the synovium. Exogenous pro-inflammatory cytokines initiate and amplify specific signaling cascades, transmitting signals to the nucleus. Following nuclear translocation, transcription factors (TFs) directly bind to specific DNA sequences and recruit epigenetic modifying enzymes, thereby altering chromatin structure and modulating the expression of target genes. This process leads to an increased production of pro-inflammatory mediators while concurrently suppressing the secretion of anti-inflammatory cytokines. The subsequent release of these endogenous inflammatory mediators further activates related signaling pathways, establishing a vicious cycle that ultimately compromises the functionality of RA-associated cells (Fig. 2).

2.1.1. JAK–STAT signaling pathway

The Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway serves as an essential mechanism for the transduction of cytokine signals, consisting of transmembrane receptors, JAKs, and STATs[21]. This signaling pathway requires specific JAK subtypes, including JAK1, JAK2, JAK3, and TYK2, to effectively activate cytokine receptors and ensure efficient signal transmission. As downstream targets of JAK, the STAT family consists of STAT1-4, STAT5A, STAT5B, and STAT6, all integral to signal transduction and gene transcription activation[22]. Recent studies indicate a close association between the progression of RA and hyperactivation of this pathway[23]. Different cytokines and chemokines contribute to the accumulation of immune cells within the synovial region, instigating inflammation and tissue damage. The interaction of cytokines with their specific receptors activates JAK kinases, which promote the phosphorylation of STAT proteins *via* their tyrosine kinase activity. Once phosphorylated, STAT proteins dimerize and translocate to the nucleus to modulate the expression of genes associated with inflammation, thereby perpetuating the inflammatory response[24]. Suppressor of cytokine signaling (SOCS) proteins act as critical negative regulators within the JAK/STAT pathway, functioning to inhibit JAK kinase activity and diminish the phosphorylation of STAT proteins, thus preserving the equilibrium of immune responses and cellular functions[25]. However, in the case of RA, the negative regulatory mechanisms that govern STAT protein activation, particularly those mediated by SOCS, have proven to be markedly inadequate.

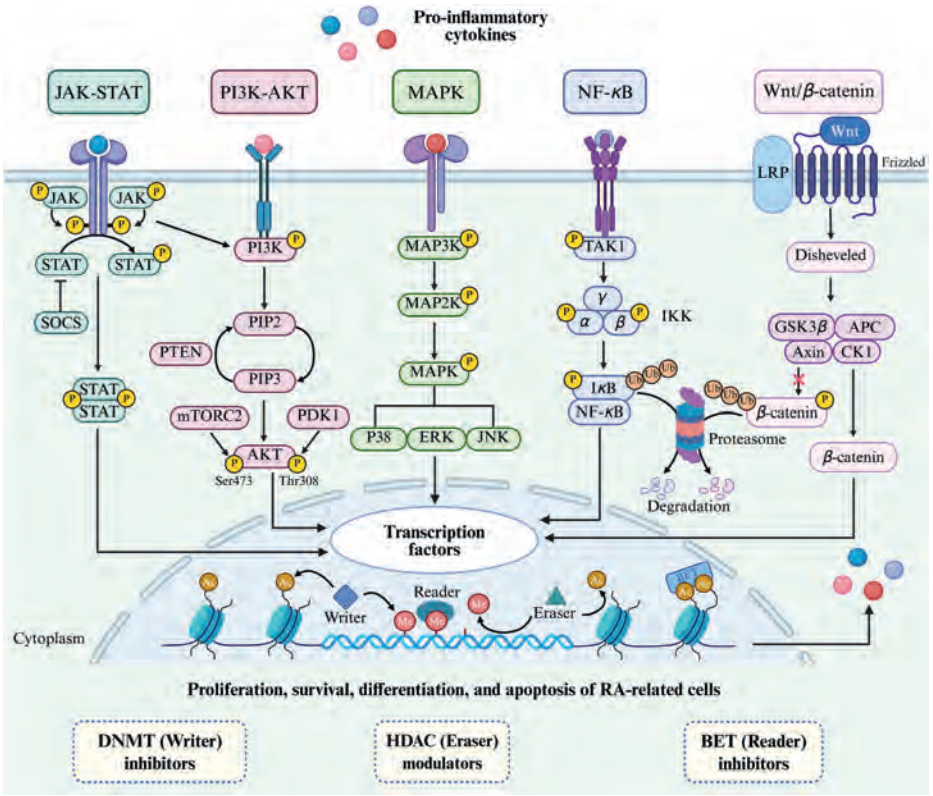


Figure 2 Key signaling pathways and epigenetic targets involved in RA. The activation of pathways including JAK−STAT, PI3K−AKT, MAPK, NF-κB, and Wnt/β-catenin facilitates the translocation of transcription factors into the nuclear through various signaling cascades. These factors interact with epigenetic regulatory proteins to regulate chromatin states and the transcription of inflammation-related genes, thereby influencing the behavior of cells related to RA and exacerbating disease progression.

2.1.2. MAPK signaling pathway

As a crucial intracellular signal transduction mechanism, the activation of the mitogen-activated protein kinase (MAPK) signaling pathway depends on a three-tiered enzyme cascade that facilitates signaling through a series of phosphorylation events[26]. The binding of signaling molecules to surface receptors initiates the phosphorylation and activation of mitogen-activated protein kinase kinase kinase (MAP3K), which subsequently phosphorylates and activates mitogen-activated protein kinase kinase (MAP2K). Following this, MAP2K further phosphorylates and activates MAPK. Upon activation, MAPK localizes to the cell nucleus, where it phosphorylates various TFs, thereby modulating cellular behavior and function. Dysregulation of this signaling pathway is strongly associated with joint damage and inflammation[27]. This pathway encompasses three main MAPK subfamilies: p38, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK). Among these, p38 MAPK is particularly crucial in RA-related inflammation, as it regulates the production of several pro-inflammatory cytokines, including IL-6, IL-1β, and TNF-α. Activation of p38 MAPK is implicated in various cellular processes, including apoptosis, cell cycle regulation, and cell differentiation, exerting multifaceted influences on the pathological progression of RA[28]. The JNK subfamily primarily mediates MMP-induced cartilage degradation in RA, whereas the ERK family is crucial for cellular proliferation and differentiation, significantly contributing to synovial tissue remodeling during the progression of RA[29,30]. Consequently, inhibition of MAPK signal transduction is increasingly recognized as a promising therapeutic strategy.

2.1.3. PI3K−AKT signaling pathway

The phosphoinositide 3-kinase (PI3K)-protein kinase B (AKT, also known as PKB) signaling pathway regulates vital cellular processes in response to external cues, including growth, metabolism, survival, and angiogenesis[31]. Upon activation, PI3K phosphorylates phosphatidylinositol (4,5)-bisphosphate (PIP2) to generate the second messenger phosphatidylinositol (3,4,5)-trisphosphate (PIP3), while phosphatase and tensin homolog deleted on chromosome ten (PTEN), a tumor suppressor, counteracts PI3K by dephosphorylating PIP3. PIP3 facilitates AKT activation, which requires additional phosphorylation at Thr308 and Ser473 by PDK1 and mammalian target of rapamycin complex 2 (mTORC2), respectively[32]. Once activated, AKT dissociates from the plasma membrane and translocates to the cytoplasm and nucleus, phosphorylating multiple downstream effector molecules, including mTOR, glycogen synthase kinase-3 (GSK-3), NF-κB, and forkhead box O (FOXO) signaling[33]. Abnormal proliferation and apoptosis imbalance of synovial cells are major pathological features observed in RA. Research has demonstrated that the PI3K−AKT signaling cascade is abnormally activated in RA synovial cells. Activated AKT promotes mTOR activation and inhibits autophagy in FLSs, leading to persistent abnormal proliferation of synovial cells[34,35]. Furthermore, this pathway increases the levels of several inflammatory cytokines, including IL-6, IL-1β, IL-17, and TNF-α, which are critical in the

development of RA. It also boosts the production of VEGF and HIF-1α, facilitating angiogenesis[36–38]. This not only hinders the nutrition supply to bones through the synovial tissue but also enhances the secretion of various inflammatory mediators, which exacerbates RA conditions.

2.1.4. *Wnt/β-catenin signaling pathway*

The initiation of the Wnt signaling pathway occurs when Wnt proteins attach to cell membrane receptors through autocrine and paracrine mechanisms, thereby activating both canonical and non-canonical Wnt signaling pathways. These pathways are critical for regulating cell proliferation, renewal, embryonic development, and bone metabolism[39]. Classical Wnt signaling is contingent upon the phosphorylation state of β-catenin and its subsequent degradation in the cytoplasm. In the resting state, β-catenin is targeted for degradation by a multiprotein complex that includes adenomatous polyposis coli (APC), GSK-3β, casein kinase 1 (CK1), and axis inhibition protein (Axin). When the Wnt protein binds to the Frizzled/LRP receptor complex on the cell surface, dishevelled (Dvl/Dsh) is activated. This activation enables the multiprotein complex to dissociate and inactivates GSK-3β, thereby preventing the phosphorylation of β-catenin. The accumulated β-catenin then moves into the nucleus, where it interacts with T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) to transcriptionally activate specific target genes[39]. The Wnt signaling pathway exhibits a complex dual role in RA. On one hand, overexpressed Wnt proteins contribute to synovial inflammation, promote the proliferation of FLSs, and exacerbate the inflammatory response. On the other hand, activation of the Wnt signaling facilitates the differentiation and proliferation of osteoblasts while inhibiting the generation of osteoclasts and bone erosion in the pathological process of RA by increasing the expression of osteoprotegerin (OPG)[40,41]. Recent studies indicate that during the progression of RA, especially in bone formation, the level of the Wnt antagonist dickkopf-1 (DKK1) is upregulated. This upregulation inhibits the activation of the Wnt signaling pathway, resulting in increased bone resorption and decreased bone formation, ultimately leading to joint destruction[42]. Therefore, different components of the Wnt signaling pathway may have varying and even opposing roles at different stages and in various cell types involved in RA. A deeper exploration of the specific mechanisms of the Wnt signaling pathway in RA is crucial for developing effective treatments.

2.1.5. *NF-κB signaling pathway*

The NF-κB signaling pathway is integral to the processes of inflammation and autoimmune diseases, serving as a key regulator between cytokines and inflammatory responses. Comprising NF-κB, inhibitors of NF-κB (IκB), and IκB kinase (IKK), this pathway engages NF-κB as a dimeric TF that regulates the expression of genes related to inflammation, apoptosis, and immune functions by binding to κB sites on DNA. In its inactive state, NF-κB is retained in the cytoplasm by forming a complex with IκB. When activated, IKK degrades IκB, enabling NF-κB to move to the nucleus and initiate transcription[43]. It has been found that NF-κB levels are markedly increased in the synovial tissue of RA patients, triggering the generation of pro-inflammatory cytokines that further stimulate the NF-κB pathway, thus perpetuating a destructive cycle[44,45]. Activated NF-κB also promotes the proliferation of FLSs, enhances their invasive and infiltrative capabilities, and reduces apoptosis in these cells by regulating the expression of anti-apoptotic proteins[46]. Additionally, this pathway interacts with other signaling pathways such as MAPK and PI3K−AKT, working collaboratively to mediate the inflammatory response and regulate cellular functions in RA.

2.2. *Epigenetic mechanisms in the pathogenesis of RA*

Epigenetic dysregulation stands at the forefront of pathogenic mechanisms underlying widespread autoimmune diseases, including rheumatoid arthritis (RA), systemic sclerosis (SSc), systemic lupus erythematosus (SLE), Sjögren syndrome (SS), and type 1 diabetes (T1D). Although aberrant differentiation and dysfunction of immune cells are key factors in these conditions, the precise etiology of most autoimmune diseases involves complex gene-gene and gene-environment interactions[47,48]. Epigenetic mechanisms are integral to autoimmune diseases at multiple stages, encompassing disease etiology, pathogenic mechanisms during disease development, and as biomarkers for disease activity and progression[49]. This comprehensive involvement of epigenetics throughout the disease course highlights its critical role in understanding and managing autoimmune diseases.

The organization and function of chromatin are closely linked to the macromolecules DNA and histones[50]. Epigenetics refers to heritable changes in gene expression and function that occur independently of alterations in the DNA sequence, mainly including DNA methylation, histone modification patterns, and non-coding RNAs. The corresponding epigenetic proteins have been identified as ‘writers’ or ‘erasers’ that modify DNA and histones by adding or removing specific functional groups, thereby modulating gene expression[51,52]. Moreover, ‘reader’ proteins are responsible for recognizing these epigenetic marks to regulate gene activity.

Research into epigenetic alterations in RA has focused on immune cells derived from peripheral blood samples, particularly T cells and B cells. Of relevance, the epigenetic modifications observed in rheumatoid arthritis synovial fibroblasts (RASF) have also garnered particular attention due to their aggressive phenotype[53,54]. These specific epigenetic alterations can influence key signaling pathways critical for the pathogenesis of RA. For example, DNA methylation changes in RA lead to the hypermethylation-induced downregulation of PTEN, promoting the survival and proliferation of FLSs by activating the PI3K−AKT signaling pathway[55]. The histone code alters the interaction between histones and DNA, affecting the accessibility of TFs to DNA and regulating gene expression. Histone modifications do not merely act as switches for gene transcription; they work in concert with TFs to modulate signaling pathways, thereby contributing to the pro-inflammatory phenotype in RA[56]. In an inflammatory microenvironment, the acetylation of NF-κB p65 reduces its affinity for IκB, allowing p65 to dissociate and enter the nucleus. Additionally, acetylated p65 enhances its binding to DNA, promoting the transcription of inflammatory factors[57,58]. The JAK/STAT signaling pathway also relies on histone acetylation. Specifically, STAT3, a direct target of histone acetyltransferases (HATs) and HDACs, requires HAT-induced acetylation for its dimerization, DNA binding, and transcriptional activation in response to cytokine stimulation[59,60]. As another important regulatory mechanism in the epigenetics of RA, miRNAs can directly target components of inflammatory signaling pathways to exert their effects. Currently, miR-146 and miR-155 have emerged as the most frequently studied miRNAs in RA, often associated with biomarkers and targeted therapies for the disease[61]. Importantly, epigenetic changes are reversible and can

be affected by environmental factors such as diet, stress, and exposure to toxins. This plasticity establishes a critical link between environmental stimuli, gene expression, and the disease manifestation in RA. Collectively, DNA methylation, histone modifications, and miRNA-mediated mechanisms are regarded as the key epigenetic factors in RA.

