Impacts of epigenetic reprogramming on innate immunity

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Abstract

The innate immune response is the host's first line of defense, promptly activated upon pathogen invasion. Its precise and rapid activation relies on innate immune cells (IICs). Upon recognizing danger signals postinfection or injury, they release various innate immune effectors to eliminate invading pathogens or damaged cells, thus supporting the host's immune homeostasis. Epigenetic modifications, by shaping chromatin structures, orchestrate specific gene transcription patterns to regulate the lineage development, differentiation, and activation of IICs. This intricate process ultimately contributes to effective pathogen clearance and IICs' healthy development and differentiation. To thoroughly elucidate the epigenetic mechanisms underlying the development and differentiation of IICs, this review first introduces the fundamental concepts and latest advancements in this field. We then delve into how the immune microenvironment or other signaling molecules shape the epigenetic landscapes of distinct IIC subsets during their lineage development and differentiation. Furthermore, we summarize how different epigenetic modification profiles mediate specific transcriptional patterns, thereby influencing the lineage development, differentiation, and activation of IICs in response to infections or injuries. Finally, we discuss several unresolved critical issues from the perspective of targeting epigenetic modifications to modulate the innate immune response. In summary, this review aims to uncover the molecular mechanisms underlying the development, differentiation, and activation of IICs from an epigenetic perspective, providing theoretical foundations for scientific and medical researchers pursuing disease treatments.

Introduction

During differentiation, development, infection, stress, and damage repair, innate immune cells (IICs), including macrophages, dendritic cells (DCs), neutrophils, and innate lymphoid cells (ILCs), assume specific gene expression patterns in response to the modulation of the local immune microenvironment (Zhang and Cao 2021). These patterns confer distinct phenotypes and biological functions to the IICs. IICs recognize danger signals following infection or injury through pattern recognition receptors (PRRs), which can detect pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). Subsequently, IICs release various innate immune effectors to eliminate invading pathogens and damaged cells, contributing to the resolution of inflammation and the lineage development and differentiation of IICs (Guo et al. 2022; Li and Wu 2021).

The phenotypes of IICs exhibit a remarkable degree of plasticity, and specific phenotypes confer corresponding functions on IICs (Locati et al. 2020; Tsioumpekou et al. 2023). Upon pathogen invasion, IICs swiftly convert from inactivated to activated phenotypes to facilitate the eradication of microbial invaders. Conversely, following pathogen clearance, activated IICs transition to a suppressed state, resulting in reduced inflammatory levels (Cao 2016; Zhang and Cao 2021). However, pathogen invasion or sterile inflammatory signals employ diverse strategies to disrupt the defensive capabilities of the innate immune system. The disruption results in compromised lineage development, differentiation, and subsequent activation of IICs, thereby allowing pathogens to parasitize the host. Dysregulation of innate immune responses can lead to outbreaks of organismal inflammation, subsequent diseases, and even mortality (Amatullah and Jeffrey 2020; Martins et al. 2019). Therefore, deciphering the molecular mechanisms underlying the lineage development, differentiation, and activation of IICs, along with identifying effectors of innate immune responses, would help identify promising therapeutic targets to address dysregulated innate immune responses in infections and inflammatory diseases.

Epigenetic reshaping serves as the dynamic foundation to regulate gene expression (Fig. 1). Chromatin status changes, mediated by histone modifications in enhancer and promoter regions, play a crucial role in the development, differentiation, and activation of IICs
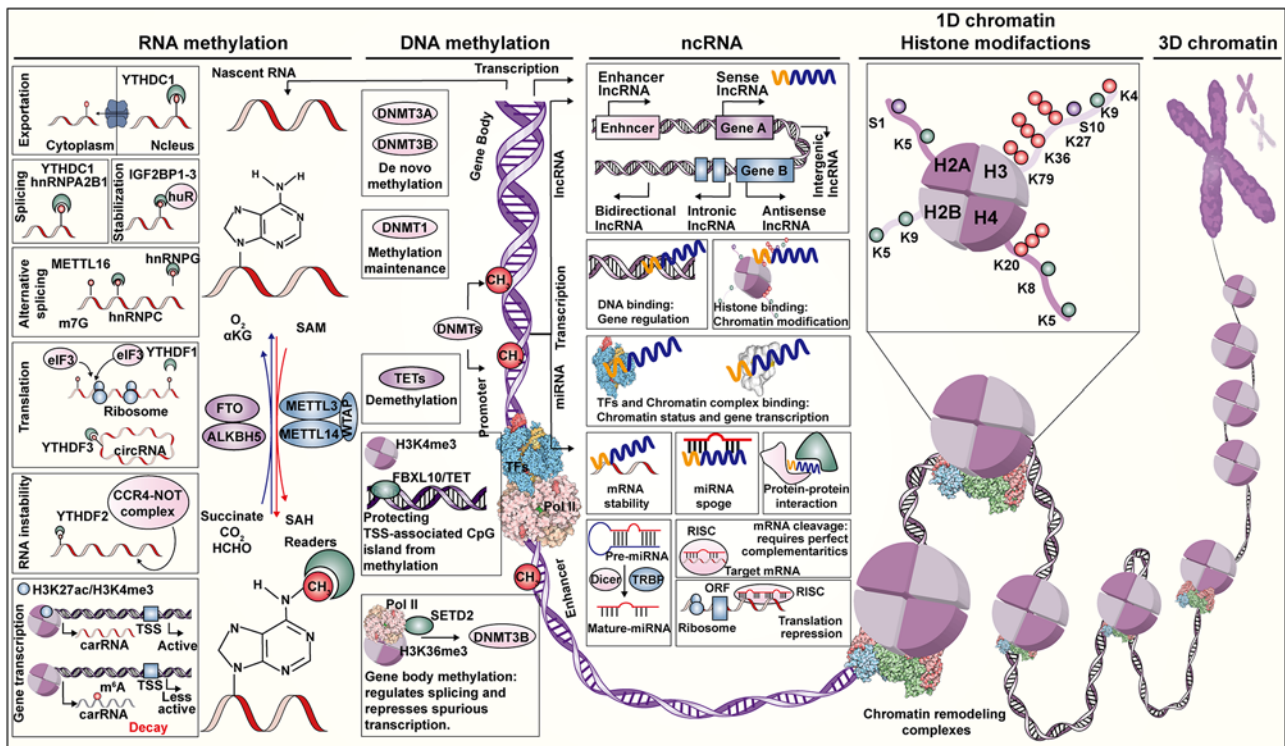
Figure 1. Introduction to epigenetics. Notes: RNA methylation. RNA is transcribed from DNA and subsequently undergoes reversible methylation modifications catalyzed by METTL3/METTL14/WTAP and FTO/ALKBH5. RNA containing m[6]A modifications is recognized by reader proteins, mediating diverse biological functions. DNA methylation. Methylation modifications are written by the DNMTs family on gene promoters, enhancers, and gene bodies. These methylation modifications influence neighboring genes' transcription or chromatin's openness. ncRNAs. lncRNAs and miRNAs are transcribed from DNA. lncRNAs, classified according to their transcriptional sites, influence gene transcription, chromatin accessibility, and mRNA stability through various mechanisms. miRNAs primarily affect mRNA cleavage and translation. Histone modifications and 3D chromatin structure.

