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REVIEW ARTICLE

Application of methylation in the diagnosis of ankylosing spondylitis

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Abstract

Ankylosing spondylitis (AS) is a chronic inflammatory autoimmune disease, mainly characterized by perifibrocartilage osteitis of the sacroiliac joints and spinal enthesitis. To date, the exact pathogenesis of AS remains elusive. It is generally believed that AS is a multifactorial disease involving genetics, infection, environment, and immunity. Among them, genetic factors are the primary determinants of disease risk and severity. In recent years, epigenetic mechanisms such as DNA methylation have been extensively surveyed with respect to the pathogenesis of AS. This review summarizes the latest research progress of methylation in AS, from whole-genome sequencing to individual differentially methylated gene. And finally, the role of methylase in AS inflammation, autophagy, and osteogenic differentiation was explored. In summary, the results of this review attempt to explain the role of methylation in the occurrence and development of AS and point out the shortcomings of current methylation research, providing directions for subsequent methylation research in AS.

Keywords Ankylosing spondylitis · Biological process · CPG island · Epigenetics · Methylation

Ankylosing spondylitis (AS) is a chronic inflammatory arthropathy that predominantly affects the axial skeleton, especially the sacroiliac joints and spine, resulting in low back pain clinically [1, 2]. The pathogenesis of AS is complicated and involves multiple risk factors, both genetic and environmental [3]. AS is widely regarded as an inherited disease, and the association of human leukocyte antigen B27 (HLA-B27) alleles with AS has been well-established. HLA-B27 positivity is present in over 90% of AS patients, while the frequency of this gene in the general population is less than 9% [4, 5]. However, it is also reported that only 5–7% of HLA-B27-positive individuals have AS [6], indicating that other genetic factors contribute to the genetic susceptibility of AS [7]. Exploring the genetic mechanism of AS has always been a hot research topic [8]. The effective treatment methods available to AS patients are still limited, partly due to the complexity of the disease and insufficient understanding of its etiology. In recent years, the role of epigenetic modifications including DNA methylation in the pathogenesis of AS has gained remarkable interest [9, 10].

DNA methylation is an epigenetic modification [11] that changes gene expression by adding methyl groups to the cytosine ring of DNA molecules [12, 13]. In 1997, methyltransferase-like protein 3 (METTL3) was found to have the ability to synthesize all N6-methyladenosine (m^6A) in the mRNA transcriptome, forming a major breakthrough in methylation research [14, 15]. Subsequently, further studies reveal that this modification is a dynamic and reversible process, which can be catalyzed by methyltransferases ("writers"), removed by demethylases ("erasers"), and recognized by m^6A-binding proteins ("readers") [16–18]. These studies confirm that m^6A is a regulatory modification required for specific developmental processes [19]. Although the sequence of methylation processes does not alter the fundamental principle of complementary base pairing, they functionally determine the direction of RNA molecules (including RNA splicing, transport, and translation) [20]. Aberrant m^6A modification can lead to serious pathological changes, including cancer [21], metabolic diseases [22, 23], and abnormal immune system activation [23, 24].

Increasing evidence has correlated DNA methylation dysregulation with AS and other autoimmune diseases [24–26].

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Genome-wide association studies have found that the heritability of some non-HLA susceptibility genes in AS is lower than the estimated heritability. The current research on methylation modifications in AS is still insufficient compared with immune system diseases such as rheumatoid arthritis (RA). Most of the studies in this field are limited by experimental design, methylation typing, and statistical methods. Indeed, exploring the methylation mechanism is expected to provide new insights into the pathogenesis of AS, which may also lead to the development of innovative diagnostic or prognostic tools, as well as the identification of new drug targets. In this review, we summarize and discuss current research and knowledge on epigenetic modifications that occur in AS.

Genome-wide DNA methylation analysis

HLA-B27 has been suggested to be important in the pathogenesis of AS [27]. However, recent studies have shown that HLA-B27 only explains 20% of the overall genetic susceptibility to AS, suggesting the implication of other susceptibility genes undiscovered [28, 29]. Emerging evidence has suggested that dysregulation of DNA methylation is pivotal in the context of rheumatic immune diseases [30]. Comparative studies of genome-wide DNA methylation profiles are considered powerful tools for interrogating methylation changes associated with the disease status [31].