2.2.1. *DNA methylation*

DNA methylation mainly takes place at cytosine residues within CpG dinucleotides. Such CpG sequences cluster within CpG islands, with about 70% of islands located in gene promoter regions[62]. This modification determines gene activation or silencing, with hypomethylation typically associated with activation and hypermethylation with silencing. The methylation process is mediated by DNMTs, which transfer a methyl group from *S*-adenosylmethionine (SAM) to the fifth carbon of the cytosine ring within the DNA sequence. In terms of structure and function, DNMTs can be classified into maintenance (DNMT1) and *de novo* methyltransferases (DNMT3a, DNMT3b). DNMT3L, although catalytically inactive, serves as a regulatory protein that enhances the activity of DNMT3a and DNMT 3b[63]. While DNA methylation was once considered a stable epigenetic feature, new research indicates that it is, in fact, dynamic and subject to modification by environmental influences, such as pharmacotherapy and variations in dietary methyl donor intake[64]. DNA demethylation occurs through two distinct mechanisms: active and passive. Active DNA demethylation involves the enzymatic removal of the methyl group from 5-methylcytosine (5 mC), whereas passive DNA demethylation transpires in the absence of DNMTs and their associated activities. This absence results in a decrease in the quantity of methylated cytosines, ultimately leading to a decline in overall DNA methylation levels[65]. Nevertheless, there remains a paucity of information regarding the relationship between DNA demethylation and the progression of RA.

Research on DNA methylation in RA mainly focuses on FLSs and peripheral blood mononuclear cells (PBMCs), including T and B lymphocytes. These investigations have evolved from initial findings of genome-wide hypomethylation to sophisticated epigenomic analyses employing contemporary technologies, which enable the identification of differentially methylated genes and the discovery of novel candidate genes implicated in the pathogenesis of RA[66,67]. Early studies indicated that the long interspersed nuclear elements 1 (LINE-1) transposable element in RASF is silenced due to methylation of its promoter region. Subsequent research has shown that the observed increase in LINE-1 expression within RASF is attributed to global DNA hypomethylation[68]. Indeed, RASF do not exhibit consistent hypomethylation. A comparative examination of the methylomes in RA and osteoarthritis (OA) FLSs identified unique patterns across 1859 loci. Among these loci, 732 were found to be hypomethylated, while 1127 exhibited hypermethylation[69]. Intriguingly, the levels of SAM, 5 mC, and DNMT1 were significantly downregulated in RASF, whereas components involved in polyamine metabolism were upregulated. This indicates that global DNA hypomethylation may result from a deficiency in DNMT1 and high consumption of SAM[70,71]. Similar to RASF, PBMCs also exhibit a global trend toward hypomethylation[72]. It is noteworthy that, despite global hypomethylation levels among various cell types in RA patients, the methylation patterns in specific gene promoter regions differ between these cell types. This suggests that distinct epigenetic profiles may be associated with different cellular phenotypes in RA. Hypomethylation of the promoter region of the peptidyl arginine deiminase 4 (PADI4) gene in RA patients is closely related to its increased expression and subsequent autoantigen production, contributing to the progression of RA[73]. Conversely, forkhead box protein P3 (Foxp3), a key TF for regulatory T (Treg) cells, exhibits increased methylation levels, potentially leading to impaired Treg function and weakened regulation of RA inflammatory responses[74]. Additionally, the altered methylation status of individual gene promoters for DR3, IL-6, IL-10, CXCL12, and TBX5 has been delineated in PBMCs and RASF[75]. Recent investigations have unveiled a new methylation signature present in both B cells and T cells of RA patients, significantly augmenting the potential for identifying novel therapeutic targets through specific modulation of these immune cell populations[76]. The DNA methylation patterns associated with RA are depicted in Table 1[55,66,73,77–102], highlighting the epigenome as a promising target for therapy and as reliable indicators for predicting disease progression and treatment.

2.2.2. *Histone modifications*

Histones are basic proteins that facilitate the packaging of DNA into nucleosomes, which are key structural units that further assemble into chromosomes within the nucleus. The N-terminal tails of histones undergo several post-translational modifications (PTMs), including methylation, acetylation, phosphorylation, ubiquitination, sumoylation and ADP-ribosylation modifications[103,104]. These PTMs can alter the interaction between histones and DNA, thus influencing chromatin structure and gene expression. Notably, acetylation and methylation have been strongly implicated in RA aetiology.

2.2.2.1. Histone acetylation and deacetylation. The acetylation level of lysine ε-amino groups is determined by the balance between acetylation and deacetylation, catalyzed by the opposing action of HATs and HDACs, respectively[105]. Acetyl groups are added to histones by HATs, leading to a more accessible chromatin configuration that encourages transcriptional activation. Conversely, HDACs remove these acetyl tags, restoring positive charges on lysine residues and stabilizing a condensed chromatin structure that inhibits transcription[106]. Beyond their role in histone modification, HATs and HDACs also modulate the acetylation levels of non-histone proteins, including TFs, signaling molecules, and structural proteins, to alter protein activity, stability, subcellular localization, and protein-protein interactions (Fig. 3A)[107]. Early investigation of RA synovial tissue revealed that a tendency toward hyperacetylation, particularly of histone H3 in the IL-6 promoter region compared to OA synovial cells, thereby promoting IL-6 production and accelerating RA progression[108]. However, studies on the relationship between histone acetylation dynamics and RA show many discrepancies, emphasizing the need for further exploration into the precise role of acetylation-related enzymes in the pathogenesis of RA.

Based on the mechanism of lysine deacetylation catalyzed by HDACs, deacetylases are classified into two categories: zinc-dependent HDACs (HDAC1-11), which utilize water as a nucleophile to hydrolyze amide bonds, and NAD^+-dependent sirtuins (SIRT1-7), which employ NAD^+ as a cofactor to transfer the acyl group to ribose. Furthermore, according to cellular localization and sequence homology, HDACs are divided into classes I-IV, where class II can be further subdivided into IIa and IIb (Fig. 3A)[109]. Each HDAC isoform has specific roles in

Table 1 DNA methylation status of RA-associated genes.

Gene	Alias	DNA methylation level	Effect	Ref.
PTEN	Phosphatase and tensin homolog deleted on chromosome 10	↑	Contributes to FLSs inflammation and activation	55
PARP9	Poly (ADP-ribose) polymerase family member 9	↓	Alters cell cycle and proliferation to enhance RA immunoreactions	66
PADI4	Peptidyl arginine deiminase type 4	↓	Increased PAD enzyme expression enhances citrullination, causing excessive ACPA production	73
CTLA-4	Cytotoxic T-lymphocyte associated protein 4	↑	Impairs Treg cell function	77
CASP1	Caspase 1	↓	Involved in inflammatory response, apoptosis, and programmed cell death	78
MAP3K5	Mitogen-activated protein kinase kinase kinase 5	↓	Implicated in regulating inflammatory pathways	78
STAT3	Signal transducer and activator of transcription 3	↓	A key signaling protein in IL-6 function	78
MEFV	Mediterranean fever	↓	Associated with inflammation and apoptosis	78
WISP3	WNT1 inducible signaling pathway protein 3	↓	Encodes growth factors	78
CHI3L1	Chitinase 3-like 1	↓	Acts as a cartilage-specific antigen	78
FOXO1	Forkhead box O1	↑	Involved in cellular stress and metabolic regulation	78
TGFBR2	Transforming growth factor β receptor 2	↑	Linked to TGF-β activity and articular cartilage degeneration	78,79
IL-6	Interleukin-6	↓	Increased IL-6 levels	80,81
TBX5	T-box transcription factor 5	↓	Enhances expression of IL-8, CXCL12, and CCL20	82
CXCL12	C-X-C motif chemokine ligand 12	↓	Upregulates CXCL12 and induces MMPs expression	83
SMAD7	Mothers against decapentaplegic homolog 7	↑	Disrupts Th17/Treg balance due to decreased SMAD7	84
UBASH3A	Ubiquitin associated and SH3 domain-containing A	↓	Promotes antigen presentation to autoimmune T cells	85
FOXP3	Forkhead box protein P3	↓	Restores the function of Treg cells, reflecting the therapeutic effect of methotrexate	86
JUN	Jun proto-oncogene	↑	Regulates cell proliferation, cell cycle and apoptosis	87
STAT1	Signal transducer and activator of transcription 1	↑	A key signaling protein in IL-6 function	87
CD44	Homing cell adhesion molecule	↑	Increases incidence as a cell adhesion factor	87
DUSP22	Dual specificity phosphatase 22	↑	Negatively regulates STAT3 and the IL-6/STAT3 signaling pathway	88
KRAS	Kirsten rat sarcoma viral oncogene homolog	↓	Lowers the threshold for T-cell activation and tolerance to self-antigens	89
CD40L	CD40 ligand	↓	Contributes to higher RA incidence in females	90
PTPN11	Protein tyrosine phosphatase non-receptor type 11	↑	Promotes the invasive phenotype of RASF	91
ZBTB38	Zinc finger and BTB domain containing 38	↓	Inhibits the expression of the anti-inflammatory factor IL1r2	92
LBH	Limb bud and heart development	↓	Enhances expression to influence the pathogenesis of RA	93
GALNT9	Polypeptide *N*-acetylgalactosaminyltransferase 9	↓	Increases mucin biosynthesis	94
TNFAIP8	TNF alpha induced protein 8	↓	Negatively regulates innate and adaptive immunity	95
SFRP2	Secreted frizzled-related protein 2	↑	Activates Wnt signaling and promotes FLSs proliferation	96
DPP4	Dipeptidyl peptidase 4	↑	Inhibition of DPP4 enhances cartilage invasion by RASF	97
CCR6	C−C motif chemokine receptor 6	↑	Involved in migration, proliferation, and MMPs production	98
EBF3	Early B-cell factor 3	↑	Mediates TNF-β pathway and affects RASF invasive behavior	99
IRX1	Iroquois homeobox 1	↑	Leads to abnormal proliferation and apoptosis resistance of RASF	99
AHR	Aryl hydrocarbon receptor	↑	Links to germinal center formation in B cells	100
IL-10	Interleukin-10	↓	Associated with higher IL-10 mRNA and serum levels	101
DR3	Death receptor 3	↑	Confers apoptosis resistance in RA synoviocytes	102

↑, hypermethylation; ↓, hypomethylation

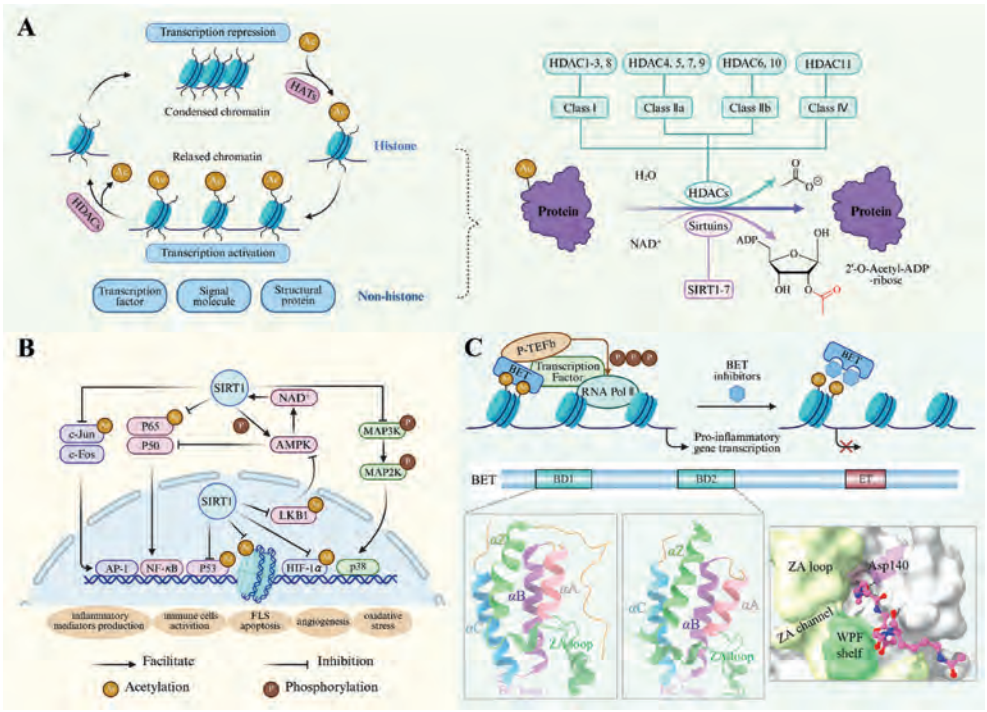


Figure 3 The regulatory mechanisms of histone acetylation in the pathogenesis of RA. (A) (Left) The regulation of gene transcription by histone acetyltransferases (HATs) and histone deacetylases (HDACs) through the equilibrium of histone and non-histone protein acetylation. (Right) The classification of the HDACs based on their catalytic mechanisms. (B) The regulatory mechanism of SIRT1 involvement in the pathogenesis of RA. (C) (Top) Transcriptional regulation by BET proteins and the mechanism of BET inhibitors. (Middle) Distinctive functional domains of BET proteins, including the two bromodomains (BD1 and BD2) and the extra-terminal domain (ET). (Bottom) Cartoon representations of BRD4−BD1 (PDB: 6KEJ) and BRD4−BD2 (PDB: 2OUO). Top-down surface representation of the BRD4−BD1 complex bound to H4K8AcK12Ac (PDB: 3UW9). The ZA loop is highlighted in cyan, the WPF motif in green, and the conserved Asn140 responsible for recognizing acetylated lysine is indicated in purple.

inflammation, manifesting either pro-inflammatory or anti-inflammatory effects[110]. The expression of Class I HDACs was found to be elevated in RA FLSs, correlating with the expression of inflammatory mediators. Interestingly, PBMCs from RA patients exhibited distinct histone acetylation patterns compared to FLSs, showing reduced expression and activity of class I HDACs in PBMCs[111,112]. Class I HDAC1 expression was elevated in RASF compared to that in OASF. HDAC1 knockdown in RASF decreased proliferation, invasion, and migration *in vitro*. In a collagen-induced arthritis (CIA) model, HDAC1 inhibition reduced joint swelling, tissue damage, and TNF levels[113]. Similarly, HDAC3, another class I member, shows potential as a therapeutic target. It is essential for the induction of type I interferons (IFN) and the subsequent activation of STAT1. HDAC3 inhibition reduced the expression of IFN-dependent gene subsets in RASF and impaired activation of nearly half of the inflammatory gene expression programs in macrophages upon lipopolysaccharide (LPS) stimulation[114,115]. Unlike class I HDACs, multiple studies have reported anti-inflammatory functions of some class II HDACs[116]. HDAC5 expression in RA synovial tissue was inversely related to IL-6 expression and disease activity, suggesting an anti-inflammatory role in RA[112]. HDAC6, classified as a Class IIb cytoplasmic enzyme, is upregulated in RA synovial tissues and deacetylates non-histone proteins like α-tubulin, MyD88, and cortactin. Its overexpression is associated with increased inflammatory responses[117,118]. Thus, HDAC6 could potentially be targeted for RA treatment. Studies on sirtuins in RA are limited and inconsistent, leading to no clear consensus on their role in this disease[119]. SIRT1, the most extensively studied sirtuin, predominantly resides in the nucleus but can also shuttle to the cytoplasm, performing regulatory roles in both compartments[120]. It modulates the release of inflammatory mediators and immune cell function through deacetylating histone and non-histone targets, including NF-κB, AP-1, and HIF-1α[121,122]. Interestingly, some inhibitory effects of SIRT1 occur independently of its deacetylase activity; for instance, it can inhibit P38 MAPK phosphorylation, thereby preventing downstream signaling pathways activation. Additionally, SIRT1 indirectly influences NF-κB *via* the phosphorylation that activates AMP-activated protein kinase (AMPK), a crucial NF-κB inhibitor. Simultaneously, activated AMPK enhances cellular NAD^+ levels, boosting SIRT1 activity and establishing a positive feedback loop (Fig. 3B)[123]. Discrepancies in reports on HDACs expression in synovial tissue under pathological conditions indicate that not all HDACs exhibit pro-inflammatory properties. Consequently, targeted inhibition of specific HDAC isoforms may offer a more precise therapeutic approach with fewer side effects compared to pan-HDAC inhibitors.