(Fraschilla et al. 2022; Hoeksema and de Winther 2016; Liotti et al. 2022). DNA methylation modifications at specific sites can either hinder or promote the binding of key transcription factors (TFs) responsible for fate conversion in IICs, inducing or impeding the transcription of critical genes involved in lineage development, differentiation, and activation (Bonder et al. 2017; Dekkers et al. 2019; de la Rica et al. 2013; Saeed et al. 2014). Histone modifications open up new chromatin accessibility regions, allowing DNA binding by specific transcription complexes or TFs, thereby initiating the transcriptional pattern of particular phenotypes in IICs (Álvarez-Errico et al. 2015; Li et al. 2022a). Noncoding RNAs (ncRNAs) impact chromatin accessibility and downstream gene expression by interacting with promoters, enhancers, histones, and transcription complexes (Chen et al. 2020; Han and Chang 2015; Li et al. 2014a). RNA m[6]A methylation not only regulates chromatin accessibility and transcription rate through co-transcriptional mechanisms but also influences the development and differentiation of IIC lineages and the activation of their key genes by mediating nascent RNA splicing and mRNA metabolism (Boulias and Greer 2023; Sendinc and Shi 2023; Tang et al. 2023). Relying on the transcriptional patterns of innate immunity-specific phenotypes mediated by epigenetic reshaping, IICs assume distinct phenotypes and biological functions to facilitate pathogen elimination, reduction of inflammatory levels, and healthy development and differentiation. High-throughput sequencing technology quantitatively reveals various epigenetic modification landscapes in different developmental stages and different activation states of IICs, enabling studies on the impact of epigenetics on the development

and activation of IIC lineages (Table 1). Epigenetic modifications allow precise and dynamic reversible control of IICs, and dysregulation in this process can lead to various diseases. Hence, considerable clinical potential resides in harnessing the power of epigenetic modifier enzymes, along with their specific inhibitors and activators, to manipulate the epigenetic modification profile of IICs for the treatment or prevention of infection and inflammation-related diseases.

This review aims to explore how the local ecological microenvironment shaped by the host following the development, differentiation, infection, or inflammatory damage of IIC lineages, modulates the gene expression of epigenetic modification groups and their modifying enzymes. Additionally, it investigates how the epigenetic modifications shaped by the local microenvironment and their modifying enzymes reciprocally mediate specific gene expression patterns in IICs, contributing to the regulation of lineage development, differentiation, and timely response to infections and inflammation.

Components and recent advances in epigenetic modifications

DNA methylation

DNA methylation primarily refers to the methylation of the fifth carbon atom of cytosine in CpG dinucleotides, forming 5-methylcytosine (5mC) (Yang et al. 2023). Two protein families directly participate in DNA methylation pathways:

Table 1. Epigenomic techniques

Method	Description
DNA methylation	
WGBS, RRBS, oxBS-seq	Bisulfite treatment by sequencing method to identify genome-wide peaks of DNA methylation
MeDIP-seq, MDB-seq, hmeDIP-seq, 6mA-IP-seq	Specific antibody IP sequencing methods to identify genome-wide peaks of DNA methylation
Histone modifications	
Chip-seq, Cut-tag/Cut-Run	The study of intracellular protein–DNA interactions is also used to identify specific sites on the genome associated with histone modification, that is, the targets of histone-modifying enzymes
Three-dimensional genome	
3C, 4C,5C, Hi-C	Detect the high-level structure of chromosomes and specific regions of the genome and detect the interactions between different regions of the genome
Chip-loop, chIA-PET	To detect the interaction of genomic regions mediated by the target protein
Chromatin	
ATAC-seq, DNase-seq, FAIRE-seq	The detection of open areas on chromatin directly reflects the accessibility of chromatin.
KAS-seq	N3-kethoxal directly marks the single-strand DNA being transcribed, and extraction of enriched DNA can reflect changes in chromatin accessibility, enhancer activity, and DNA topology
Mnase-seq	MNase-seq is a method that indirectly reflects chromatin accessibility by sequencing nucleosome-protected DNA
RNA m^{6}A methylation	
miCLIP	Antibody-based method for locating m^{6}A in the whole transcriptome
MeRIP-seq	This method can locate m^{6}A residues in the 100–200 nt transcription region, and the exact location of m^{6}A cannot be identified at the full transcriptome level.

DNA methyltransferases (DNMTs), which promote and maintain DNA methylation, and the ten-eleven translocation (TET) family proteins, which catalyze multiple steps to remove DNA methylation (Fig. 1). Both two families work coordinately to maintain the transcriptional state, exhibiting different site specificity and dependency (Ginno et al. 2020; Lyko 2018; Wu and Zhang 2011). DNMT3A and DNMT3B, aided by DNMT3L, establish de novo DNA methylation (Schmidl et al. 2018). Once established, DNA methylation patterns are stably inherited through cell division by DNMT1, endowing DNA methylation with genuine epigenetic modification capabilities (Allis and Jenuwein 2016; Schmidl et al. 2018). DNA methylation modulates gene expression primarily by altering DNA accessibility for transcription, leading to downstream recruitment of proteins that regulate chromatin remodeling. On one hand, DNA methylation can obstruct the binding of TFs to promoters (Schübeler 2015). On the other hand, TFs can recognize methylated DNA and recruit other TFs

to remodel chromatin and initiate transcription (Yin et al. 2017). Thus, methylated DNA can be recognized by proteins like methyl-CpG binding domain proteins and recruit histone deacetylases (HDACs), thus instigating changes in chromatin structure (Jones et al. 1998). DNA methylation at CpG islands, particularly in promoters, leads to transcriptional inhibition. One example is high methylation levels support processes like X chromosome inactivation and imprinting (Schmidl et al. 2018; Schübeler 2015). However, methylation within CpG islands in the gene body is positively correlated with gene expression (Arechederra et al. 2018; Han et al. 2011; Mittelstaedt et al. 2021). Recently, DNA N6-methyldeoxyadenosine (6mA) within the human genome has come to light (Kong et al. 2022; Xiao et al. 2018). Numerous studies underscore the unique biological and pathological significance of 6mA in modulating gene transcription, chromatin structure, and disease progression (Boulias and Greer 2022; Feng and He 2023). Current data suggest a potentially conserved function of 6mA in recognizing and clearing exogenous DNA, thereby participating in immune regulation (Boulias and Greer 2022; Xiao et al. 2018).

With the continual advancement in DNA methylation sequencing and molecular biology techniques, our understanding of the functions of DNA methylation is challenging and overturning prior simplistic understandings. Epigenetics mediated by DNA methylation represents a crucial pathway governing the development and activation of the innate immune system. Notably, DNA methylation can undergo rapid changes, especially in response to dynamically shifting environments during pathogenic infections (Qin et al. 2021a). Remarkably, mounting evidence suggests that pathogens possess the capacity to manipulate DNA methylation or regulate the transcription and activity of DNA methylation-modifying factors like TET and DNMT, leading to transcriptional changes in core gene clusters associated with immune responses (Lutz et al. 2021; Pacis et al. 2019, 2015). These shifts in DNA methylation or its modifying agents can play opposite roles, contributing to host immune defense against pathogens or providing pathogens with the means to evade immune responses. Studies indicate that DNA methylation plays a role in regulating monocyte-to-macrophage differentiation, DCs maturation, macrophage polarization, and in controlling T cell differentiation along with memory responses (Dekkers et al. 2019; Jain et al. 2019; Lau et al. 2018; McErlean et al. 2021; Zhang et al. 2022). Consequently, the utilization of candidate genes and epigenome-wide association studies to profile DNA methylation in infected, injured, and immunologically compromised individuals is being employed to elucidate the biological mechanisms underlying disease susceptibility and severity.