The initial study adopted Illumina Infinium HumanMethylation 450 BeadChip to compare the genome-wide DNA methylation profiles of peripheral blood mononuclear cells (PBMCs) from 5 cases of AS and healthy controls (HCs) in China. It was observed that there were 1915 differentially methylated CpG sites between AS and HCs, which mapped to 1214 differential genes. Among them, HLA-DQB1 was the most differentially expressed gene with lower expression in AS and participated in the regulation of antigen processing and intestinal tract immunity pathways [32]. These findings pinpointed the advantages of HLA gene in the diagnosis of AS and also provided a basis for subsequent research.

Immediately, Patrick Coit from Europe conducted a stratified analysis on 24 AS patients based on whether the HLA-B27 allele was positive (12 cases were HLA-B27-positive and 12 cases were HLA-B27-negative) to explore the genetic characteristics of HLA gene in AS. Through Infinium MethylationEPIC array, a 10% methylation expression difference was determined. It was found that gene enrichment mostly existed in highly expressed genes, and the average methylation level of HLA-B27-positive AS patients (41%) was significantly lower than that of HLA-B27-negative patients (60%). HLA-B27 may exert potential pathophysiological effects in an allele-specific manner by affecting DNA methylation at CpG sites surrounding the HLA-B locus. HLA-B27 may contribute to the pathogenesis of ankylosing spondylitis disease. The contribution of HLA-B27 to the pathogenesis of AS may include surrounding regions (e.g., HCP5) or other potential disease-related genetic loci [33]. Genome-wide DNA methylation analysis of whole blood DNA samples from 24 AS patients and 12 OA patients revealed significant differences in methylation levels [33]. This study fills the gap in the comparison of methylation differences between AS and other diseases and once again confirms that the diagnostic effect of HLA genes in AS may be partially achieved by inducing epigenetic dysregulation.

MIN [34] used high-throughput microarray to analyze epigenome-wide DNA methylation and mRNA expression in PBMCs of 45 individuals (AS:HC = 30:15). Genome-wide methylation analysis on 12 independent cohorts identified 2526 differentially methylated positions (DMPs) and 1753 differentially methylated genes (DMGs), and the biological processes are enriched in leukocyte activation, immune response, lymphocyte activation, immune system processes, and immune effect processes. The expression data of 31 AS patients and 20 age- and gender-matched HCs were detected, yielding a total of 4144 differential genes. Through meta-analysis, it was found that the inverse correlation between methylation and expression is more common, with genes exhibiting high methylation and low expression, which supports the "switching" role of DNA methylation in gene silencing. It is confirmed that strong changes in DNA methylome and gene transcriptome underlie the susceptibility of PBMCs to AS, and these changes may regulate the cellular mechanisms in AS.

The above results provide a theoretical basis for the possible involvement of abnormal methylation in the pathogenesis of AS. Through stratified research, not only have the genetic characteristics of HLA genes in AS been determined, but also the phenomenon of high methylation and low expression of most genes in AS has been revealed. It is confirmed that changes in methylation are important factors in regulating the process of AS. These existing studies have laid the foundation for the subsequent screening of methylation genes, which have the potential to serve as diagnostic tools and therapeutic targets for AS (Table 1).

Diagnosis and mechanism of AS based on individual differentially methylated gene

Screening of whole-genome methylation suggests the differences in DNA methylation between AS patients and normal individuals, linking abnormal DNA methylation to the pathogenesis and development of AS. Subsequently, scholars focus on the expression of abnormal gene promoters in AS and the factors affecting gene methylation levels to find diagnostic biomarkers for AS. It is generally believed that

Table 1 Genome-wide DNA methylation analysis

Research method	Source sample group	Group	DMP	DMG	Biological pathway	Year	Cite
Illumina Infinium HumanMethylation 450 BeadChip	PBMC	AS:5	1611	1045 UP	Antigen processing and presentation dysfunction	2017	32
		HC:5	304	169 DOWN			
Infinium MethylationEPIC array		AS:24	37	33 UP	GTPase activator activity, GTPase modulator activity, and Rab/Ras GTPase binding	2020	33
		OA:12	30	22 DOWN			
		B27 + :12	85	48 UP	Potassium ion binding and alkali metal ion binding molecular functions		
		B27 − :12	74	43 DOWN			
High-throughput array		AS:30	3294	2256	Regulation of T cell activation, immune responses, and intercellular adhesion	2022	34
		HC:15	1500				
		AS:12	13385	8531	Leukocyte activation, immune response, lymphocyte activation, immune system processes, immune effector processes		
		HC:12	18,356				

DMGs differentially methylated genes, *DMPs* differentially methylated positions.

the methylation levels of genes are related to gender and treatment methods [35, 36].