2.2.2.2. Histone acetylation recognition by 'reader' proteins. Histone acetylation levels are regulated by the activities of writers and erasers. However, the functional outcomes of these acetylation marks ultimately rely on specific 'reader' proteins. Bromodomains (BDs) are protein−protein interaction modules

that have been evolutionarily conserved and exclusively identify acetylation marks[124]. BDs adopt a characteristic left-handed antiparallel four-helix bundle (αZ, αA, αB, αC), with ZA and BC loops forming a hydrophobic binding pocket essential for recognizing acetylated lysine[125]. This distinctive 'BD fold' structure underpins its role in epigenetic regulation. The BET family is a subset of bromodomain proteins, distinguished by their unique structure with two tandem N-terminal bromodomains (BD1 and BD2) and a C-terminal extra-terminal (ET) domain. Variations in the sequence and length of the ZA and BC loops between BD1 and BD2 provide a structural basis for designing selective inhibitors either domain[126]. In mammals, the BET family comprises four members: BRD2, BRD3, BRD4, and the testis-specific BRDT. These proteins serve as crucial scaffolds that bridge acetylated chromatin with the transcriptional machinery. They recruit TFs and P-TEFb to the promoters and enhancers of target genes, forming transcriptional complexes and phosphorylating RNA polymerase II, thereby driving the transcription of pro-inflammatory genes (Fig. 3C)[127]. Moreover, inhibition of BET proteins has been shown to affect the functionality of multiple RA-relevant cell types, including T cell differentiation into distinct subsets, polarization of macrophages, and activation of pathogenic phenotypes in B cells, synovial fibroblasts, and osteoclasts[128−130]. Consequently, targeting BET proteins presents a valuable therapeutic approach worthy of further exploration for the treatment of RA.

2.2.2.3. Histone methylation and demethylation. As another important PTM of histones in the pathophysiology of RA, research on histone methylation remains limited. Methylation of lysine and arginine residues can either promote or inhibit transcription, depending on the specific site and extent of methylation. For instance, trimethylation of lysine 4 on histone H3 (H3K4me3) is generally associated with active transcription, while the trimethylation of lysine 27 on histone H3 (H3K27me3) indicates transcriptional silencing[131]. In RASF, dysregulation of histone methylation is characterized by altered activities of histone methyltransferases (HMTs) and demethylases (HDMs). Specifically, the enhancer of zeste homolog 2 (EZH2), a HMT, is overexpressed in RASF, leading to gene silencing through increased H3K27me3 levels. The epigenetic alteration of secreted frizzled-related protein 1 (SFRP1), mediated by EZH2, significantly reduces the inhibition of the Wnt signaling pathway, contributing to the activation of RASF. Downregulation of EZH2 effectively suppresses the proliferation, migration, and invasion of RASF[132−134]. Conversely, Jumonji domain-containing protein 3 (JMJD3), a HDM that specifically removes the repressive histone mark H3K27me3, is expressed at higher levels in RA FLSs compared to healthy FLSs, with further upregulation induced by platelet-derived growth factor (PDGF). This upregulation of JMJD3 promotes both proliferation and migration in FLSs[135,136]. Therefore, a deeper investigation into the role of histone methylation in RA could not only unveil the molecular mechanisms of the disease but also provide crucial insights for the development of new therapeutic strategies.

2.2.3. MicroRNAs

MiRNAs are endogenous single-stranded non-coding RNAs that are transcribed from DNA but not translated into proteins. Typically around 22 nucleotides long, these small RNAs are biosynthesized through a multi-step process involving the key ribonucleases Drosha and Dicer in the nucleus and cytoplasm, respectively[137]. Mature miRNAs regulate gene expression by interacting with the 3′ UTR of target mRNAs to either suppress protein translation or induce mRNA degradation (Fig. 4A)[138]. While individual miRNAs may exert subtle regulatory effects, mammalian mRNAs often contain binding sites for multiple miRNAs. This multi-layered regulation means that even minor changes in a single miRNA's expression can significantly alter the expression of numerous genes[139,140]. MiRNAs also mediate intercellular communication by secretion and transport to target cells *via* vesicles, including exosomes. These functional miRNAs form a complex regulatory network that engages in extensive signaling of inflammatory pathways and is essential for key biological processes such as proliferation, differentiation, apoptosis, and metabolism[141]. Given their tissue-specific and temporal expression patterns, miRNAs have emerged as critical regulators in the pathophysiology of RA (Fig. 4B)[142]. Altered levels of specific miRNAs have been observed in diverse pathogenic cell types, affected tissues, and biological fluids throughout different stages of RA progression, positioning them as promising diagnostic biomarkers and therapeutic targets for this chronic autoimmune disorder.

FLSs, immune cells, and bone-related cells are the main cell entities affected in RA that undergo alterations in response to the dynamic synovial microenvironment. MiRNAs modulate this microenvironment through cell-specific regulation of target genes in key signaling pathways, particularly the MAPK, PI3K/AKT, Wnt/β-catenin, and NF-κB cascades[143]. Notably, the dysregulation of FLSs, largely attributed to altered miRNA expression, contributes substantially to synovial inflammation and joint erosion[144]. For instance, miR-146a, a widely examined epigenetic regulator, shows increased expression in immune cells upon IL-1β stimulation[145]. This upregulation inhibits the proliferation of RASF by targeting TNF receptor associated factor 6 (TRAF6), a key protein in the NF-κB pathway[146]. Conversely, elevated levels of miR-21 and miR-26a-5p promote aberrant FLSs behavior. MiR-21 overexpression enhances nucleoprotein NF-κB levels, activating the NF-κB pathway and accelerating RASF proliferation[147]. Likewise, miR-26a-5p directly targets PTEN, thus promoting the proliferation and invasion of FLSs and enhancing their resistance to apoptosis[148]. Downregulation of certain miRNAs also significantly impacts RA progression. Najm et al.[149] demonstrated that miR-17-5p acts as an anti-inflammatory agent in RA FLSs and CIA mouse models by targeting JAK1 and STAT3, thereby suppressing the JAK/STAT pathway and reducing IL-6 secretion. These examples illustrate the complex interplay of dysregulated miRNAs in modulating aggressive phenotypes, mediating inflammatory responses, and influencing the integrity of synovial joint tissue in RASF. However, the impact of miRNAs extends beyond FLSs, affecting immune cell function and bone metabolism.

MiRNAs exert significant influence on immune homeostasis, particularly in modulating the differentiation of $CD4^+$ T cells. The balance between Treg and Th17 cells, mediated by the TFs Foxp3 and RORγt, is crucial for immune regulation[150]. In RA pathogenesis, miRNA dysregulation contributes to an increased Th17/Treg ratio, promoting inflammation and disease progression through enhanced Th17 differentiation and concomitant suppression of the anti-inflammatory Treg population[151,152]. Meanwhile, miRNAs act as key regulators in bone metabolism[153]. Aberrant miRNA expression patterns disrupt the functional equilibrium of osteoblasts, osteoclasts, and chondrocytes, ultimately contributing

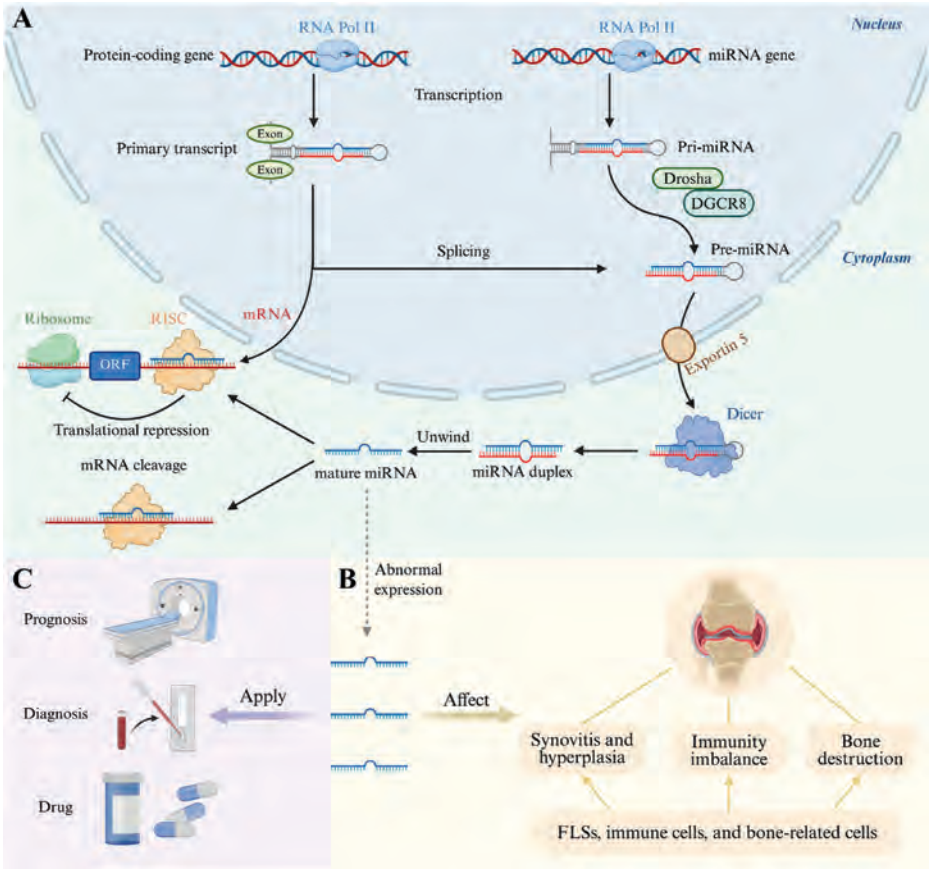


Figure 4 The role of microRNAs (miRNAs) in the pathogenesis of RA. (A) Mechanism of action and biogenesis of miRNAs. (B) Aberrantly expressed miRNAs disrupt the function of synovial fibroblasts, immune cells, and bone-related cells, impacting the primary pathological features of RA. (C) Changes in specific miRNA levels can serve as indicators for early diagnosis, prognosis, and therapeutic intervention of RA.

to the characteristic bone destruction seen in RA. Owing to the limited sensitivity and specificity of existing serological markers such as RF and ACPA, identifying distinct miRNA alterations in RA may provide valuable insights for early diagnosis, prognosis, and predicting responses to treatment (Fig. 4C)[20]. Taken together, elucidating the roles of differentially expressed miRNAs in the dysfunction of FLSs, immune cells, and bone-related cells may yield valuable biological targets for both diagnostic and therapeutic interventions in RA.

3. Targeting epigenetic mechanisms as a promising therapy for RA

3.1. Targeting DNMTs

Dysregulated DNA methylation in RA provides potential targets for epigenetic therapies. DNMT inhibitors (DNMTis) effectively reverse the hypermethylation of RA-related causative genes by either directly or indirectly inhibiting the catalytic activity of DNMTs. These inhibitors are primarily classified into two categories: nucleoside analogs and non-nucleoside analogs. Nucleoside analogs mimic natural cytidine and are phosphorylated in cells to form active triphosphate derivatives. During replication, they are incorporated into DNA, resulting in the irreversible binding of DNMTs and effective inhibition of aberrant methylation[154]. Non-nucleoside analogs inhibit the activity of DNMTs by binding to the enzyme or altering its conformation. They exhibit reduced cytotoxicity as their mechanism of action does not rely on DNA incorporation. However, achieving effective inhibition may require higher concentrations, and the ability of non-nucleoside analogs to selectively target specific DNMT types may be limited[155]. An overview of the structures of DNMTis is presented in Fig. 5.

3.1.1. Nucleoside DNMT inhibitors

The first nucleoside DNMTis, namely 5-azacytidine (**1**) and 5-aza-2′-deoxycytidine (**2**), were initially identified as anti-metabolite and cytotoxic agents. Subsequent research has revealed their capacity to induce DNA hypomethylation, with a predominant emphasis on their potential applications in hematologic malignancies[156]. Building upon this foundation, many researchers have begun to acknowledge these compounds as promising candidates for RA intervention, thereby expanding their therapeutic scope beyond oncology. It has been confirmed that low-dose administration of **1** has a remarkable effect on proteoglycan-induced arthritis in a murine model of RA[100]. As reported by Fu et al.[101], treatment of PBMCs with **1** resulted in the demethylation of upstream hypermethylated CpG motifs, leading to an increased expression of the immunosuppressive cytokine IL-10 and subsequently the inhibition of RA progression. In a murine model of CIA, the administration of **2** was found to significantly ameliorate clinical symptoms, decrease the production of Th1 and Th17 inflammatory cytokines, and reduce levels of anti-type II collagen autoantibodies[157]. Similar studies have demonstrated that in

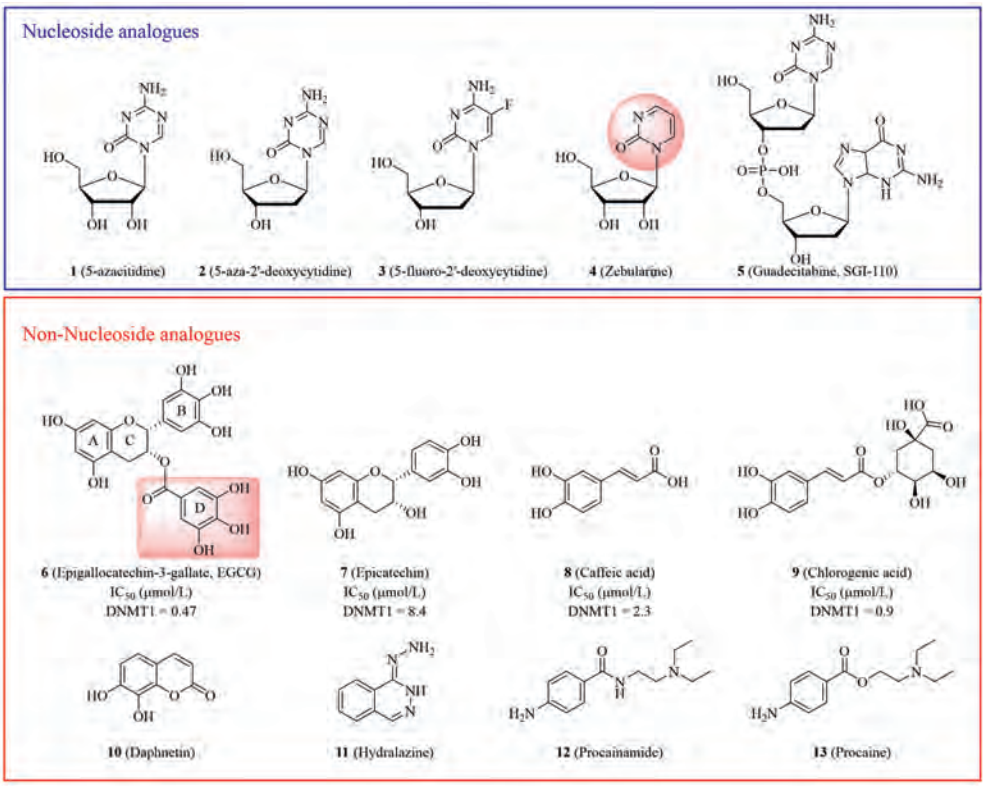


Figure 5 Structures of nucleoside and non-nucleoside DNMT inhibitors in preclinical RA studies, with some key structural units highlighted in orange.

validated RA models, transient treatment with **2** generates anti-arthritic Treg cells, depletes effector T cells, and induces indoleamine 2,3-dioxygenase (IDO)-dependent lasting disease remission[158]. These findings provide compelling evidence to support the preclinical evaluation of **2** as a treatment for RA. Although not yet in clinical trials for RA therapy, these drugs have been approved for treating myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), and chronic myelomonocytic leukemia (CMML)[159]. Nevertheless, their therapeutic use is constrained by metabolic instability, a short half-life, and insufficient specificity.