Histone modification

Histones, a group of alkaline proteins found in the nucleus of eukaryotic cells, bind DNA to form nucleosomes, the fundamental structural units of chromatin (Skvortsova et al. 2018) (Fig. 1). Generally, a nucleosome comprises 147 base pairs coiled around an octamer consisting of four pairs of histones (H2A, H2B, H3, and H4). Most histones contain a globular domain and an N-terminal tail protruding outside the nucleosome. Under specific enzymatic action, the amino acid residues in the N-terminal tail covalently attach to corresponding biochemical functional groups such as acetyl, methyl, ubiquitin, etc., leading to subsequent posttranslational modifications such as acetylation, methylation, ubiquitination, etc. Notably, covalent modifications of histone N-terminal

residues known so far include acetylation, ubiquitination, sumoylation, and biotinylation of lysine; methylation of lysine and arginine; phosphorylation of serine, threonine, and tyrosine, etc. Compared to DNA methylation, histone modifications do not alter the DNA sequence but are more intricate, influencing chromatin structure and transcriptional activity. For instance, histone acetylation serves to diminish the bond strength between histone molecules and DNA or neighboring nucleosomes, relaxing chromatin structure and facilitating accessibility by TFs and chromatin remodeling factors, thus promoting gene transcription and expression. Hence, histone acetylation is often linked to gene activation (Chen et al. 2020; Pradeepa et al. 2016). The role of histone methylation varies, potentially leading to transcription repression or activation based on the placement of amino acid residues on histone N-termini and the quantity of covalently attached methyl groups. For instance, trimethylation of lysine 4 on histone H3 (H3K4me3) activates transcription, while dimethylation of lysine 9 on histone H3 (H3K9me2) suppresses it (Bernstein et al. 2005; Comer et al. 2015). Overall, histone modifications can influence gene expression by altering chromatin structure or recruiting biochemical functional groups. Recently, more and more data suggest the vital importance of histone modifications in gene transcription related to the differentiation and maturation of IIC lineages. These modifications profoundly affect how IICs detect and respond to pathogens, shaping the landscape of associated diseases (Daskalaki et al. 2018; Li et al. 2022a; Zhao et al. 2020).

Chromatin remodeling

The condensed state of chromatin hinders processes such as gene transcription, DNA replication, and damage repair at the corresponding chromosomal loci. Consequently, eukaryotes have evolved a set of chromatin remodeling enzymes and associated proteins to regulate chromatin structure through modulating nucleosome assembly, disassembly, and rearrangement on chromatin (Kuzelova et al. 2023; Narlikar et al. 2013; Yang et al. 2022). One class of proteins involved in this process is the adenosine triphosphate (ATP)-dependent chromatin remodeling complexes (CRCs). These proteins utilize the energy generated by ATP hydrolysis to facilitate the "sliding" of nucleosomes along DNA or mediate the "exchange" between histone variants and canonical histones within the nucleosome. CRCs can be broadly categorized into four major families based on their distinct functional domains: SWI/SNF, ISWI, CHD, and INO80. Despite similarities in protein structure and enzymatic activity among different CRCs, each family exhibits its own specificity. Chromatin remodeling, mediated by CRCs, plays a crucial role in facilitating specific gene transcription, conferring immune cells with the capability to respond to pathogenic infections. For instance, SWI/SNF is involved in chromatin remodeling at the Il-6 gene promoter, thereby promoting Il-6 transcription (Liu et al. 2019b). Furthermore, BRG1, an ATPase subunit of the SWI/SNF, is indispensable for the transcription of STAT2-dependent pro-inflammatory cytokine genes during TLR4 activation (Seeley et al. 2018).

RNA m⁶A methylation

The discovery of RNA m^6A modification dates back to 1974 in murine Novikoff hepatoma cells (Desrosiers et al. 1974). However, it wasn't extensively studied until 1997 when Bokar et al. isolated the methyltransferase-like 3 (METTL3) protein from Hela cells (Bokar et al. 1997). RNA m^6A modification constitutes a dynamic and reversible process that is primarily regulated by three types of enzymes – methyltransferases (writers), demethylases (erasers), and binding proteins (readers) – which collectively modulate post-transcriptional RNA modifications (Fig. 1). The demethylation of RNA m^6A primarily relies on the catalysis of demethylases FTO and α-ketoglutarate-dependent dioxygenase ALKBH5 (Jia et al. 2011; Zheng et al. 2013). The RNA m^6A modification is added to RNA by the multi-subunit writers complex consisting of the METTL3-METTL14 heterodimer and numerous additional adaptor proteins. The methyltransferase complex mainly comprises the catalytic subunit METTL3 (Yao et al. 2018), the RNA-binding platform METTL14 (Kobayashi et al. 2018), and the auxiliary factors Wilms tumor-associated protein WTAP and KIAA1429 (Scholler et al. 2018; Schwartz et al. 2014). The functional effects of m^6A modification on target RNAs are believed to be mediated by "readers" (Riquelme-Barrios et al. 2018). Among the many "readers," the YTH domain-containing (YTH) protein family has been well studied (Meyer et al. 2012; Patil et al. 2016), including cytoplasmic members YTHDF1, YTHDF2, and YTHDF3 (Dominissini et al. 2012; Shi et al. 2017; Wang et al. 2014b, 2015), as well as nuclear proteins YTHDC1 and YTHDC2 (Hsu et al. 2017; Xu et al. 2014). In addition to YTH family members, other proteins have been identified to recognize and bind to m^6A. The eukaryotic initiation factor 3 complex interacts with m^6A-containing 5′UTRs through multi-subunit interfaces, directly recruiting the 40S pre-initiation complex to the 5′UTR of target mRNA to facilitate translation initiation (Meyer et al. 2015). hnRNPA2/B1 and hnRNPG can bind to m^6A-modified RNAs to regulate splicing and microRNA maturation (Alarcon et al. 2015).

RNA m^6A modification participates in various biological processes. Recent studies demonstrate that RNA m^6A modification not only engages numerous aspects of RNA metabolism, such as splicing, nuclear export, stability, and translation efficiency (Zhou et al. 2022), but also dynamically regulates gene transcription directly in a co-transcriptional manner through diverse RNA types, including nascent RNA, long noncoding RNAs (lncRNAs), chromatin-associated regulatory RNAs (carRNAs), endogenous retroviral RNAs, and R-loops (Akhtar et al. 2021; Cen et al. 2020; Li et al. 2020c; Liu et al. 2020; Sendinc and Shi 2023; Xu et al. 2022b, 2021). On January 31, 2020, the collaborative research team of Chuan He, Dali Han, and Yawei Gao published a groundbreaking study in *Science*, proposing for the first time that m^6A on carRNA regulates chromatin status and transcription (Liu et al. 2020). They discovered that carRNA can be methylated by METTL3, resulting in m^6A modifications. A portion of these m^6A-modified carRNAs is recognized by YTHDC1 and degraded through the NEXT complex (Liu et al. 2020). The m^6A modification serves as a switch that affects the abundance of these carRNAs, thereby regulating the chromatin status and downstream transcription nearby. In addition, the absence of m^6A leads to the enrichment of certain TFs and an increase in active histone markers, inducing transcriptional activation and an increase in chromatin accessibility (Liu et al. 2020).