Most of the differentially methylated genes studied are associated with bone differentiation, inflammation, and methylases. Among them, endoplasmic reticulum aminopeptidase 1 (ERAP1), B-cell lymphoma/leukemia 11b (BCL11B), Forkhead box class O3a (FOXO3a), Dickkopf-1 (DKK-1), interferon regulatory factor 8 (IRF8), DNA methyltransferase 1 (DNMT1), and tumor necrosis factor (TNF) receptor-associated factor 5 (TRAF5) are all weakly expressed in AS serum. Except for the hypomethylation of CpG-4 and CpG-5 detected in FOXO3a, the methylation levels of other genes are negatively correlated with mRNA levels. FOXO3a, prickle1, IRF5, Runt-related transcription factor 2 (RUNX2), and leucine-rich repeat domain-containing G protein-coupled receptor 6 (LGR6) present hypomethylation in AS serum, and their mRNA levels are often negatively correlated with their methylation levels. Notably, CD279 (PDCD1/B7-H3) shows opposite methylation and mRNA trends in two studies in AS serum. Despite the dual role of CD279 in regulating innate and adaptive immunity [37], this opposite trend remains unexplained, which may be attributed to the small sample size.

Bone differentiation-related genes play an important role in AS methylation, among which the methylation of prickle1 is negatively correlated with inflammatory indicators (erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)) and HLA-B27 [38], suggesting that aberrant methylation status may aggravate patient indicators. As a potential regulator of the Wnt/β-catenin signaling pathway [39], prickle1 in a hypomethylation state may promote bone formation by regulating Wnt/β-catenin. DKK-1, a key regulatory factor of bone mass, plays a synergistic role with prickle1 to inhibit the Wnt/β-catenin pathway [40]. Hypermethylation and low expression of DKK-1 are observed in AS synovial bursa [41]. Hence, it is speculated that the downregulation of DKK-1 may be partially attributed to hypermethylation. After confirming the diagnostic value and ossification-inducing function of DKK-1, scholars have found that the DKK-1 methylation rate is higher in the serum of AS patients with advanced radiological progression, reflecting the possibility of DKK-1 methylation level for the diagnosis of AS with advanced radiological progression. Similarly, the methylation level of ERAP1 is also specific in imaging. The methylation level of ERAP1 is positively correlated with the X-ray classification of sacroiliac joints [42] and also significantly related to back pain in AS patients. These findings suggest the association between ERAP1 methylation and joint ossification. The effect of nonsteroidal anti-inflammatory drugs (NSAIDs) on ERAP1 methylation has been observed, but it is worth noting that this correlation does not occur in patients treated with TNF inhibitors or sulfasalazine. LGR6 is also closely related to the Wnt/β-catenin pathway [43] and plays an important role in coordinating inflammation and bone homeostasis [44]. LGR6 exhibits methylation differences between AS patients and HCs, but no association is observed with disease activity, gender, and HLA-B27 [45]. RUNX2 [46] is critically involved in osteogenic differentiation, which regulates osteoblast progenitor cell proliferation and differentiation into osteoblasts through mutual regulation with various signaling molecules and transcription factors. The methylation level of RUNX2 is related to gender and use of disease-modifying antirheumatic drugs (DMARDs) [47].