To enhance the stability and efficacy of nucleoside DNMTis, new compounds like 5-fluoro-2′-deoxycytidine (**3**) and zebularine (**4**) have been created. The former has been identified as a potent DNMTi *in vitro* and is noted for its increased stability in aqueous solutions[160]. Compound **4**, a cytidine derivative lacking an amino group at the 4 position of the pyrimidine ring, is capable of inhibiting both DNMTs and cytidine deaminase (CDA) when taken orally[161]. This compound is more stable and less cytotoxic than azacytosine analogs. Its mechanism of action is distinguished by a unique 2-(1*H*)-pyrimidinone ring (highlighted in orange), a structural feature that facilitates the formation of covalent bonds with DNMTs upon incorporation into DNA[162]. Compound **4** has also been shown to possess immunosuppressive activity and can synergize with IFN-γ to induce the expression of IDO1, an immunosuppressive enzyme, through demethylation of its gene promoter[163]. Recently, a prodrug strategy has been employed with the goal of improving cellular uptake of drugs. Guadecitabine (SGI-110, **5**) represents a second-generation DNMTi that functions as a prodrug of **2**. Unlike earlier nucleoside DNMTis that are prone to inactivation by CDA, compound **5** exhibits resistance to the deamination by CDA[164]. Its molecular structure consists of a dinucleotide formed by the linkage of decitabine and deoxyguanosine through a phosphodiester bond. The stepwise cleavage of this bond by phosphorylases and other enzymes facilitates a gradual release of its active metabolite, **2**, thereby achieving a longer half-life and enabling sustained drug action *in vivo*[165]. A phase I study demonstrated that subcutaneous administration of **5** is well tolerated and shows both biological and clinical activity in patients with AML and MDS[166]. Nevertheless, its potential as a DNMTi for treating RA still requires additional development and optimization.

3.1.2. Non-nucleoside DNMT inhibitors

Non-nucleoside DNMTis have been identified through high-throughput screening (HTS) and include natural products such as the tea catechins epigallocatechin-3-gallate (**6**) and epicatechin (**7**), as well as coffee polyphenols like caffeic acid (**8**) and chlorogenic acid (**9**). In addition, several repurposed drugs have emerged as promising therapeutic options for RA, including hydralazine (**11**), an antihypertensive agent, procainamide (**12**), used as an antiarrhythmic drug; and procaine (**13**), a local anesthetic. Despite their approval for various conditions, including cancer, idiopathic pulmonary fibrosis, Alzheimer's disease, hypertension, and arrhythmia, these non-nucleoside inhibitors have not yet been rigorously tested in clinical trials specifically for RA. However, their repurposing for RA treatment is supported by their mechanisms of action on epigenetic regulation. Compound **11** inhibits DNMT activity by interacting with the Lys162 and Arg240 residues of the enzyme through its nitrogen atoms, while **12** functions as a competitive inhibitor, showing a preference for binding to DNMT1[167]. Both compounds have demonstrated notable efficacy in reducing the levels of pro-inflammatory cytokines in preclinical

models of RA[168]. In RA, the pro-apoptotic gene DR3 exhibits hypermethylation levels, which may be a significant reason for the development of apoptosis resistance in synovial cells[102]. According to Shu et al.[169], daphnetin (**10**), a DNMTi extracted from *D. marginata*, downregulated the expression of DNMTs in synovial cells from CIA rats, thereby reducing methylation levels. Concurrently, it upregulated the expression of the DR3 gene, facilitating apoptosis in a dose-dependent manner.

Catechol-containing dietary polyphenols (epicatechin, **7**, caffeic acid, **8** and chlorogenic acid, **9**) indirectly inhibit DNMTs by acting as substrates for COMT-mediated O-methylation. This process, which employs the methyl donor SAM, leads to the methylation of these polyphenols and the generation of *S*-adenosylhomocysteine (SAH). As a potent inhibitor of DNMTs, SAH subsequently suppresses DNMT-mediated DNA methylation[170,171]. In contrast, compound **6** functions as a direct inhibitor with a lower IC_{50} of 0.47 μmol/L, specifically targeting the catalytic site of DNMTs. From a structural perspective, compound **6** contains a gallic acid moiety at the C_3 position of its C-ring (highlighted in orange), which facilitates the formation of at least four hydrogen bonds with DNMT1. This interaction effectively obstructs the incorporation of the DNA nucleotide cytosine into the active site of DNMT1, thus hindering the methylation process[171]. In light of its distinctive antioxidant and anti-inflammatory characteristics, compound **6** has attracted increasing attention for RA therapy[172]. Fechtner et al.[173] discovered that **6** can reduce the generation of cytokines (IL-8 and IL-6), cellular proteins (COX-2), and matrix-degrading enzymes (MMP-2) in FLSs stimulated by IL-1β, suggesting its superior efficacy in managing inflammation compared to other catechins. In the context of bone health, compound **6** is known to inhibit osteoclasts formation, induce the differentiation of mesenchymal stem cells into osteoblasts, and restore the balance of bone metabolism[174]. Additional studies have indicated that administration of **6** significantly decreases both the incidence and severity of arthritis in animal models[175]. However, compound **6** as a treatment for RA remains limited due to its inability to specifically target inflamed tissues, resulting in widespread distribution and inadequate accumulation within the affected joints.

In conclusion, the strategic use of DNMTis represents a promising approach to treat RA. Unfortunately, covalent nucleoside DNMTis, in particular, tend to have poor selectivity, potentially interfering with DNA methylation in normal cells and leading to significant toxic side effects. Additionally, in the treatment of solid tumors, DNMTis often trigger genome-wide demethylation, which not only affects the expression of target genes but also activates a large number of off-target genes, including certain potential oncogenes or pro-inflammatory genes, increasing the risk of secondary cancers and immune system abnormalities. Given the complex regulation of immune and inflammatory pathways in RA, similar off-target effects may impact the safety and efficacy of DNMTis in the treatment of RA. Therefore, it is crucial to thoroughly evaluate the therapeutic potential and safety of this epigenetic therapy. The development of highly active and selective non-nucleoside DNMTis has become a direction for future research.

3.2. Targeting HDACs

Considering the modified activity and expression of HDACs observed in PBMCs, synovial tissue, and FLSs derived from RA patients, synthetic and natural HDAC inhibitors (HDACis) restore acetylation levels, regulate both histone and non-histone proteins, and influence RA-related inflammatory mediator release as well as immune cell function. As of now, the FDA has approved five HDACis for various medical conditions, including epilepsy, refractory or relapsed cutaneous T-cell lymphoma (CTCL), peripheral T-cell lymphoma (PTCL), and multiple myeloma (MM). These approved agents include valproic acid (VPA, **14**), vorinostat (SAHA, **15**), romidepsin (FK228, **16**), belinostat (PXD101, **17**), and panobinostat (LBH-589, **18**). Notably, Chidamide (**19**) is regarded as the first subtype-selective benzamide HDACi, targeting HDAC1, HDAC2, HDAC3, and HDAC10, and has been approved in China for the treatment of PTCL (Fig. 6A)[176]. However, clinical studies have shown that, with the exception of **19** being approved for use in combination therapies for solid tumors (such as breast cancer, NCT02482753), other HDACis have shown limited efficacy in treating non-hematological tumors. For instance, the clinical trials of **15**, **16**, and **18** for breast cancer were terminated due to insufficient efficacy and the toxicity associated with their metabolic byproducts (NCT01194427, NCT01938833, NCT00777335). Currently, most available HDACis lack selectivity, resulting in limited effectiveness in treating solid tumors, as well as issues related to drug resistance, toxicity, and severe adverse events. Despite the fact that over 270 clinical trials have explored the application of HDACis in various immune system diseases[177], the majority of studies pertaining to RA remain at the preclinical stage.

3.2.1. Zn^{2+}-dependent HDAC inhibitors

Apart from class III HDACs (sirtuins), classes I, II, and IV HDACs possess a zinc-dependent catalytic domain essential for their enzymatic activity. The typical pharmacophore model designed for targeting Zn^{2+}-dependent HDACs comprises three components: (i) a variable hydrophobic cap group that engages with the amino acid residues at the rim of the HDAC active site; (ii) a linker that traverses the hydrophobic channel of the enzyme pocket with the appropriate length and flexibility; and (iii) a zinc-binding group (ZBG) that chelates the catalytic zinc ion, commonly including hydroxamic acid, *O*-aminoanilides, thiols, and carboxylic acid[178,179]. As an example, the initial crystal structure of the SAHA-HDLP complex reveals that the hydroxamic acid moiety engages in typical *O*,*O*′-bidentate chelation with the zinc ion situated within the active site, forming hydrogen bonds with residues His131, His132, and Tyr297. The six-carbon aliphatic linker fits snugly into the tubular hydrophobic pocket, while the phenyl-amino ketone group acts as the cap group, facilitating binding interactions with key residues at the rim of the enzyme, namely Tyr91, Pro22, Phe141, and Leu265 (Fig. 6B)[180]. This synergistic structural feature provides a theoretical basis for designing effective HDACis through modifications of the cap structure, variations in the linker, or alterations to the ZBG. Although established HDACis are primarily used in clinical research related to oncology, they also exhibit considerable anti-inflammatory effects at lower doses[181,182]. Both clinical and preclinical studies indicate that HDACis exert anti-rheumatic effects though multiple mechanisms, as summarized in Table 2[183−218].

3.2.1.1. Pan-HDAC inhibitors. Most HDACis used in RA research are pan-inhibitors that target multiple HDAC isoforms. One notable example is SAHA (**15**), which was developed based on studies of dimethyl sulfoxide and its effects on the growth and differentiation of transformed cells[219]. *In vitro* studies have

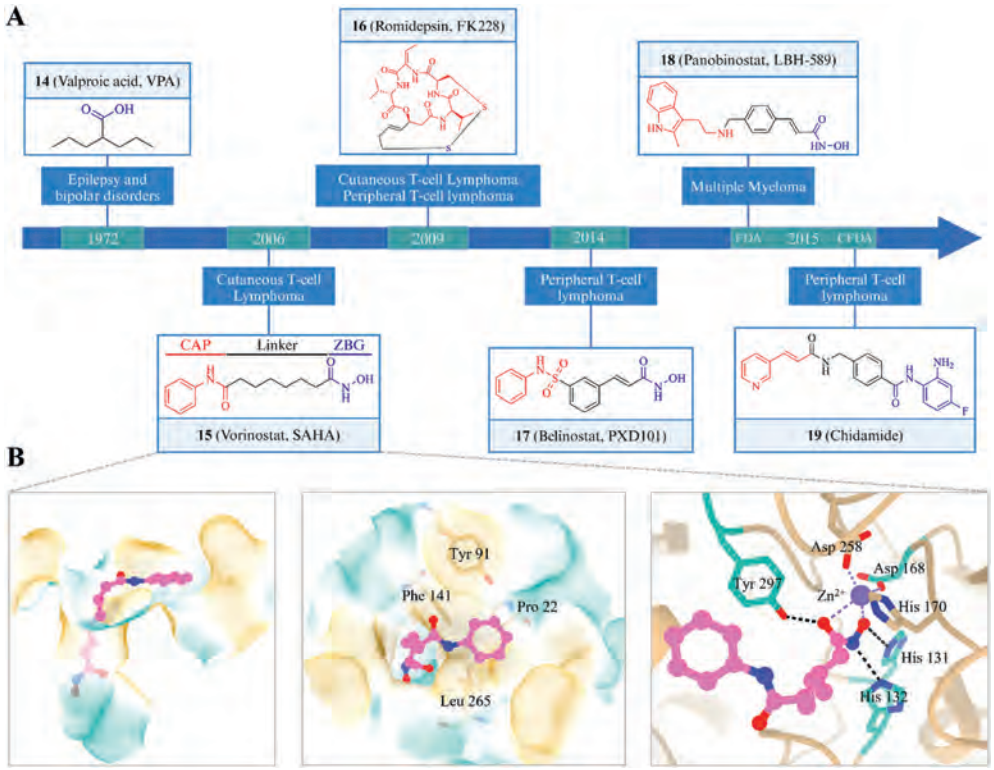


Figure 6 Overview of currently approved HDAC inhibitors and structural representation of **15** (SAHA) in complex with HDAC homolog (HDLP). (A) Approved HDAC inhibitors are used in the treatment of epilepsy and hematological malignancies. Common pharmacophore models of HDAC inhibitors include three key components: Cap (red), linker (black), and ZBG (blue). (B) (left) The hydrophobic surface of **15** (hot pink, PDB: 1C3S) in complex with the HDLP. (Middle) A top-down view of the active site of HDLP. (Right) A close-up view of the co-crystal interaction between **15** and HDLP. Key residues that form hydrogen bonds, including Tyr297, His131, and His132, are displayed in light sea green, with hydrogen bonds illustrated by black dashed lines. Metal coordination is indicated by purple dashed lines. Purple spheres represent zinc ions.

demonstrated that **15** maintains the balance between Th17 and Treg cells while inducing apoptosis in RA FLSs. *In vivo*, it alleviated arthritis symptoms and reduced the expression of inflammatory cytokines[183,184]. In addition to **15**, Lai et al.[220] discovered a novel class of 1-arylsulfonyl-5-(*N*-hydroxyacrylamide) indoles as effective HDACis. The lead compound MPT0E014 (**20**) exhibited remarkable inhibitory activity against HeLa nuclear HDAC, with an IC_{50} value of 29.5 nmol/L. Structure−activity relationship (SAR) analysis indicated that the 5-*N*-hydroxyacrylamide moiety and the 1-arylsulfonyl group in the indole structure are crucial for its inhibitory potency. Building on the structure of **20**, Lee et al.[221] made subtle modifications by converting the indole ring into an indolyl ring and introducing a methoxy group onto the aromatic ring, resulting in the synthesis of MPT0G009 (**28**). This new compound demonstrated a remarkable 7-fold enhancement in HDAC inhibitory activity in HeLa nuclear cells when compared to its precursor. Importantly, compound **28** exhibited superior potency relative to SAHA, both *in vitro* and *in vivo*, effectively inhibiting the release of inflammatory factors induced by LPS. In a rat model of oral administration, compound **28** (25 mg/kg) displayed anti-inflammatory effects comparable to those of SAHA (200 mg/kg) (Fig. 7). However, limited selectivity for isoforms may result in a wide array of side effects and heightened cytotoxicity, especially owing to the varying levels of HDAC expression in the synovial tissue of individuals with RA. Thus, selective HDAC inhibition, rather than pan-inhibition, may enhance treatment safety and shows promise for clinical application in RA.