The presence of RNA m^6A modification has been demonstrated to sustain cellular self-recognition of endogenous RNA, while its absence can lead to the generation of aberrant endogenous double-stranded RNA in hematopoietic stem cells (HSCs) and progenitor cells, triggering robust innate immune responses and necrosis within the hematopoietic system (Gao et al. 2020b). The myeloid cell-specific RNA m^6A modification promotes differentiation of monocytes into macrophages and granulocytes (Yu et al. 2021), enhancing their capacity to combat pathogen invasion

(Tong et al. 2021). Deletion of METTL14 in macrophages impairs the functionality of CD8+ T cells (Dong et al. 2021). In the latter part of this review, we will delve deeper into how RNA m^{6}A modification regulates the differentiation and plasticity of IICs.

Noncoding RNA

Over 70% of the genetic sequences can be transcribed into RNA, yet only 2% are protein-coding sequences (Carninci et al. 2005; Djebali et al. 2012). ncRNAs are categorized into small miRNAs and long lncRNAs based on their length (Fig. 1). miRNAs primarily function as posttranscriptional inhibitors, estimated to regulate over 60% of protein-coding genes. The seed region of miRNA (2–8 nt of the 5′ end) guides the RNA-induced silencing complex to degrade or inhibit mRNA translation in the ribosome by complementarily binding to the target gene's mRNA (Bartel 2009) (Fig. 1). Notably, miRNAs can bind and regulate multiple target genes, modulate various components of the same signaling pathways, and facilitate rapid responses during infections and immune reactions (Chen et al. 2020). XIST, one lncRNA that drives X chromosome inactivation, was first discovered in 1991 (Brown et al. 1991). With the advancement of sequencing technologies, comprehensive lncRNA profiles in different diseases and cell types have been established, revealing hundreds of disease-regulating lncRNAs. While these lncRNAs have diverse transcription sites, their functions and mechanisms remain similar (Fig. 1). For instance, numerous lncRNAs suppress RNA polymerase II or mediate chromatin remodeling and histone modifications, thus influencing downstream gene expression (Chen et al. 2020; Han and Chang 2015). Some lncRNAs form RNA-protein complexes with TFs, altering their structure and activity upon binding, thereby regulating gene expression (Li et al. 2014a). Additionally, lncRNA's self-transcription can interfere with the transcription of neighboring protein-coding genes. Upstream lncRNAs, during transcription, can selectively relocate to the promoter or enhancer regions of nearby genes, occupying the binding sites for TFs and inhibiting gene transcription (Chen et al. 2022; Ferrè et al. 2016; Statello et al. 2021). LncRNAs also modulate mRNA expression in various disease microenvironments (Chen et al. 2017b; Zhang and Cao 2016). However, only a limited number of lncRNAs are involved in regulating infections and immune responses (Castellanos-Rubio et al. 2016; Gomez et al. 2013; Ranzani et al. 2015). Recent research highlights the indispensable role of lncRNAs in controlling immune cell activation (Atianand et al. 2016; Wang et al. 2014a). A series of lncRNAs such as lincRNA-Cox-2 (Carpenter et al. 2013), lincRNA-PACER (Krawczyk and Emerson 2014), lincRNA-THRIL (Li et al. 2014b), lnc-13 (Castellanos-Rubio et al. 2016), lincRNA-EPS (Atianand et al. 2016), lncRNA-ACOD1 (Wang et al. 2017), lncRNA-Mirt2 (Du et al. 2017), and linc-AAM (Chen et al. 2021b) have been reported to regulate macrophage development or activation.

Epigenetic modifications orchestrate phenotypes of IICs

Epigenetic modifications regulate lineage development and polarization of macrophages

The role of epigenetic modification in lineage development of macrophages

Macrophages serve as the first line of defense against invading pathogens and are pivotal in immune responses. They participate in tissue homeostasis, either facilitating or resolving inflammation that can lead to tissue damage or contribute to tissue repair.

Saeed et al. investigated the epigenetic modifications and transcriptional dynamics during the monocyte-to-macrophage differentiation (Saeed et al. 2014) and found that the epigenetic alterations during this process primarily occurred at promoters and distal regulatory elements. Among these, 1240 promoters showed decreased H3K27 acetylation while 1307 promoters exhibited increased H3K27 acetylation (Saeed et al. 2014). This finding suggests a nearly equal number of opened or closed promoter modifications during the monocyte-to-macrophage differentiation process. Further analysis revealed a positive correlation between the existence of H3K27ac elements and the transcriptional activity of adjacent genes (Saeed et al. 2014). Additionally, H3K4me1 was found to provide epigenetic memory during this process (Saeed et al. 2014). These findings suggest a positive correlation between histone H3 modifications (H3K4me3/H3K27ac) at promoters and enhancers' distal regulatory elements (H3K4me1/H3K27ac) during monocyte-to-macrophage differentiation (Fig. 2A). Similarly, Dekkers et al. investigated the genome-wide DNA methylation changes during the differentiation of monocytes into macrophages (Dekkers et al. 2019). They found that during the differentiation process, there were 4283 upregulated differentially methylated CpGs (DMCs) and 1493 downregulated DMCs. Interestingly, these DNA methylation changes were highly localized, typically affecting individual CpGs that are predominantly within enhancer regions bound by specific TFs (H3K4me1) and in active enhancer regions (H3K4me1/H3K27ac) (Fig. 2B). However, this study did not provide sufficient evidence to establish DNA methylation changes as the direct cause driving monocyte differentiation into macrophages. The observed DNA methylation changes might result from downstream impacts of histone modifications or TF binding (Bonder et al. 2017; de la Rica et al. 2013). Furthermore, occupancy of TF binding sites by TFs inhibits local DNA methylation or vice versa. In addition, Rodríguez et al. observed epigenetic dynamic changes during the differentiation of pre-B cells into macrophages (Rodríguez-Ubreva et al. 2012) (Fig. 2C). Despite distinct DNA methylation states before and after differentiation were observed, crucial differentiation genes did not exhibit significant changes in DNA methylation. However, C/EBPα was discovered to induce histone modifications in genes associated with macrophage differentiation, by means of binding to highly methylated promoters of macrophage-specific genes and recruiting p300, a transcriptional co-activator and acetyltransferase. This action activated macrophage-specific gene expression, thereby regulating the differentiation of pre-B cells into macrophages (Rodríguez-Ubreva et al. 2012). This study emphasizes the role and mechanisms of epigenetic modifications in this reprogramming process, highlighting the importance of epigenetic reprogramming in regulating cell fate transitions.