Among the genes studied, inflammation-related genes are mostly analyzed, fulfilling a vital role in controlling cell signaling and gene transcription, as well as stimulating the expression of cytokines (TNF-α, IL-6, IL-12, and IL-23), chemokines, and type I interferons. IRF5 and IRF8 can regulate the pro-inflammatory polarization of macrophages [48]. IRF5 [49] is positively correlated with CRP in AS serum. The methylation level of IRF8 is lower in treated AS patients compared with that in untreated AS patients [50]. In addition, a definite correlation has

been observed between the methylation level of IRF8 and disease course, Bath Ankylosing Spondylitis Functional Index (BASFI), ESR, and CpG-2 methylation. IL12B [51] gene is one of the most important genes affecting the IL23 signaling pathway in the pathogenesis of AS. The methylation level of IL12B [52] is negatively correlated with CRP and positively correlated with thoracic expansion, but no correlation with disease activity. TRAF5 is expressed in a variety of immune cells such as macrophages and lymphocytes. The methylation and transcriptome analysis of TRAF5 suggests that anti-rheumatic drugs can reduce the methylation level of TRAF5 [53], and this difference is independent of clinical indicators and B27 + . As a key tumor suppressor, FOXO3a [54] is involved in immune responses by regulating the proliferation and viability of immune cells. Through subgroup analysis of AS patients, it is found that the methylation level of FOXO3a [55] is related to HLA-B27(+) and the use of biological agents, and the CpG-4 island in AS patients is negatively correlated with ESR, CRP, and ankylosing spondylitis disease activity score (ASDAS). Suppressor of cytokine signaling-1 (SOCS-1) [56] is the target of signal transducer and activator of transcription 3 (STAT3). Compared with healthy subjects, elevated methylation levels of SOCS-1 can be observed not only in patients with severe spondyloarthritis and sacroiliitis but also in AS patients with higher IL-6 and TNF-α levels, suggesting that the SOCS-1 methylation level has unique advantages in the diagnosis of AS [57]. BCL11B [58] gene is located on human chromosome 14q32.2 and plays an important role in the development, differentiation, and proliferation of T cells. BCL11B has a variety of functions in inhibiting cell apoptosis and is lowly expressed in AS, indicating that BCL11B is sensitive in the diagnosis of AS. For AS patients with differences in B27, there is no significant difference in BCL11B methylation sites and gene levels, as well as no significant correlation with clinical indicators [59]. CD279, also known as PDCD1 or B7-H3, is critically implicated in inhibiting T cell signal transduction, mediating tolerance mechanisms, and maintaining immune homeostasis [60]. Chen believes that the hypomethylation of the B7-H3 promoter is irrelevant to HLA-B27 status or gender, and moreover, the clinical manifestations of AS patients have no significant correlation with the methylation level or mRNA level of B7-H3 gene [61]. Wu believes that the DNA methylation level of PDCD1 promoter is positively correlated with ESR, CRP, and ASDAS in AS patients [62]. DNMT1 [63] is an essential enzyme for maintaining genomic DNA methylation. The detection of the methylation sites of DNMT1 [64] reveals that DNMT1 has higher methylation and lower gene levels, which is independent of gender, HLA-B27(+), and clinical indicators.

The methylation levels of genes with different functions from different sources vary in AS [65], and the methylation levels observed in some genes are related to treatment, gender, and HLA-B27. The methylation levels of these genes have significant differences in disease progression and are correlated with inflammatory markers. The heritability of methylation levels of these genes may be lower than the estimated heritability. The genes for which these characteristics have not been observed may be related to sample selection and race [66] (Table 2).

Table 2 Differentially methylated gene loci in AS

Gene	mRNA expression	Methylation expression	GPG	DMP	AOC area	Influence factor
PRICKLE1	Up	Down	CPG1, CPG3, CPG4, CPG5	49	0.6382	
DKK-1	Down	Up			0.788	
ERAP1	Down	Up		31	0.779	Biological agents, HLA-B27
LGR6	Down	Down	CpG-1, CpG-2	21	0.676	Male
RUNX2	Up	Down	CpG-2, CpG-4	18	0.689	Medication
IRF5	Up	Down		9	0.81	
IRF8	Down	Up	CpG-1, CpG-2, CpG-3, CpG-4			Medication
IL12B	Up	Up	CpG 3, CpG 8, CpG 10, CpG 13, CpG 15, CpG 18, CpG 23, CpG34	37	0.5675	Male
TRAF5	Down	Up		3		DMARD
FOXO3a	Down	Down	GpG-2, CpG-4, CpG-5	19	0.776	HLAB27, medication
SOCS-1		Up				TNF-a, IL-6
BCL11B	Down	Up	CPG3, CPG5			
B7-H3	Up	Down	B7-H3-1, B7-H3-2	25		
PDCD1	Down	Up			0.764	
DNMT1	Down	Up	CpG 2, CpG5, CpG11, CpG12, CpG14, CpG16			

Methylase regulates disease biological processes in AS

The interaction between genetic factors, mechanical influences on the spine and peripheral joints, and innate immunity is considered to be the culprit in the pathogenesis of AS [67]. The systematic analysis of m^6A modification has unveiled a close relationship of this epigenetic modification with the pathogenesis and development of AS [68, 69].