3.2.1.2. Class I-selective HDAC inhibitors. The expression of class I HDAC is elevated in the synovial compartment and correlates with RA activity. This has sparked interest in the distinct roles of HDAC members in RA pathology, resulting in the creation of HDACis that are specific to class I[119]. Natural non-ribosomal cyclic peptides represent a valuable source of HDACis, including FK228 (**29**), which is isolated from the bacterium *Chromobacterium violaceum*, and Largazole (**31**), derived from the marine cyanobacterium *Symploca* sp.[222,223]. Both compounds are prodrugs that undergo either disulfide bond reduction or thioester side chain hydrolysis within the body, leading to the formation of active structures with thiol groups (Fig. 8A)[224]. In the crystal structure of the HDAC8-Largazole thiol (**32**) complex, the thiol side chain penetrates the active site groove, coordinating with the catalytic zinc ion *via* interactions with Asp178, His180, and Asp267. Compound **32** forms direct hydrogen bonds with residues Asp101 and Tyr306, and engages in van der Waals interactions with Phe208. Notably, HDAC8 undergoes significant conformational changes upon inhibitor binding, aiding in the accommodation of the bulky substrate. The presence of disulfide or thioester protecting groups enhances the stability and bioavailability of thiols (Fig. 8B). In contrast to **29**, compound **31** incorporates a thiazole-thiazoline moiety that rigidifies its structure and

Table 2 Biological effects of HDAC inhibitors and their implications in RA.

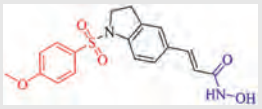
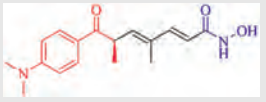
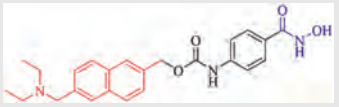
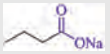
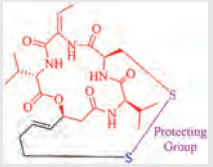
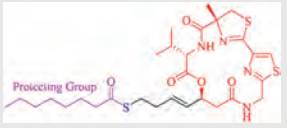
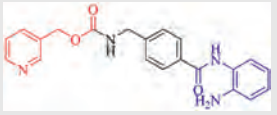
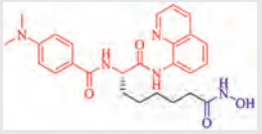
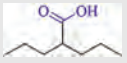
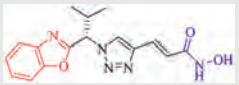
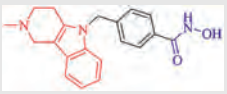
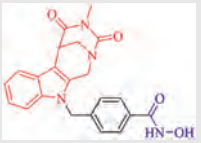
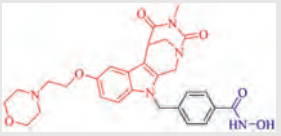
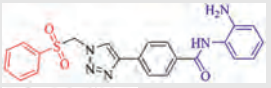
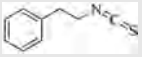
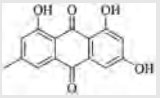
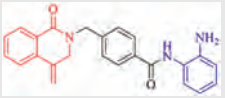
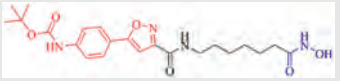
Category	Compound	Structure	Cell culture (*in vitro*)	Animal study (*in vivo*)	Ref.
Pan-HDAC inhibitors	SAHA (**15**)		Maintains Treg cells, reduces Th17 differentiation, induces apoptosis in FLSs	Alleviates arthritis and reduces inflammatory cytokines in CIA mice	183,184
	MPT0G009 (**28**)		Inhibits key inflammatory mediators and prevents osteoclastogenesis	Mitigates inflammation, reduces cartilage and bone destruction in AIA rats	185
	Trichostatin A (**54**)		Inhibits RASF and Th1 cell proliferation, induces apoptosis, and reduces IL-6 mRNA stability and MMP expression	Alleviates joint inflammation and cartilage destruction in CIA and CAIA mice	186−192
	ITF-2357 (**55**)		Modulates mRNA stability of inflammatory mediators in RA FLSs	Alleviates joint inflammation and protects bone in RA animal models	187,193,194
	Sodium phenylbutyrate (**56**)		Induces $p21^{Cip1}$ and $p16^{INK4}$ expression to inhibit synovial cell proliferation	Inhibits TNF-α expression in adjuvant arthritis rat tissues	195
	Sodium butyrate (**57**)		Controls of Th17/Treg balance, inhibits osteoclastogenesis	Improves RA symptoms in CIA mice, effects dependent on IL-10	196
Class I-HDAC inhibitors	FK228 (**29**)		Reduces hypoxia-induced HIF-1α and VEGF expression, inhibits osteoclast differentiation and RASF proliferation	Suppresses VEGF expression in CAIA synovial tissue, prevents disease progression and bone destruction in AIA rats and AMA mice	197−199
	Largazole (**31**)		Co-admin with **44** reduces TNF-α-induced adhesion molecules expression and completely blocks MMP-2 activity	–	200
	MS-275 (**58**)		Inhibits CCR6 upregulation under Th17-skewing conditions	Decreases serum IL-6 and IL-1β levels in CIA mice	201,202
	NW-21 (**59**)		Suppresses osteoclast formation and MCP-1, MIP-1α mRNA expression	Reduces inflammation and bone loss in CAIA model	203
	VPA (**60**)		Enhances the suppressive function of Treg cells and increases their numbers	Reduces disease incidence and severity in CIA mice, decreases joint inflammation	204
Isoform-HDAC inhibitors	NK-HDAC-1 (**37**)		Induces G2/M arrest in FLSs, enhances apoptosis, increases caspase activity, suppresses IL-6 production	Enhances synoviocytes apoptosis, slows CIA progression	205

Table 2 (*continued*)

Category	Compound	Structure	Cell culture (*in vitro*)	Animal study (*in vivo*)	Ref.
	Tubastatin A (**44**)		Suppresses TNF-α, IL-6, and NO production, induces α-tubulin hyperacetylation	Ameliorates synovial inflammation, protects against joint destruction	206,207
	Marbostat-100 (**45**)		Potent HDAC6 inhibition, high selectivity, and favorable cell compatibility	Alleviates arthritis symptoms and histopathological changes in CIA model	208
	5f (**53**)		Highly selective inhibition of HDAC6	Ameliorates disease severity and reduces clinical arthritis score in CIA model	208
	TTA03-107 (**61**)		Inhibits macrophage polarization and Th17 cell differentiation	Mitigates inflammation and joint damage in CIA and CAIA models	209
	PEITC (**62**)		—	Alleviates inflammation and bone erosion, reduces TNF-α and HDAC1 levels in CFA-induced arthritis model	210
	Emodin (**63**)		Inhibits HDAC1, reduces pro-inflammatory cytokines	—	211
	MI192 (**64**)		Inhibits TNF and IL-6 production dose-dependently in RA PBMCs	—	212
	BML-281 (**65**)		Suppresses IL12p40, IL6 at low concentrations; amplifies TNF, IL1β at higher concentrations	Anti-inflammatory activity inversely correlates with dose in CIA rats	213
	CKD-506 (**66**)	Undisclosed	Reduces TNF-α, IL-6 in RA PBMCs, suppresses MMPs in FLSs, inhibits effector T cell proliferation	Improves clinical arthritis in a dose-dependent manner	214
	M-134 (**67**)	Undisclosed	—	Significant therapeutic effects in monotherapy and with tofacitinib	215
	CKD-L (**68**)	Undisclosed	Enhances Treg cell suppressive function, regulates cytokine secretion in RA PBMCs	Alleviates arthritis symptoms, protects joints in CIA model	216
	M808 (**69**)	Undisclosed	Suppresses FLSs inflammation and invasiveness, reduces ICAM-1 and VCAM-1 expression	Improves clinical arthritis scores, reduces synovial inflammation, alleviates joint damage in AIA model	217
	ITF3056 (**70**)	Undisclosed	Inhibits HDAC8, reduces LPS-induced IL-1β, TNF-α, and IL-6 in PBMCs	Reduces serum TNF-α and IL-1β levels induced by LPS	218

Red, Cap group; Black, linker; Blue, ZBG group; —, not applicable

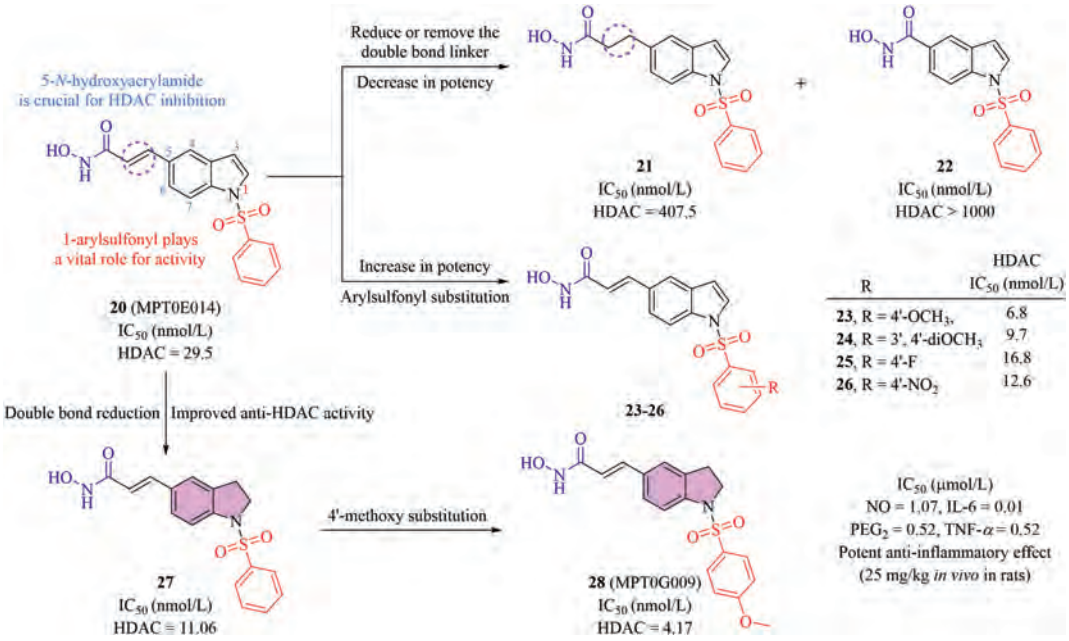


Figure 7 Structure–activity relationship (SAR) analysis of **20** (MPT0E014) and the optimization process leading to the discovery of **28** (MPT0G009). The HDAC enzyme assays were performed using purified nuclear extracts from HeLa cells.

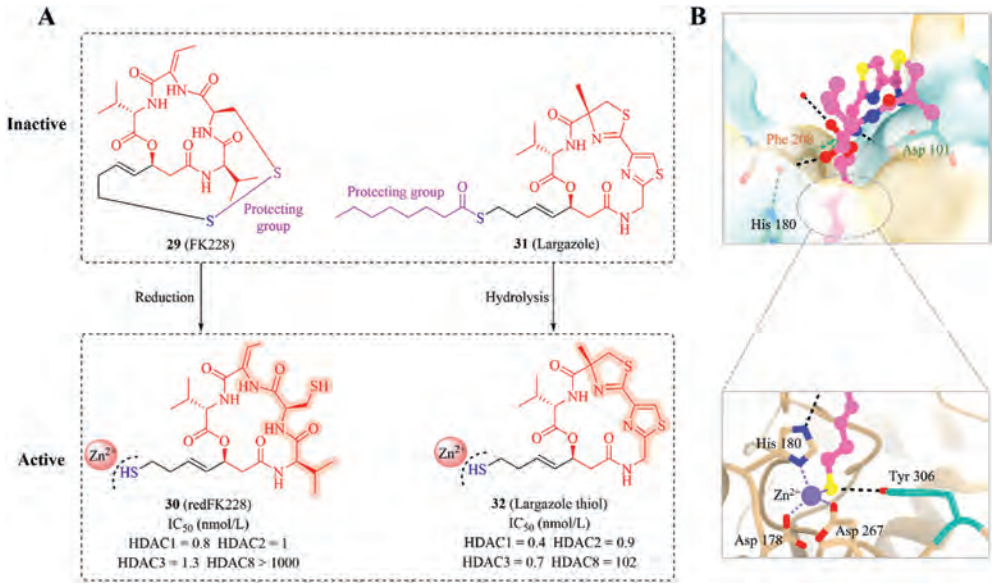


Figure 8 Mechanisms of action and structural insights of prodrugs **29** (FK228) and **31** (Largazole). (A) Mechanism of action of **29** and **31**. The distinct segments in the structures of their active components are highlighted in light orange. The thiol groups chelate with the zinc ion at the active site of HDACs. (B) The cocrystal structure of **31** (hot pink) with HDAC8 (PDB: 3RQD). Key residues that directly form hydrogen bonds are depicted in light sea green, with hydrogen bonds represented by black dashed lines. van der Waals interactions with residue Phe208 are depicted as green dashed lines, while metal coordination is indicated by purple dashed lines. Red and purple spheres represent structural water molecules and zinc ions, respectively.

effectively inhibits class I selective HDACs at low nanomolar concentrations[225]. Besides their anti-neoplastic activities, both prodrugs exhibit potential anti-rheumatic effects in RA. As reported by Manabe et al.[197], compound **29** was found to inhibit the expression of HIF-1α and VEGF mRNA and protein stimulated by cytokines in a dose-dependent manner. When administered intravenously at a dosage of 2.5 mg/kg, compound **29** reduced VEGF expression and hindered angiogenesis within the synovial tissue of the collagen antibody-induced arthritis (CAIA) model. Moreover, studies on rats with adjuvant-induced arthritis (AIA) indicated that **29** not only prevented disease progression in a prophylactic setting but also mitigated bone damage in a therapeutic model[198]. Synovial fibroblasts facilitate immune cells infiltration *via* ICAM-1 and VCAM-1. Interestingly, in RASF, the administration of **31** inhibited Class I HDACs, while unexpectedly increasing HDAC6 expression, which further enhanced the TNF-α-induced

expression of these adhesion molecules. However, the combination of the selective HDAC6i Tubastatin A with **31** effectively suppressed Largazole-induced expression of ICAM-1 and VCAM-1 and completely blocked MMP-2 activity[200]. While class-selective HDACis show a degree of target specificity compared to pan-inhibitors, certain HDACs (*e.g.*, HDAC5) may exert opposing effects on RA inflammation[112]. Fortunately, compounds with enhanced selectivity for specific HDAC family members, particularly HDAC6, are currently under development.