A report by Sakai focused on the transcriptomic and epigenetic features of newly settled liver macrophages, contributing valuable insights into the mechanisms through which precursor cells develop tissue-specific phenotypes (Sakai et al. 2019). By characterizing the transcriptomic and epigenetic alterations of macrophages resettled in the liver post-acute Kupffer cells (KCs) (liver resident macrophage) depletion, they proposed insights into signaling pathways and TFs that promote KCs differentiation. Post-depletion of KCs, recruited monocytes rapidly differentiated into KCs, and the liver environment reprogrammed the enhancer landscape of the recruited monocytes (Fig. 2D). Newly differentiated liver macrophages assumed more accessible chromatin, similar to the observed pattern in KCs. Mechanistic studies revealed that DLL4 activation in sinusoidal endothelial cells triggers the Notch
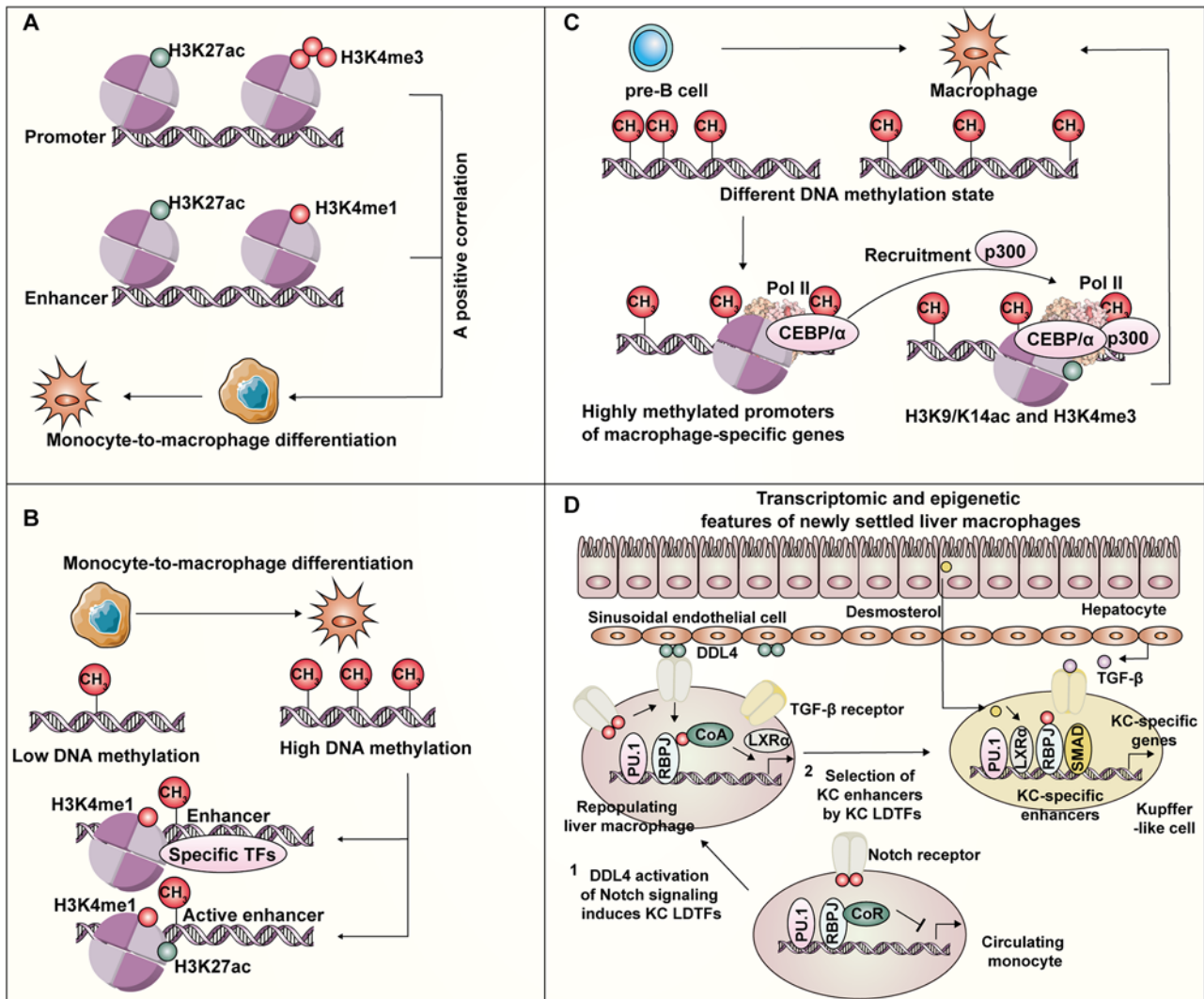
Figure 2. The role of epigenetic modification in lineage development of macrophages. A. The role of H3K27ac and H3K4me3 in guiding monocyte-to-macrophage differentiation. B. Increased DNA methylation during monocyte-to-macrophage differentiation promotes the binding of TFs and active histone elements to relevant differentiation genes. C. Epigenetic patterns during pre-B cell-to-macrophage differentiation. D. Epigenetic characteristics and mechanisms underlying the differentiation of newly settled hepatic macrophages.

signaling pathway in circulating monocytes. This, in turn, stimulates the expression of KC-specific genes and suppresses the activity of monocyte-specific TFs, thereby giving rise to repopulating liver macrophages (RLMs) (Fig. 2D). Subsequently, the Notch signaling pathway and TGF-β further activate RLMs at KC-specific gene H3K27ac enhancers, inducing the expression of genes that promote differentiation toward KCs, ultimately leading to the formation of KCs.

Taken together, these findings collectively suggest that ecological signals under physiological and pathological environments have the capability to induce specific differentiation or phenotypic transitions in tissue-resident macrophages, precursor cells, and monocytes by reconfiguring their epigenomes.