Methylase mediates inflammation development in AS

Persistent inflammatory response and tissue damage caused by T cell immune imbalance are prominent features of AS [70]. The early stage of AS is always dominated by inflammatory response, manifested as elevation of CRP and ESR, but these indicators do not have specificity and sensitivity for the diagnosis of AS [71]. Methylase has been correlated with the regulation of immunity and inflammation and considered a favorable diagnostic factor. Lipopolysaccharide can induce inflammation in in vitro models. Previous studies have found that METTL3 knockdown reduces the accumulation of inflammatory cytokines; RNA sequencing reveals that the deletion of many inflammatory genes is regulated by METTL3. GO and KEGG results also indicate that methylase points to the inflammatory pathway [72]. In autoimmune diseases [73], METTL3 can prevent the proliferation of macrophages and reduce the production of inflammation-related cytokines, namely, IL-6 and TNF-α. The inhibitory effect of METTL3 on LPS-induced macrophage inflammation depends on the NF-κB signaling pathway, and the prediction model was consistent with the clinical indicators of AS severity including ASDAS-CRP, CRP, WBC, PLT, MPV, PCT, L, L%, PLR, M, LMR, N, N%, NLR, dNLR, and MNR. It is suggested that YTHDF2 in PBMCs may be involved in the pathogenesis of AS, and the prediction model based on the combination of YTHDF2 and SII can be used as a marker for disease diagnosis and progression [74]. In summary, m^6A methylase affects inflammation in AS. Although most current studies on the relationship between m^6A modification and inflammation are based on specific diseases and signaling pathways, the study of epigenetic changes in inflammation has promoted the diagnosis and treatment of AS.

Methylase mediates autophagy development in AS

Autophagy is a key mechanism to maintain cellular homeostasis by delivering cellular components to lysosomes/vacuoles for degradation and reuse of the resulting metabolites. Autophagy is a highly conserved biological process involving 30 types of autophagy-related proteins and is regulated by multiple signaling molecules [75]. Each step of autophagy is regulated by different genes related to autophagy. Therefore, the relationship between autophagy and rheumatic diseases characterized by immune system dysfunction has attracted increasing attention [76]. m^6A DNA regulates autophagy-related genes by affecting the accessibility of transcription factors to target gene promoters [77]. Autophagy may be a key missing link in the pathogenesis of AS [78].

Chen found that the methylation level of m^6A gene in T cells was significantly reduced in AS patients compared with that in normal people, while the expression level of METTL3 was significantly increased. Moreover, the scholars performed transcription in Jurkat cells and found that the transcription of ATGs was downregulated in METTL14-silenced cells, while the number of LC3 loci was increased in METTL14-overexpressing cells, indicating that METTL14 positively regulated autophagy in Jurkat cells. It was also observed that the expression of FOXO3a in T cells of AS patients was significantly reduced and had a positive correlation with the expression of METTL14. Functional rescue experiments confirmed that METTL14 affected autophagy by regulating the expression of FOXO3a. Mechanistically, METTL14 regulated the m^6A level of T cells and induced the upregulation of FOXO3a expression in an m^6A-dependent manner, thereby alleviating AS inflammation by activating the amount of autophagy [79]. In summary, m^6A methylase promotes the occurrence and development of AS by affecting autophagy.

Methylase mediates osteogenic differentiation in AS

Methylase not only mediates inflammation and autophagy in AS but also plays a role in osteogenic differentiation. m^6A methylation has been demonstrated to regulate osteoblast differentiation and osteoclast bone resorption [80]. AS is characterized by chronic inflammation and ectopic ossification of entheses. The osteogenic differentiation ability of mesenchymal stem cells in AS patients (AS-MSCs) is abnormally enhanced, leading to pathological osteogenesis and syndesmotic osteophyte formation [81].