3.2.1.3. Isoform-selective HDAC inhibitors. In recent years, the role of individual HDAC enzymes in the pathogenesis of RA has become increasingly clear, prompting the development of selective HDACis as promising options for targeted RA therapy. According to research executed by Hou et al.[226], the hit compound NSC746457 (**33**) was optimized by substituting the potentially carcinogenic *trans*-styryl moiety with a 2-substituted benzo-hetero ring. Additionally, a side chain was introduced at the methylene group between the cap and linker regions, ultimately yielding the promising chiral HDACi NK-HDAC-1 (**37**). In HDAC enzyme assays, the *S*-isomer (**37**) demonstrated enhanced potency with an IC_{50} of 7 nmol/L, in contrast to the *R*-isomer (**36**), which exhibited an IC_{50} of 31 nmol/L (Fig. 9). Utilizing the reported crystal structure of human HDAC2, docking studies were conducted to position both enantiomers into the active pocket of the enzyme. Notably, the *S*-isomer had a tighter interaction with HDAC2 compared to its *R* counterpart. Another study showed that **37** reduced pro-inflammatory cytokine production, induced obvious growth arrest and apoptosis in RA FLSs, and inhibited CIA progression with approximately an order of magnitude greater potency than SAHA[205]. This high HDAC inhibitory activity thus contributes to an increased therapeutic safety margin.

Unlike other HDACs, selective inhibition of HDAC6 is associated with fewer side effects, as evidenced by the viability and overall health of HDAC6 knockout mice throughout their lifespan[227]. The first reported HDAC6i was Tubacin (**38**); however, its high molecular weight and lipophilicity render it unsuitable for therapeutic use[228]. A comparative analysis of the active sites in the crystal structures of HDAC6 and HDAC1 revealed that the rim region of the HDAC6 pocket is wider (17.5 Å) than that of HDAC1 (12.5 Å), featuring a broad and shallow catalytic channel. To enhance the selectivity of HDAC6 inhibition, a design incorporating a large and rigid cap group is essential to adequately occupy the rim region of HDAC6, along with a short and wide linker that fits snugly within the catalytic channel[229,230]. Building on these structural insights, Butler et al.[229] successfully synthesized the HDAC6i Tubastatin A (**44**), utilizing a para-tolyl group as a linker instead of the conventional alkyl chain. They characterized compounds with tetrahydro-γ- and tetrahydro-β-indole cap structures, demonstrating exceptional HDAC6 inhibitory activity and high selectivity, being over 1000-fold selectivity for HDAC6 over HDAC1 (Fig. 10A). In contrast to the bidentate chelation observed in SAHA, compound **44** employs a monodentate interaction between its hydroxamic acid moiety and the active zinc ion. Furthermore, the unsaturated para-tolyl linker enhances binding stability through $\pi-\pi$ stacking interactions with Phe583 and Phe643 in the hydrophobic pocket (Fig. 10B)[231]. Studies have shown that **44** ameliorates synovial inflammation in CAIA mice and protects joints from damage when administered *via* intraperitoneal injection at a dosage of 100 mg/kg, potentially through the modulation of IL-6 expression[206]. However, it is important to note that **44** also inhibits HDAC10[232], which complicates the understanding of HDAC6 as the only drug target for drug intervention in the treatment of RA. Taking **44** as a lead compound, Sellmer et al.[208] incorporated a hydantoin increment into the tetrahydro-β-carboline framework to develop the highly selective HDAC6i Marbostat-100 (**45**). This compound, which includes both (*R*)- and (*S*)-isomers, exhibited a K_i value of 0.7 nmol/L for HDAC6, corresponding to approximately 645-fold selectivity over HDAC10. SAR studies indicated that substitution at the 8-position of the aromatic moiety or replacement of a carbonyl group in the hydantoin scaffold with a sulfonyl or methylene group was tolerated at the

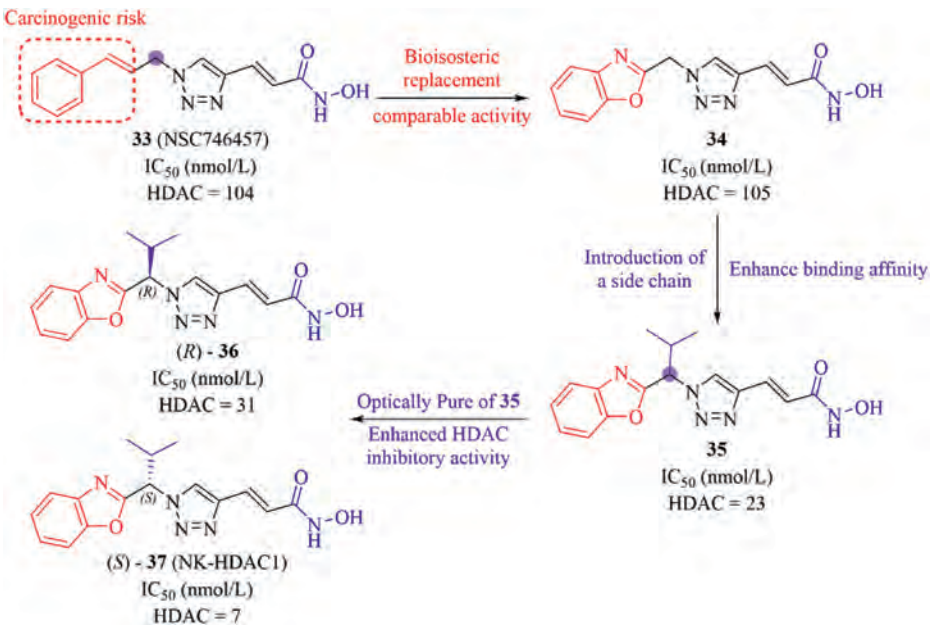


Figure 9 Optimization process leading to **36** and **37** (NK-HDAC-1). The HDAC enzyme assays were conducted using purified nuclear extracts from HeLa cells.

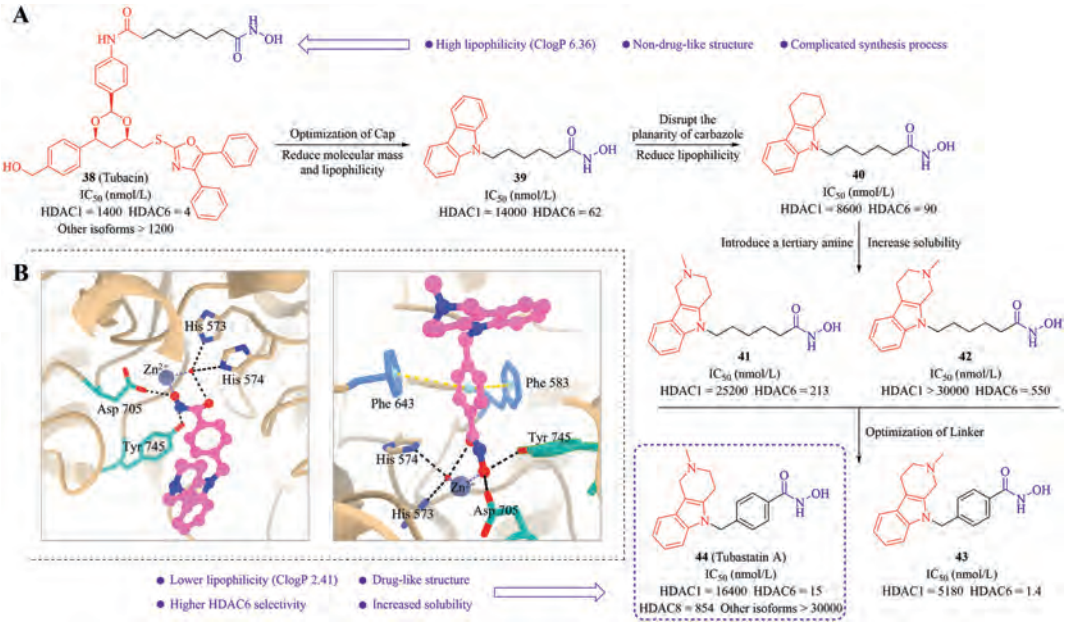


Figure 10 SAR analysis and structural insights of **44** (Tubastatin A). (A) Optimization process leading to **43** and **44**. (B) Co-crystal structure of **44** (hot pink, PDB: 6THV) with the catalytic domain 2 of *Danio rerio* HDAC6. Key residues Asp705 and Tyr745, which directly form hydrogen bonds, are shown in light sea green. Residues Phe583 and Phe643, involved in $\pi-\pi$ stacking interactions with the phenyl ring of **44**, are displayed in cornflower blue. Hydrogen bonds are represented by black dashed lines, $\pi-\pi$ stacking interactions by yellow dashed lines, and metal coordination by purple dashed lines. Red and purple spheres represent structural water molecules and zinc ions, respectively.

enzyme level. Specifically, the incorporation of morpholineethoxy at this 8-position in **45** notably enhanced solubility while preserving selectivity and activity (Fig. 11). Moreover, treatment of CIA with **45** (30 mg/kg) and **53** (42.3 mg/kg) significantly reduced clinical scores and disease activity without major safety concerns, suggesting both compounds warrant further clinical evaluation. Additionally, other structurally undisclosed selective HDACis have also been reported to exhibit potential anti-rheumatic effects, including CKD-506, M-134, CKD-L, M808, and ITF3056 (an analog of ITF-2357)[214–218]. These results bolster the idea that

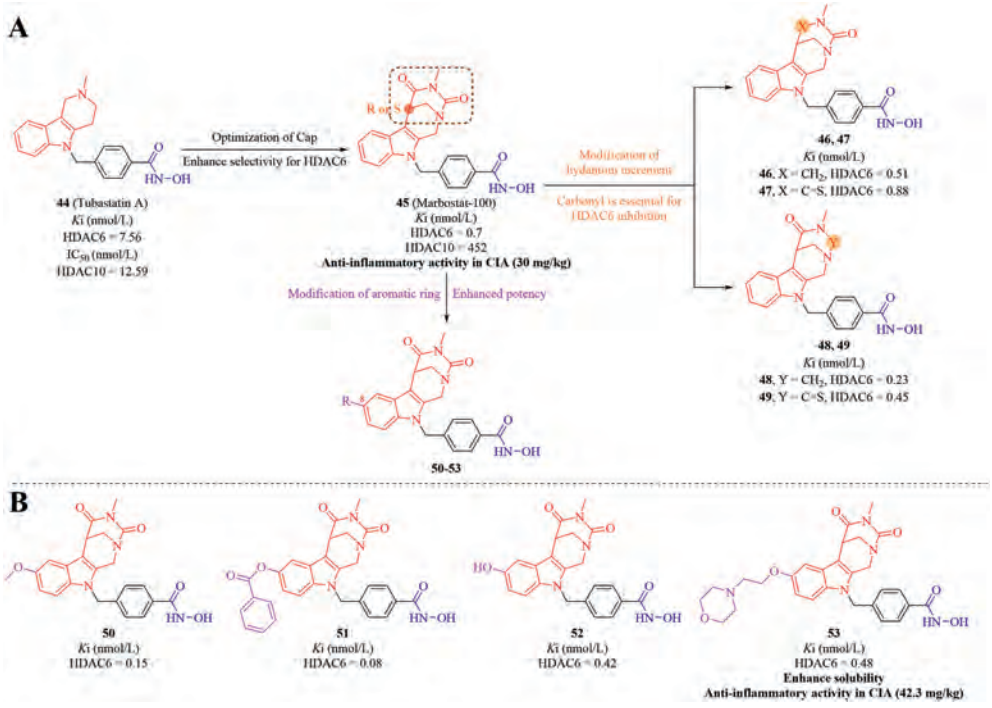


Figure 11 The optimization process that led to the identification of **45** (Marbostat-100) involved a SAR analysis. Treatment of CIA with **45** (30 mg/kg) and **53** (42.3 mg/kg) showed significant anti-inflammatory activity.

targeting specific HDAC isoforms with small-molecule inhibitors is a viable alternative to conventional RA treatments.

3.2.2. NAD^+-dependent sirtuins modulators

Considering the intricate involvement of SIRT1 in the pathology of RA, the regulation of SIRT proteins, with an emphasis on SIRT1 activity, emerges as a critical area of interest. Both naturally occurring and synthetic SIRT modulators may serve as valuable tools for understanding sirtuin function and potentially providing therapeutic options for RA. Indeed, depending on the specific RA-related models and underlying pathologies, it may be necessary to either activate or inhibit specific SIRT isoforms to achieve optimal therapeutic outcomes (Fig. 12). Nicotinamide (**71**), a product of deacetylation, acts as an endogenous inhibitor that disrupts the deacetylation process. It attenuated inflammation by suppressing cytokine production and promoting apoptosis in macrophages derived from RA patients[189]. Paradoxically, the majority of studies suggest that SIRT1 agonists could offer therapeutic advantages for inflammatory disorders like RA through their antioxidant and anti-inflammatory properties.

Resveratrol (**72**), a naturally occurring polyphenolic compound located in the skins of red grapes and in *Polygonum cuspidatum*, has demonstrated both preventive and therapeutic potential in preclinical studies of RA. These beneficial effects are largely attributed to its capacity to modulate a variety of SIRT1 targets that are crucial in inflammation and oxidative stress pathways[233−240]. Other plant-derived compounds, such as Quercetin (**73**), and Sweroside (**74**), have also shown promise as SIRT activators with potential benefits in RA. Shen et al.[241] found that arthritis is reduced in CIA mice through the action of **73**, particularly by improving dysfunctional mitochondrial biogenesis and activity. Similarly, Wang et al.[242] investigated **74**, an iridoid glycoside obtained from *P. hookeri*, which ameliorates LPS-induced inflammation in RAW264.7 cells. The mechanism underlying this anti-inflammatory effect involves the activation of SIRT1, which subsequently modulates the NF-κB and FOXO1 signaling pathways. Despite these promising results, the mechanisms of action of these natural compounds often extend beyond mere SIRT1 activation. To address this limitation, recent efforts have resulted in the discovery of a novel category of synthetic SIRT1 activators that exhibit superior potency and selectivity compared to **72**. Among these, SRT1720 (**75**) and SRT2104 (**76**) have emerged as noteworthy compounds. While direct studies of these activators in RA are currently lacking, research in related inflammatory conditions has yielded considerable findings. In a study by Nishida et al.[243], the administration of **75** through intraperitoneal injection attenuated the progression of experimental OA in mice. The anti-inflammatory effects observed in OA model are particularly relevant, as inflammation is a key driver of RA pathogenesis. Compound **76**, identified through HTS, is the leading SIRT1 agonist advancing into the clinical translation phase. Its exceptional potency and specificity make **76** a promising candidate for exploring SIRT1 activation in inflammatory conditions[244]. However, the heterogeneity of RA and its distinct immunopathological features necessitate careful evaluation of these compounds in RA-specific contexts. Additional studies are needed to directly assess the impact of **75** and **76** in RA models, as well as their potential application in clinical trials.