The role of epigenetic modification in macrophages polarization

Macrophages exhibit remarkable heterogeneity and plasticity, with their phenotype and function regulated by the surrounding environment, a process referred to as macrophage polarization (Ginhoux and Jung 2014; Sica and Mantovani 2012). Typically, macrophages sense and engulf the host, presenting fragmented peptides to helper T cells (Th) when pathogens invade the host. Simultaneously, macrophages release pro-inflammatory cytokines and chemokines to eradicate the pathogens, while simultaneously secreting anti-inflammatory cytokines and chemokines to protect the organism. Two discernible polarization states are observed in macrophages: M1, which releases pro-inflammatory cytokines, represents the classically activated macrophages, while M2, releasing anti-inflammatory cytokines, represents alternatively activated macrophages (Gordon and Taylor 2005; Patel et al. 2017; Steinman and Idoyaga 2010). Upon exposure to pathogenic inflammatory stimuli, gene transcription in macrophages undergoes significant changes, leading to the activation of macrophages (Fig. 3). Activation enables them to respond to infection and stimuli more effectively, thus establishing immune homeostasis. However, if the transcriptional pattern activated by inflammation persists, macrophages can become
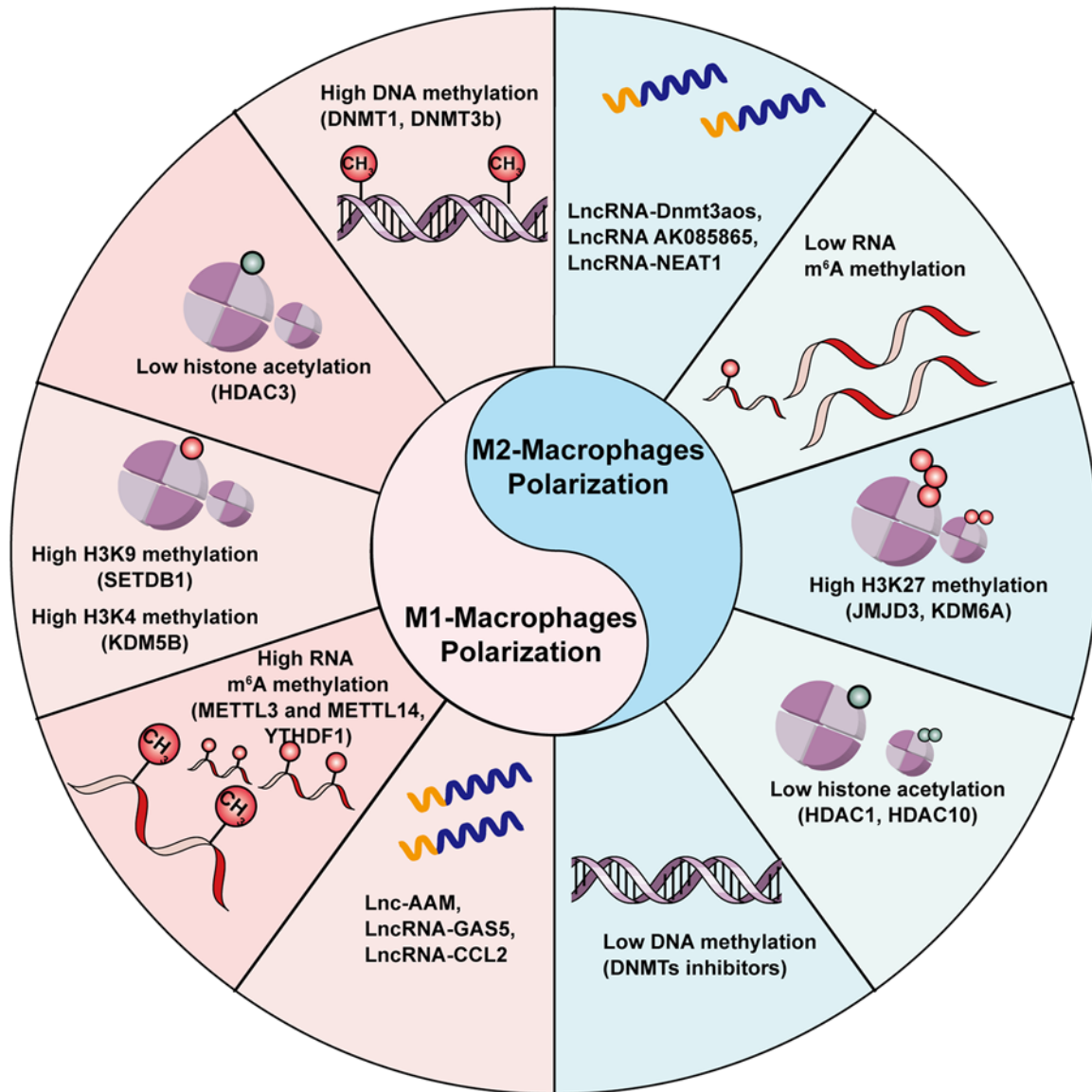
Figure 3. Epigenetic modifications and macrophage polarization. Notes: DNMT1- and DNMT3b-mediated DNA methylation favors M1 macrophage polarization, whereas DNMTS inhibitors-induced DNA demethylation typically promotes M2 macrophage polarization. HDAC3-mediated histone deacetylation commonly enhances M1 macrophage polarization, whereas HDAC1 and HDAC10-mediated histone deacetylation typically favors M2 macrophage polarization. SETDB1-mediated H3K9 methylation and KDM5B-mediated H3K4 methylation often promote M1 macrophage polarization, while JMJD3 and KDM6A-mediated H3K27 demethylation typically favors M2 macrophage polarization. METTL3/METTL14 and YTHDF1-mediated RNA m6A modification contributes to M1 macrophage polarization. Lnc-AAM, LncRNA-GAS5, and LncRNA-CCL2 enhance M1 macrophage polarization, while LncRNA-Dnmt3aos, LncRNA-AK085865, and LncRNA-NEAT1 promote M2 macrophage polarization.

excessively activated, thereby compromising host health (Fu et al. 2023). Substantial evidence suggests macrophage polarization is a reversible and adjustable dynamic process that participates in numerous immune-inflammatory diseases' onset, progression, and outcomes. Consequently, macrophages have emerged as attractive therapeutic targets and research focal points in recent years. The "reprogramming" of macrophage states represents a promising new therapeutic strategy.

DNA methylation modulates macrophage polarization. DNA methylation has been demonstrated to modulate gene transcription in macrophages in responding to the pathogenic mechanisms of various diseases including inflammation (McErlean et al. 2021). Jain et al. found that during Lipopolysaccharides (LPS)-induced

polarization of macrophages toward the M1 phenotype, there was an overall decrease in 5mC levels, along with an increase of non-methylated CpG sites, suggesting a notable reduction in DNA methylation associated with macrophage M1 polarization (Jain et al. 2019). DNMT3a-mediated Pstpip2 methylation enhances macrophage activation and inflammation in liver injury by modulating the STAT1 and nuclear factor kappa B (NF-κB) pathways (Xu et al. 2022a). Additionally, studies indicate that DNMT3b also regulates macrophage polarization and inflammation. Elevated levels of DNMT3b, associated with pro-inflammatory M1 macrophages, were observed in obese mice; knocking out DNMT3b promoted macrophage polarization toward an alternative M2 state (Yang et al. 2014). The methylation of the tumor necrosis factor (TNF)-α gene, mediated

by Uhrf1, controls pro-inflammatory macrophage polarization in experimental colitis models, resembling inflammatory bowel disease (Qi et al. 2019). Promoting macrophage M2 polarization can be facilitated by inhibiting DNA methylation at the peroxisome proliferator-activated receptor γ1 (PPARγ1) promoter using 5-azacytidine (DNMT inhibitor) or through DNMT1 deficiency (Wang et al. 2016; Yu et al. 2016). These instances provide explicit evidence that inhibiting DNMTs can facilitate the transcriptional activation of M2 macrophage-associated genes. Inhibitors targeting DNMTs may enhance anti-inflammatory responses, thus alleviating damage.

Histone modification regulates macrophage polarization. Epigenetic modifiers such as histone methyltransferases and acetyltransferases exhibit differential expression in macrophage M1/M2 states, suggesting they play a role in maintaining and regulating macrophage M1/M2 polarization (Zhou et al. 2017). For instance, HDAC10 is upregulated in macrophages and the upregulation promotes activation of mouse M2 macrophages (Zhong et al. 2023). Inhibiting HDAC6 and HDAC8 suppresses macrophage M2 polarization (Li et al. 2020a; Shi et al. 2022; Zhou et al. 2023). Moreover, inhibition of HDAC6 and HDAC3 substantially suppresses LPS-induced macrophage M1 polarization and reduces pro-inflammatory cytokine production (Yan et al. 2014). Epigenetic regulation by H3K4 and H3K27 methylation influences M2 macrophage polarization genes. For instance, the STAT6-dependent induction of JMJD3, an H3K27 demethylase, reduces H3K27 methylation in the promoter regions of genes associated with M2 macrophage polarization, thus maintaining their transcriptional activity (Ishii et al. 2009). Histone H3K27 demethylase KDM6A-dependent demethylation regulates Ire1α expression, which enhances M2 macrophage polarization (Chen et al. 2021a). Histone demethylase JMJD1C upregulates miR-302a to promote M1 macrophage polarization (Zhong et al. 2021). Knockout of Setdb1, a macrophage-specific H3K9 methyltransferase, in mice upregulated interleukin-6 (IL-6) levels upon LPS stimulation and increased its susceptibility to endotoxic shock, indicating that H3K9 methyltransferase SETBD1 is an epigenetic regulator of pro-inflammatory cytokine expression (Hachiya et al. 2016). Genome-wide analysis of KDM5B binding peaks revealed its selective recruitment to the Nfkbia gene promoter, associated with activated macrophages. KDM5B-mediated erasure of H3K4me3 reduces chromatin accessibility at the Nfkbia gene locus, resulting in reduced IκBα expression and augmented macrophage activation mediated by NF-κB pathway (Zhang et al. 2023a). Additionally, ornithine decarboxylase deficiency during bacterial infection mitigates H3K9 methylation to enhance M1 macrophage polarization (Hardbower et al. 2017).