Differences in METTL14 expression have been observed in mesenchymal stem cells between AS patients and HCs. In HC-MSCs, high-level TNF-α intervention can induce stronger migration of AS-MSCs by increasing the expression of ELMO1, and inhibiting METTL14 in MSCs can reduce the m^6A level of ELMO1, thereby upregulating the protein level of ELMO1 and eventually promoting the migration ability of MSCs in vitro and in vivo. This differentiation is due to the METTL14-mediated m^6A modification of ELMO1 3′UTR in AS-MSCs. Ultimately, it is found that

METTL14, together with YTHDF2 and YTHDF3, reduces the mRNA stability of ELMO1 and increases its degradation rate after m^6A modification, resulting in a decrease in the expression of ELMO1 in TNF-α-treated MSCs [82]. In another study, in vitro experiments revealed that in MSC, METTL14 significantly enhanced osteogenic differentiation of BMSCs by overexpression regulating m6A-modification-dependent beclin-1 expression and inducing autophagy [83]. These studies provide evidences for the role of methylases in osteogenic differentiation (Fig. 1).

Diagnostic and therapeutic potential of methylation in AS

Based on the diversity of biological processes in ankylosing spondylitis (AS), differential gene methylation, screened from biological pathways such as inflammation and bone metabolism, has the potential to serve as a biomarker of AS activity. These differential methylations are often associated with factors such as drug use [42, 47] and HLA-B27 [38], suggesting that drugs may modulate the extent of gene methylation. In studies targeting tumors, drug resistance has been found to impede effective cancer treatment [84], and DNA methylation plays an important role in the development of cancer drug resistance, with certain methylation patterns potentially correlating with disease severity and prognosis [85]. The emergence of these specific targets provides potential targeted targets for new drug development. By detecting potential gene methylation markers, the progression and prognosis of AS can be better monitored. However, there are no studies using DNA methylation for intervention or decision-making [86]. Disease progression in AS is still largely dependent on the detection of non-specific indicators such as ESR and CRP [87], and the discovery of differential methylation is expected to break through this limitation and provide AS patients with more sensitive and specific biomarkers. In addition to its great potential in diagnosis, by analyzing methylation patterns, high-risk patients who may develop severe joint injuries or other complications can be identified for early intervention and monitoring, which can help improve long-term prognosis [88].