3.3. Targeting BET proteins

In multiple arthritis-relevant cell types and mouse models of arthritis, inhibitors targeting BET proteins have demonstrated anti-inflammatory efficacy[128]. Within the ZA loop, there exists a hydrophobic region referred to as the WPF shelf (W, Trp; P, Pro; F, Phe), which also contributes to the formation of the ZA channel. These structural characteristics are crucial for small molecule inhibitors to achieve both selective binding and high affinity for BET proteins[245,246]. In the BC loop, a conserved asparagine (Asn) establishes a direct hydrogen bond with acetyl-lysine (Kac), while the lysine (Lys) located in the ZA loop interacts with Kac through a hydrogen bond that is mediated by water (Fig. 3C)[247]. By simulating the binding mode of Kac with BET proteins, numerous BET inhibitors have been designed to incorporate acetyl-lysine mimetics. These inhibitors competitively bind to the bromodomain, effectively blocking BET proteins from recognizing acetylation marks on target genes.

First-generation small molecule inhibitors (*i.e.*, (+)-JQ1 and I-BET762) share a benzodiazepine core, with the methyl triazole portion mimicking key hydrogen bonds within the bromodomain pocket. The crystal structure of the (+)-JQ1−BRD4 (BD1) complex reveals that (+)-JQ1 (**77**) exhibits excellent

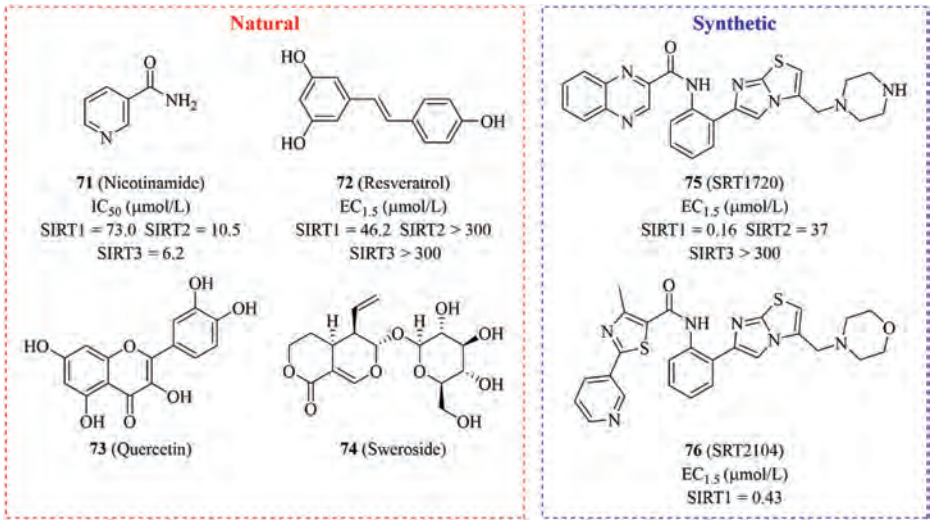


Figure 12 Chemical structures of natural and synthetic SIRT modulators (activators and inhibitors).

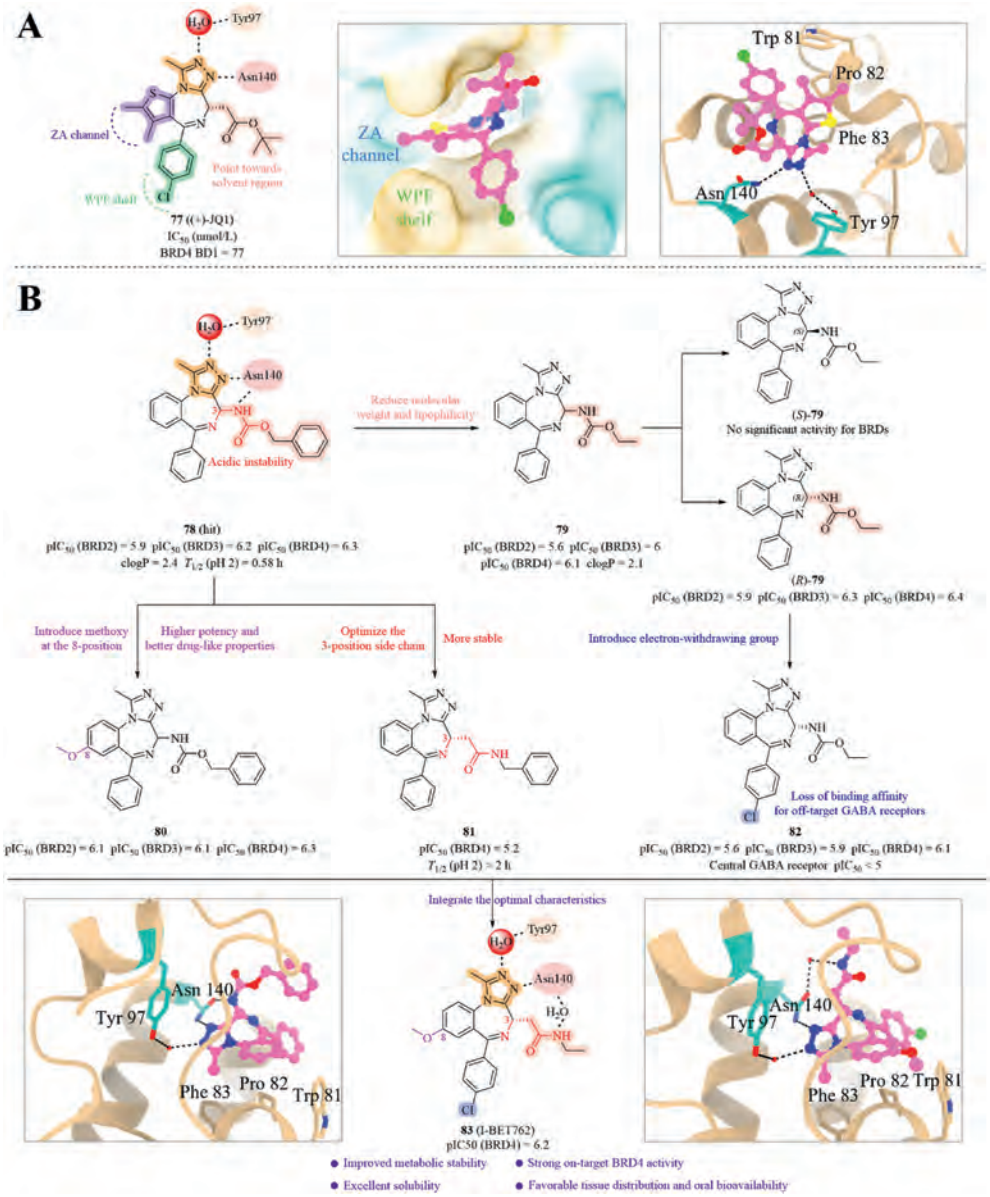


Figure 13 SAR analysis and structural insights of the triazole-based BET inhibitors **77** (JQ1) and **83** (I-BET762). (A) Chemical structure of **77** and its crystal structure (hot pink) in complex with the human BRD4 bromodomain 1 (BD1) (PDB: 3MXF). (B) Optimization process leading to **83**, with crystal structures of **78** and **83** (hot pink) bound to human BRD4 BD1 (PDB: 2YEL, 3P5O). Key residues Asn140 and Tyr97, which form hydrogen bonds, are highlighted in light sea green. Hydrogen bonds are indicated by black dashed lines, and red spheres denote structural water molecules.

complementarity with the Kac binding pocket. It establishes a direct hydrogen bond between the triazole ring and Asn140, as well as a water-mediated hydrogen bond with Tyr97. In addition, the chlorophenyl substituent and the dimethyl-substituted thiophene group occupy the WPF shelf and the ZA channel, respectively, thereby enhancing the stability of the interaction (Fig. 13A). In contrast, the enantiomer (−)-JQ1 (IC_{50} > 10,000 nmol/L) shows significantly reduced inhibitory effects on BRD4 (BD1) relative to (+)-JQ1 (IC_{50} = 77 nmol/L)[248]. In functional studies, compound **77** demonstrated its ability to inhibit IκB kinase-dependent NF-κB activation *in vitro*, subsequently lowering pro-inflammatory cytokine and MMP production[249]. In T cell differentiation assays, compound **77** selectively inhibited Th17 cell development by approximately 90%, without affecting other T cell subsets[250]. In a CIA mouse model, intraperitoneal administration of **77** significantly attenuated synovial inflammation and joint destruction as evidenced by lower arthritis scores and histological analysis[129]. However, due to its short half-life of 1 h, compound **77** failed to advance to clinical development[251]. Mirguet et al.[252] optimized lead compound **78** by modifying the carbamate side chain at the 3-position and exploring the effects of substituents on both the fused and pendant phenyl rings regarding their inhibitory activity against BET proteins. By integrating these optimized features, they successfully synthesized I-BET762 (**83**). This new compound addresses the acidic instability of **78**, eliminates off-target activity at GABA receptors, and enhances selectivity for BET proteins. Crystal structure analysis of I-BET762 bound to BRD4 reveals that the binding mode of the

triazole ring is similar to that of **77**, with a key difference: the amide NH in the side chain creates a water-mediated hydrogen bond with Asn140, replacing the direct hydrogen bond formed by the carbamate NH in **78** (Fig. 13B)[253,254]. With favorable drug-like and pharmacokinetic properties, compound **83** has successfully entered clinical trials to assess its efficacy in treating NUT midline carcinoma and other cancers. Beyond oncology, compound **83** has been shown to inhibit T-cell-mediated inflammation in a rat model with efficacy similar to that of the positive control, rapamycin[255], suggesting a potential relationship between its action and RA.

Second-generation BET inhibitors utilize a dimethyl isoxazole moiety as the Kac-binding motif, where the nitrogen atom (in PLX51107) or the oxygen atom (in I-BET151) in the isoxazole establishes a hydrogen bond with Asn140 (Fig. 14). Ozer et al.[256] found that PLX5981 (**84**), featuring a 7-azaindole core, serves as a suitable scaffold for interfacing with both the WPF shelf and the ZA channel. By substituting the 7-azaindole with a 4-azaindole and introducing a pyridine ethyl group along with a benzoic acid group, they successfully synthesized PLX51107 (**85**). The *S*-enantiomer exhibits 5- to 10-fold increased potency across all BET bromodomains compared to the *R*-enantiomer. The crystal structure of **85** in complex with BRD4 reveals that it engages with the WPF shelf and the ZA channel through its side chains of pyridyl ethyl and benzoic acid, with the benzoic acid extending into the ZA channel to form a salt bridge with Lys91. Compound **85** represents a novel class of inhibitors designed to exploit the additional binding site provided by the ZA loop to modulate BET protein functionality. It exhibited a unique mechanism by regulating Fcγ receptor function, thereby reducing inflammation in RA models[257]. I-BET151 (**86**) was initially identified as an upregulator of ApoA1. Seal et al.[258] analyzed the binding mechanism and pharmacological traits of **86**, confirming that it hinders the interaction between BET proteins and acetylated histones through a mechanism akin to that of benzodiazepines[259]. *In vitro* studies revealed that **86** significantly reduced the production of IL-6 and TNF-α in human PBMCs stimulated with LPS in a dose-dependent manner. In a mouse model of acute inflammation, a single oral dose of 10 mg/kg exhibited extensive anti-inflammatory effects of **86**[260]. Additionally, it demonstrated potent anti-inflammatory effects in RASF and inhibited osteoclast generation by downregulating NFATC1, a master regulator of osteoclastogenesis[261,262]. Noteworthy, RVX-297 (**87**), a novel BD2-selective BET inhibitor featuring a quinazolone scaffold, displays a dimethyl phenyl moiety that points toward the solvent boundary without overlapping with the WPF shelf. The presence of His433 narrows the major groove of the binding pocket, restricting the movement of the dimethyl phenyl group and consequently enhancing the binding stability of **87** in BD2, as observed in the crystal structure of BRD2 (BD2)[263]. Moreover, compound **87** has shown efficacy in preclinical models of acute inflammation and autoimmunity[264]. Despite promising results, research on BET inhibitors in RA remains nascent, with pan-BET inhibitors exhibiting dose-limiting toxicities and potential side effects[265]. Future efforts should focus on developing BD1- or BD2-selective inhibitors that offer improved safety profiles and enhanced tolerability.

3.4. *Targeting other epigenetic-related proteins*

Targeting HATs and HDMs represents a novel epigenetic therapeutic strategy for RA (Fig. 15). Unlike HDACis, HAT inhibitors (HATis) exert anti-inflammatory effects in RA by reducing the hyperacetylation of pro-inflammatory genes through the inhibition of HAT activity[266]. Curcumin (**88**), a natural polyphenol HATi, lowers the acetylation levels of histone H3 at the IL-6 promoter, thereby suppressing both mRNA expression and protein secretion of IL-6 in RASF[108]. Other HATis, such as Delphinidin (**89**), MB-3 (**90**), and Anacardic acid (**91**), also inhibit the acetylation of pro-inflammatory genes, including p65 and KAT2A, which in turn suppresses inflammatory signaling pathways and the proliferation and invasion of synovial fibroblasts[267–269]. In terms of HDM inhibitors, GSK-J4 (**92**) is a prominent compound that targets JMJD3 in both *in vitro* and *in vivo* models of RA. It increases H3K27me3-mediated histone methylation, resulting in reduced

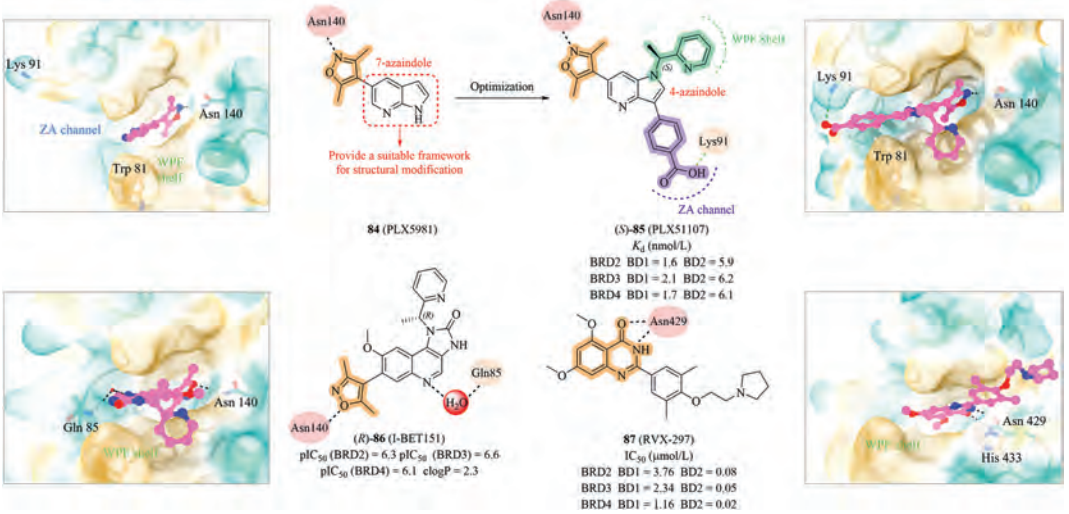


Figure 14 Chemical structures of **84**–**87**. Crystal structures of **84** (PLX5981), **85** (PLX51107), and **86** (I-BET151) (hot pink) in complex with BRD4 bromodomain 1 (BD1) (PDB: 5WMA, 5WMG, and 3ZYU). (Lower right) X-ray crystal structure of **87** (RVX-297, hot pink) bound to human BRD2 BD2 (PDB: 5DW1). Key residues involved in hydrogen bonding are highlighted in light sea green. Hydrogen bonds are indicated by black dashed lines, while the salt bridge is represented by green dashed lines.