During LPS-induced M1 macrophage polarization, HDAC3 interacts with activating TF to facilitate transcriptional activation in an enzymatic-independent manner (Nguyen et al. 2020). This suggests HDAC3 not only regulates chromatin activity through histone deacetylation but also modulates gene transcription through interacting with key macrophage TFs. Arginine methyltransferase 1 (PRMT1) regulates c-Myc-dependent transcription by altering acetyltransferase p300 recruitment to its promoter. PRMT1 inhibition decreases p300 recruitment to c-Myc target promoters and increases HDAC1 recruitment, thereby reducing transcription at these sites. Inhibiting PRMT1 disrupts induction of several c-Myc-mediated target genes, including PPARG and MRC1, highlighting the necessity of PRMT1 in c-Myc function during M2 macrophage differentiation (Tikhanovich et al. 2017).

These data indicate that various chromatin-modifying factors may interact with same TFs to regulate distinct gene subgroups. In conclusion, the relationship between histone modifications and macrophage polarization is crucial for understanding macrophages' heterogeneity and functional transition.

RNA m⁶A methylation regulates macrophage polarization. In the past 5 years, extensive evidence confirmed that RNA methylation plays a crucial role in transcription initiation, regulation of nascent RNA transcription and chromatin-associated RNA m^{6}A methylation, consequently regulating chromatin openness and activity during the development and differentiation of embryonic HSCs. However, the specific impact of RNA methylation in monocyte-to-macrophage differentiation and M0 to M1/M2 polarization remains unclear. Through transcriptomic analysis of nascent RNA, m^{6}A methylation profiling, and chromatin accessibility sequencing, we found that METTL3 regulates m^{6}A modification and transcription of nascent RNA and chromatin-enriched noncoding RNAs (caRNAs) during macrophages polarization from M0 to M1 polarization. The loss of METTL3 significantly reverses the expression of nearly 40% of genes involved in M0 to M1 polarization, including the NF-κB and Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathways. This suggests that RNA m^{6}A methylation modulates the global dynamic transcription and chromatin accessibility during the macrophage transition from M0 to M1, thereby imparting plasticity to macrophages (unpublished data).

In addition, extensive research highlighted the impact of RNA m^{6}A methylation on the stability and translation efficiency of mRNAs that are related to macrophage polarization, thereby mediating the activation of crucial pathways involved in this process. For instance, Qin and colleagues revealed that conditional METTL3 knockout in myeloid cells inhibits liver macrophage as well as T-cell differentiation. This can be attributed to the absence of METTL3 in macrophages, which leads to low levels of Ddit4 mRNA m6A modification and enhanced stability. Ddit4 subsequently suppresses mTOR and NF-κB signaling pathways mediating macrophage activation and inflammatory responses (Qin et al. 2021b). Moreover, Tong et al. established a CRISPR screening system to induce M1 polarization in LPS-stimulated macrophages, revealing METTL3 as a critical factor in macrophage activation based on differential TNF-α expression. Mechanistically, METTL3 promotes Irakm mRNA m^{6}A modification and its degradation, leading to Irakm-toll-like receptor (TLR) signaling activation and macrophage M1 polarization (Tong et al. 2021). Furthermore, research elucidated that METTL3 directly methylates STAT1 mRNA, thus enhancing its stability and STAT1 expression. STAT1 next binds to the promoters of pro-inflammatory genes to promote polarization toward M1 and inhibit M0 to M2 polarization (Li et al. 2022b; Liu et al. 2019c). The METTL3-driven m^{6}A function also stimulates the development of miR-34a-5p which, by interacting with Sirt1 mRNA in KCs to suppress its translation, affects the transcription and translation of certain genes associated with M1 polarization (Pan et al. 2023). Additionally, Han and colleagues demonstrated that the knockout of METTL3 in myeloid cells intensifies Th2 cell responses and exacerbates allergic airway inflammation by activating M2 macrophage. Mechanistically, METTL3 facilitates m^{6}A modification of PTX3 mRNA to promote its YTHDF3-dependent degradation, resulting in reduced PTX3 levels. The decreased PTX3 suppresses macrophage M2 polarization, thereby promoting allergic airway inflammation (Han et al. 2023). These studies

collectively emphasize the contribution of METTL3 to promoting macrophage transition from M0 to M1.

Similar to METTL3, METTL14 was also found to facilitate M0 to M1 polarization while inhibiting M0 to M2 polarization. For example, Zheng and colleagues found that METTL14, through m⁶A modification, enhances Myd88 mRNA stability, consequently promoting Myd88-p65 axis-mediated IL-6 transcription (Zheng et al. 2022). This process facilitates macrophage M0 to M1 polarization while suppressing M2 polarization, thus promoting foam cell formation and enhancing migration (Zheng et al. 2022). Additionally, research has indicated that METTL14, through the KAT3B-STING axis, regulates M1 polarization and triggers NLRP3 inflammasome activation in macrophages postischemic stroke (Li et al. 2023). Conversely, Wang et al. found a negative regulation of macrophage M1 polarization by METTL14. They observed that LPS-induced KAT2B-mediated acetylation of METTL14 at the K398 site enhances the stability of METTL14 protein, which next promotes m⁶A modification of Spi2a mRNA via the YTHDF1 axis. Elevated SPI2A binds to IKKβ to inhibit NF-κB pathway, thus inhibiting macrophage M1 polarization (Wang et al. 2023). Similarly, Du et al. identified METTL14-mediated m⁶A modification on Socs1 mRNA enhances YTHDF1 translation, which eventually inhibits TLR4/NF-κB signal transduction and macrophage M1 polarization (Du et al. 2020). Reintroducing SOCS1 in METTL14 or YTHDF1-deficient macrophages rescued their heightened inflammatory phenotype (Du et al. 2020). Moreover, conditional loss of METTL14 in myeloid cells exacerbated macrophages' reaction to acute bacterial infection in mice, resulting in higher mortality rates (Du et al. 2020). These findings indicate that m⁶A-mediated expression of Socs1 maintains a negative feedback loop that regulates macrophage activation during bacterial infections.

The RNA demethylase FTO has been discovered to promote both M1 and M2 polarization. This occurs through selective removal of m⁶A modifications from Stat1 and Ppar-γ mRNA, thus inhibiting YTHDF2-mediated degradation of Stat1 and Ppar-γ mRNA and hindering macrophage activation (Gu et al. 2020). Additionally, the RNA demethylase ALKBH5 diminishes the m⁶A modification on Cdca4 mRNA which leads to the reduced binding of YTHDC2 to its m⁶A site to inhibit YTHDC2-mediated degradation. The elevated CDCA4 promotes macrophage M2 polarization (Tan et al. 2024). It has also been reported that the absence of YTHDF2 in macrophages suppresses macrophage M2 polarization by m⁶A-mediated degradation of Hmox1 mRNA. The lower level of HMOX1 facilitates the release of inflammatory factors (Hu et al. 2023). Furthermore, Huangfu et al. discovered the interaction between RBM4 and YTHDF2, which leads to the degradation of m⁶A-modified Stat1 mRNA and subsequently regulates interferon (IFN)-γ-induced M1 polarization (Huangfu et al. 2020).