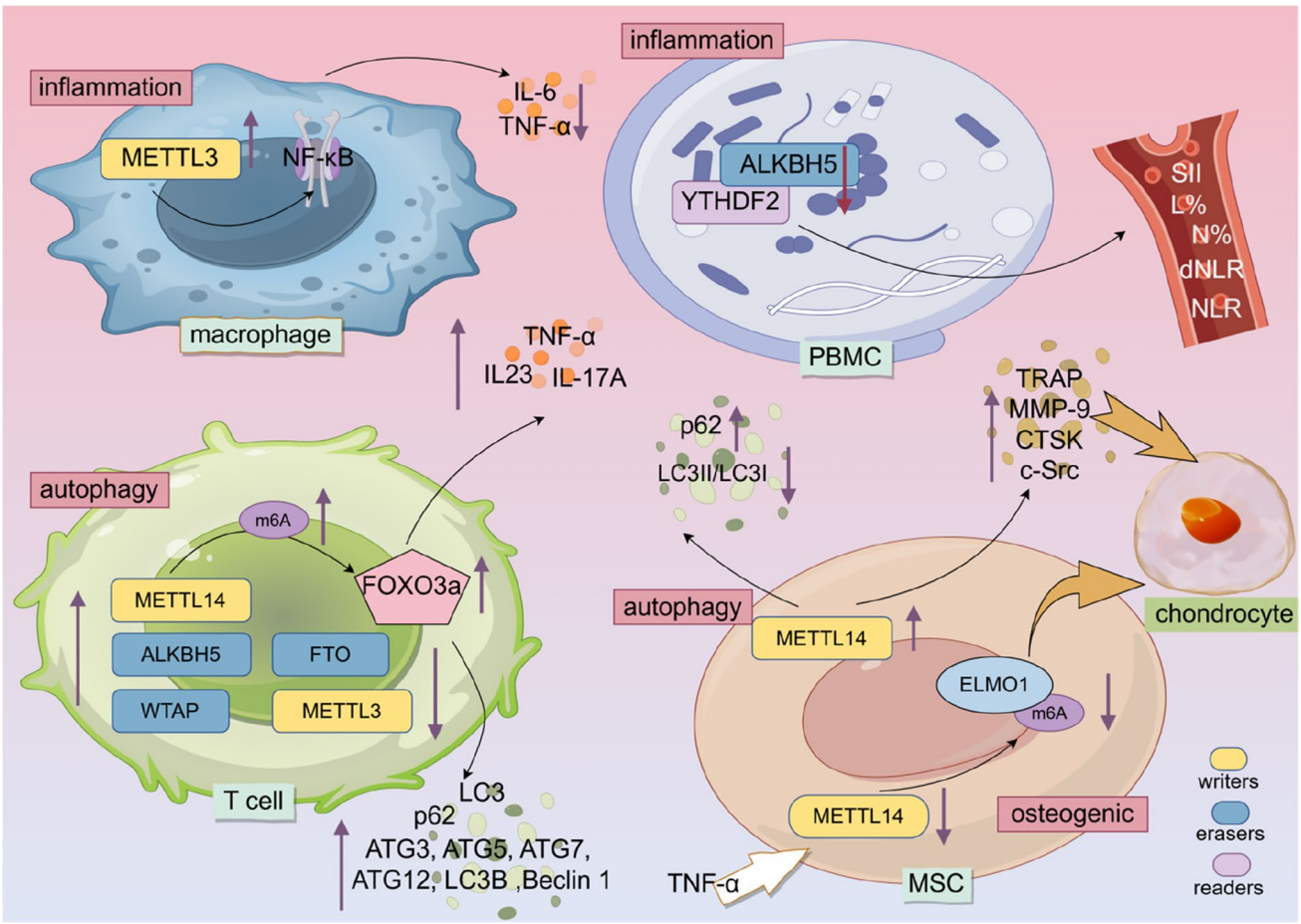

Fig. 1 Methylase regulates disease biological processes in AS

Conclusion

This review begins with an analysis of genome-wide and individual gene methylation identifications, followed by a discussion of the biological processes involved in methylated enzymes in ankylosing spondylitis and the potential for personalized treatment of methylation. In terms of genome-wide screening, not only were methylation differences identified between patients with ankylosing spondylitis and HCs and between ankylosing spondylitis and other diseases, but the genetic signature of the HLA gene in ankylosing spondylitis was also confirmed. In addition, it was observed that most ankylosing spondylitis genes showed high methylation and low expression. The differentially methylated genes were mainly involved in inflammation-related genes, bone differentiation-related genes, and methylase genes. All of these differentially methylated genes showed significant diagnostic properties in ankylosing spondylitis, and stratified analyses revealed that the use of medications, HLA-B27(+), and gender affected gene methylation in patients with ankylosing spondylitis. We then summarize the biology of ankylosing spondylitis in which methylated enzymes are involved mainly through is inflammatory, autophagic, and osteogenic differentiation pathways. Finally, we predict the potential of methylation for personalized treatment in AS.

The existing methylation research in the field of AS mainly focuses on differentially expressed genes. Through bioinformatics methods, we summarize the underlying roles and mechanisms of methylation in AS and classify the differentially expressed genes based on the different functions involved in genes, which may expand the future research methods regarding AS. However, the current research on methylation in AS also has some shortcomings. Firstly, the current research on AS methylation is still mainly based on the methylation of an individual gene, with a greater emphasis on diagnostic value and more severe homogenization. Secondly, the research samples are mostly blood samples. Although blood is the most convenient tissue to collect, the diagnostic potential of synovial cartilage tissue in AS has not been tapped. In the future, we can expand the population of methylation and broaden the types of samples to explore the role of methylation in the treatment of AS. Last but not least, the research on AS epigenetics is still in the early stage, and unified reporting of methylation sites is needed. Future work should focus on prospective sampling with appropriate control groups and replicating novel associations that have been reported.

Author contribution DX and LJ both were involved in the research design. DX participated in the data analysis, authored the first draft, and made changes to the paper. ZXH assisted in the collection of materials and data. The supervision projects of DX, YQS, and DH contributed to the manuscript revision.

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Data Availability All data included in this study are available upon request by contact with the corresponding author.

Declarations

Consent for publication Not applicable.

Disclosures None.

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