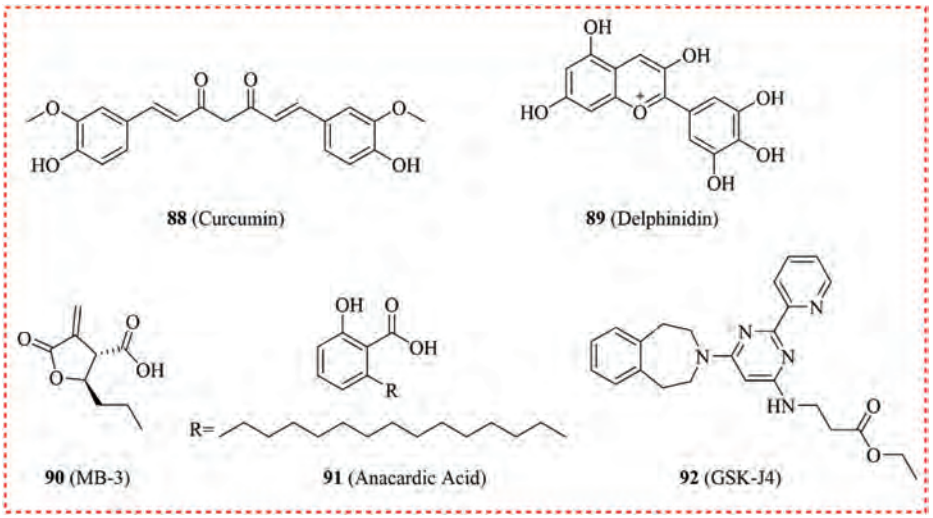


Figure 15 Chemical structures of inhibitors targeting other epigenetic-related proteins. Compounds **88**−**91** are inhibitors of HATs, while compound **92** is an inhibitor of histone demethylases (HDMs).

inflammation as well as decreased proliferation and migration of FLSs. Notably, administration of **92** markedly alleviates the severity of arthritis in CIA mice[135,136]. Although research in this field remains insufficient, the potential applications warrant attention and further development.

3.5. MiRNA-based therapies

MiRNAs are crucial post-transcriptional regulators that modulate gene expression by targeting specific mRNAs, thereby influencing cellular processes. Consequently, miRNA-based therapies seek to disrupt the activity of target miRNAs, thereby managing the expression of genes associated with RA and the synthesis of proteins by restoring dysregulated miRNA levels. These therapies predominantly utilize miRNA inhibitors and mimics. MiRNA inhibitors, or anti-miRs, are single-stranded RNA molecules designed to bind specifically to target miRNAs, thereby preventing their interaction with mRNAs. Conversely, miRNA mimics are synthetic double-stranded RNAs designed to replicate specific miRNAs, guiding the RNA-induced silencing complex to suppress target mRNAs (Fig. 16A)[270]. However, oligonucleotides are rapidly degraded by nucleases *in vivo*, and their negatively charged surface properties impede their ability to cross the cell membrane and reach target organs and tissues[270]. To address these challenges, miRNA mimics and inhibitors have been modified with common 2′-ribose modifications and phosphorothioate backbones, providing degradation protection and enhanced binding affinity, known as miRNA agomirs and antagomirs (Fig. 16B)[271]. To further improve cellular uptake and targeting efficacy, viral vectors and non-viral carriers (*i.e.*, liposomes, exosomes, and polymeric nanoparticles) have been employed as novel delivery carriers, assisting miRNAs in successfully reaching target RA-associated pathogenic cells and tissues to exert their regulatory functions (Fig. 16C)[20]. Currently, miRNA-based therapies have progressed to clinical trials for various diseases, including malignant pleural mesothelioma (MesomiR1, miR-16 mimic), wound healing and heart failure (MRG-110, anti-miR-92), hepatitis (Miravirsen, anti-miR-122), multiple solid tumors (MRX34, miR-34a mimic), and cutaneous T-cell lymphoma and mycosis fungoides (MRG-106, anti-miR-155).

Research indicates that altered miRNAs participate in the fundamental processes of RA, influencing synovial inflammation, immune dysregulation, as well as bone erosion[19]. The efficacy of specifically modified miRNA therapeutics has been investigated in various RA models through systemic delivery. Modified miR-155 inhibitors and miR-146a mimics demonstrated promise in reducing inflammation and bone erosion[272,273]. However, other interventions produced contrasting effects. For instance, intravenous injection of miR-34a agonists led to stronger disease phenotypes and Treg/Th17 imbalance in CIA mice. Similarly, miR-145-5p agonist administration exacerbated joint erosion by affecting the OPG/RANKL/RANK axis[274,275]. Localized miRNA delivery offers promising therapeutic potential beyond systemic administration. Due to low intestinal absorption and oral bioavailability, alternative delivery strategies are needed. Local administration can maintain therapeutic levels within inflamed joints, thereby minimizing the need for frequent injections or high systemic dosages. Intra-articular and intra-peritoneal injections, commonly used in chronic arthropathy treatment, are effective local delivery routes (Fig. 16D). According to Donate et al.[276], inflammatory arthritis in AIA mice was markedly alleviated by intra-articular injections of anti-miR-132, as evidenced by reduced joint swelling and lower histopathological scores of joint tissues. Moreover, Jiang et al.[277] reported that intra-peritoneal injection of miR-26a mimic alleviated arthritis symptoms and pathological changes in a pristane-induced arthritis rat model by inhibiting the TLR3 pathway. Interestingly, another intriguing idea is to employ miRNAs sourced from plants as a nutritional strategy for RA. However, critical issues remain unresolved, including the specific miRNAs present in plants, their metabolic fate within the body, and the required treatment duration[278]. Despite these advancements, miRNA-based therapeutics face several challenges, including incomplete understanding of miRNA biology, suboptimal delivery methods, and uncertainty regarding ideal administration protocols[279]. Future research endeavors will focus on addressing these issues to facilitate clinical translation of miRNA-based RA therapeutics while minimizing potential risks.

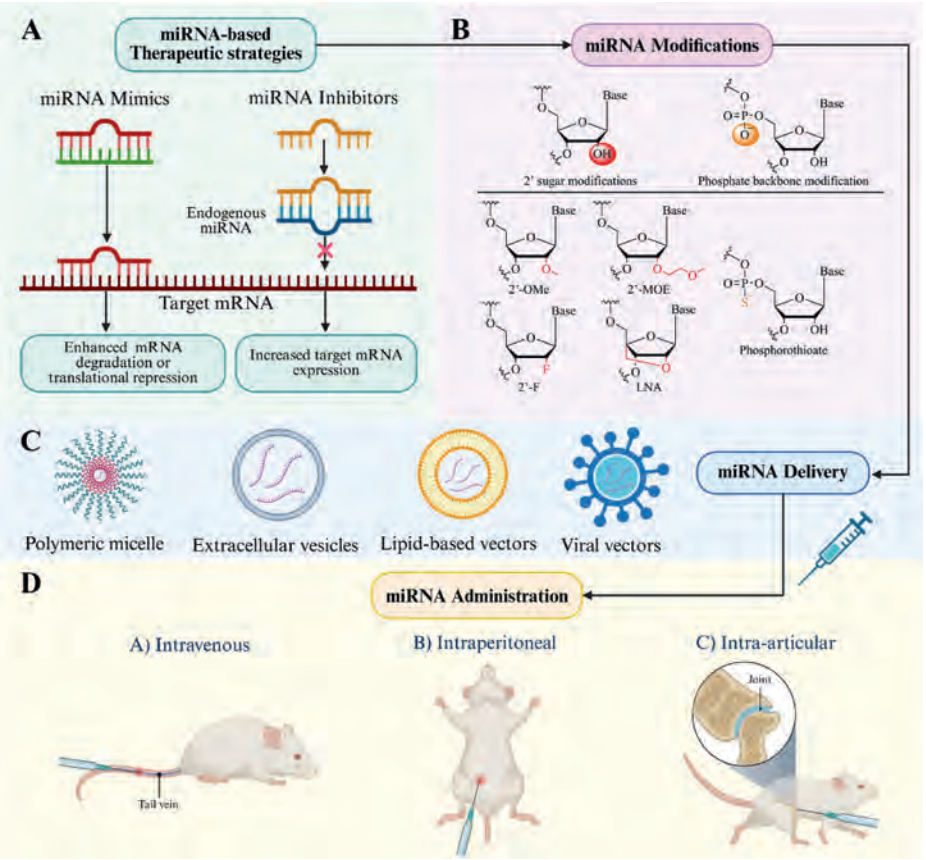


Figure 16 Potential therapeutic strategies for RA utilizing diverse miRNA-based therapies. (A) miRNA-based therapies using miRNA mimics or inhibitors. (B) Common miRNA modifications to enhance stability. (C) Main delivery methods for miRNA agomirs and antagomirs to improve cellular uptake. (D) Therapeutic delivery routes of miRNAs in RA mouse models.

4. Conclusions and prospects

4.1. Conclusions

RA is a multifaceted chronic autoimmune condition that is defined by an intricate interplay of genetic and environmental determinants. Although current therapeutic interventions, including NSAIDs, GCs, and DMARDs, demonstrate limited efficacy, they often fail to halt disease progression and are often accompanied by undesirable side effects. Recent advancements have illuminated the potential role of epigenetic mechanisms as a critical focal point in the pathogenesis of RA. Aberrant DNA methylation patterns and dysregulated histone modifications precipitate the upregulation of pro-inflammatory genes or the downregulation of anti-inflammatory counterparts. Concurrently, miRNAs participate in regulating inflammatory and immune responses pertaining to RA by targeting specific mRNAs. A comprehensive analysis of these epigenetic processes enhances our understanding of RA pathogenesis and aids in discovering new therapeutic targets, promoting the advancement of novel treatment approaches.

Within the therapeutic landscape of RA, inhibitors targeting DNMTs ('writers'), HDACs ('erasers'), and BET ('readers') proteins exhibit considerable promise. These pharmacological agents have the potential to rectify deleterious epigenetic states, reinstate normal gene expression profiles, and attenuate inflammatory mediators, thereby mitigating disease progression. Furthermore, a plethora of differentially expressed miRNAs have been identified as pivotal contributors to RA pathology. As a result, miRNA-based therapeutic strategies are undergoing rigorous investigation, with methodologies employing inhibitors and mimics designed to suppress pathogenic miRNAs with elevated expression and augment the expression of beneficial miRNAs, thereby re-establishing miRNA homeostasis. Despite the promise of epigenetic agents to advance personalized therapeutic strategies for RA, most of the current research remains predominantly in the preclinical phase. Scientific and technological advancements are expected to facilitate the translation of these insights into effective therapeutic interventions for RA.

4.2. Challenges and prospects

With an expanding understanding of immune cells, cytokines, and signaling pathways in the pathogenesis of RA, targeted therapies such as TNF-α inhibitors and JAK inhibitors have become crucial strategies for disease management. However, despite substantial progress, several challenges persist: (i) Effective RA treatment necessitates a comprehensive understanding of its complex pathogenesis, particularly regarding poorly defined epigenetic regulatory mechanisms. Epigenetic changes vary across different pathogenic cells and joint tissues, as well as at various stages of the disease, resulting in a lack of unified consensus. (ii) Although numerous murine inflammatory models have been developed to

study the *in vivo* effects of epigenetic therapies, these models do not fully replicate the human disease phenotype. This discrepancy partly explains the challenges faced in transitioning epigenetic modulators from extensive preclinical studies to clinical trials. (iii) In RA research, inhibitors targeting DNMTs, HDACs, and BET proteins are primarily pan-inhibitors. Despite demonstrating some potential for anti-inflammatory effects and symptom relief, these inhibitors have limited therapeutic efficacy and lack specificity, which can lead to side effects. Additionally, epigenetic drugs encounter physiological barriers in effectively reaching disease sites. Inadequate delivery methods often result in low bioavailability and suboptimal therapeutic outcomes.

To address these challenges, it is essential to develop more precise therapeutic strategies for RA. First, investigations into the molecular mechanisms of HDAC and BET inhibitors in specific cells and animal models should inform the design of highly selective inhibitors. Second, combination therapies that incorporate epigenetic drugs alongside inhibitors of RA-related signaling pathways can modulate relevant target networks and yield synergistic effects compared to monotherapies. Moreover, multitarget drugs based on pharmacophore fusion have the potential to significantly enhance therapeutic efficacy. In terms of drug delivery, nanocarrier-based technologies offer promising solutions to overcome conventional barriers, facilitating the efficient delivery of epigenetic modulators to affected sites. Additionally, targeted protein degradation (TPD) technology presents an innovative approach to address various diseases by selectively degrading pathogenic target proteins[280]. Only one study has indicated that proteolysis-targeting chimera (PROTAC)-mediated degradation of JAK2 can inhibit activated signaling pathways, thereby alleviating inflammatory responses in RA[281]. However, this study did not specifically target epigenetic-related proteins. In recent years, epigenetic degraders based on PROTAC, molecular glue, and hydrophobic tagging technologies have developed rapidly[282]. Nevertheless, research on applying this technology to degrade abnormal epigenetic proteins in RA remains scarce. Therefore, exploring the application of TPD technology in targeting abnormal epigenetic proteins in RA will be an important and noteworthy research direction.

Overall, while the application of epigenetic drugs in RA is still in its infancy, the potential for future development is promising. A comprehensive understanding of epigenetic modifications is essential for identifying the underlying causes of RA and developing novel therapeutic approaches. These modifications can aid in the discovery of new therapeutic targets and may serve as biomarkers for RA diagnosis, enhancing early disease detection. The reversibility of epigenetic modifications makes them ideal therapeutic targets. Despite challenges in translating laboratory research into clinical practice, epigenetics holds the potential to be integrated into standard treatment regimens, offering new avenues for the prevention and treatment of RA. We anticipate that advancements in this field will create significant opportunities to improve the quality of life for RA patients.

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Author contributions

Jifa Zhang and Liang Ouyang conceived the project and supervised the project. Chengcheng Huang, Yuxiang Liang and Yang Li summed up the literature and drafted the manuscript. Chengcheng Huang and Quan Wei were involved in drawing the figures and proofreading the structures. Jifa Zhang and Liang Ouyang revised the manuscript. All authors approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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