The cumulative findings emphasize the crucial role of RNA m⁶A in the development and polarization of macrophage lineages. It is evident that an increase in METTL3 or METTL14-mediated RNA m⁶A methylation is recognized by YTHDF1 or YTHDF2, which subsequently promotes M1 macrophage polarization. Conversely, RNA m⁶A demethylation mediated by FTO or ALKBH5 typically favors M2 macrophage polarization. Therefore, unraveling the underlying molecular mechanisms and identifying key regulatory elements or genes mediated by m⁶A modifications will aid in designing and developing small molecule inhibitors or activators targeting RNA m⁶A methylation enzymes or critical genes' m⁶A modifications involved in the development and polarization of macrophage lineages, ultimately providing potential therapies for mitigating inflammation resulting from macrophage polarization.

Regulation of macrophage polarization by ncRNAs. lncRNAs play a pivotal role in the specific regulation of macrophage polarization through various mechanisms, mediating the onset and progression of various diseases. Ma et al. reported altered expression of snRNAs during macrophage polarization, showing differences in the expression of hundreds of ncRNAs during M1 polarization of macrophages (Ma et al. 2022). The roles and mechanisms of some ncRNAs in regulating macrophage polarization have been elucidated. For instance, linc-AAM is induced early in macrophage activation, and its subsequent upregulation promotes the transcription of a series of immune response genes (IRGs), further fostering macrophage activation (Chen et al. 2021b). linc-AAM can selectively recognize the promoter sequences of IRGs. Simultaneously, the linc-AAM sequence encompasses two CACACA motifs recognized by heterogeneous nuclear ribonucleoprotein L (hnRNPL). Once interaction occurs between the two, it leads to the dissociation of hnRNPL from the hnRNPL-H3 complex, thus fostering chromatin accessibility and promoting IRG transcription (Chen et al. 2021b). It is noteworthy that the knockout of linc-AAM in mice exhibited compromised antigen-specific cellular and humoral immune responses (Chen et al. 2021b), suggesting that linc-AAM-mediated macrophage activation supports the establishment of adaptive immunity. LncRNA-GAS5 overexpression *in vitro* upregulates STAT1, promoting macrophage polarization toward the M1 phenotype (Hu et al. 2020). LncRNA-MM2P inhibits M1-polarized macrophages' excessive inflammation by interfering with SHP2-mediated STAT3 dephosphorylation (Peng et al. 2023). LncRNA-CCL2 regulates the expression of inflammatory cytokines in macrophages during sepsis (Jia et al. 2018). Li et al. discovered numerous lncRNAs with differential expression in macrophages before and after polarization, among which lncRNA-Dnmt3aos is positioned on the antisense strand of Dnmt3a. Functional experiments further confirm that lncRNA-Dnmt3aos promotes M2 macrophage polarization by regulating downstream Dnmt3a gene expression (Li et al. 2020b). LncRNA-AK085865 is markedly expressed in allergic asthma mice and drives macrophage polarization toward M2; its depletion reduces M2 macrophage polarization, suggesting that silencing lncRNA-AK085865 could ameliorate allergic asthma airway inflammation by modulating macrophage polarization (Pei et al. 2020). LncRNA-NEAT1 enhances B7-H3 expression and JAK2-STAT3 signaling activation by downregulating miR-214, promoting M2 macrophage polarization (Gao et al. 2020a). Additionally, miR-30b-5p releases HMGB1 through the UBE2D2/KAT2B/HMGB1 pathway, promoting proinflammatory polarization and macrophage recruitment (Qi et al. 2021).

Epigenetic modifications modulate differentiation and maturation of DCs

In the bone marrow, HSCs generate multipotent progenitors (MPPs), which can further differentiate into common myeloid progenitors (CMPs) and common lymphoid progenitors. CMPs expressing Flt3 differentiate into macrophage-dendritic cell progenitors (MDPs) (Belz and Nutt 2012; Boe et al. 2022; Roquilly et al. 2022). Common DC progenitors (CDPs) derived from MDPs can differentiate into conventional dendritic cell precursors (pre-cDCs) and plasmacytoid DC precursors (pre-pDCs) (Belz and Nutt 2012; Paul and Amit 2014). Pre-cDCs move from the bone marrow into the bloodstream and migrate to lymphoid and non-lymphoid organs, differentiating into cDCs. As MPPs differentiate into CDPs, the genetic profile for classical DCs (cDCs) and plasmacytoid DCs (pDCs) undergoes epigenetic activation

and loss of inhibitory histone marks (Lin et al. 2015). The process from HSCs differentiation to mature DCs (mDCs) encompasses several intermediate stages, with each stage gradually limiting their developmental and differentiation potential. This suggests that gene expression patterns encounter increasing constraints during lineage cell differentiation. However, existing research indicates that epigenetics participates in reshaping chromatin structure, thereby influencing the transition of gene expression patterns during lineage cell differentiation (Boukhaled et al. 2019).

Epigenetic modification influences the differentiation and maturation of DCs by regulating the expression and function of key TFs

Vento et al. compared the DNA methylation dynamics in the differentiation process of monocytes into DCs and macrophages, identifying distinct gene sets experiencing DC-specific or macrophage-specific demethylation. Their findings indicated the role of IL-4 in coordinating STAT6-mediated DNA demethylation, crucial for monocyte differentiation into DCs (Vento-Tormo et al. 2016). Pu.1, as a TF, holds a pivotal role in hematopoiesis and exhibits continuous expression along the lineage of DCs. Loss of the histone deubiquitinase MYSM1 impairs DCs development without affecting other myeloid cell lineages, including monocytes, macrophages, and granulocytes. Mechanistic studies revealed that MYSM1 regulates Flt3 transcription by controlling histone modifications and Pu.1 recruitment, thereby controlling the differentiation of DCs and CMPs (Won et al. 2014). To unravel the functional roles of lncRNAs in human DCs, Wang et al. employed lncRNA gene chip analysis to examine the expression profile of lncRNAs in monocyte-derived DCs and LPS-induced mDCs from peripheral blood. They discovered the significantly elevated expression of linc-DC (>100-fold) specifically in mDCs. Mechanistic insights revealed that during the maturation of DCs, the genomic regions of linc-DC gradually acquired an open and accessible chromatin structure favoring H3K4me3 and H3K27ac levels, thereby facilitating the binding of the TF PU.1, ultimately resulting in the production of linc-DC in mDCs. The transcribed linc-DC directly binds to the structural region near the phosphorylation site Tyr705 of STAT3, inhibiting SHP1-mediated dephosphorylation and enhancing STAT3 signaling, thereby promoting DC maturation and maintaining DC functionality (Wang et al. 2014a). Pacis et al. comprehensively reported the DNA methylation profile of monocyte-derived DCs for the first time. They found extensive and rapid loss of DNA methylation during *Mycobacterium tuberculosis* infection in human DCs (Pacis et al. 2015), a process dependent on TET2 (Pacis et al. 2015; Vento-Tormo et al. 2016). However, the understanding of how DNA methylation regulates the development from CMPs to DCs and the DNA methylation patterns during the gradual differentiation of DCs from HSCs remains limited. Nevertheless, studies have indicated that Pu.1 can recruit TET2 and DNMT3b to target genes, as observed during osteoclast differentiation from monocytes (de la Rica et al. 2013). Based on this, it can be speculated that in the development of DCs, Pu.1 might interact with TET and DNMT, inducing DNA methylation of certain regulatory differentiation genes. These interactions can then lead to the recruitment of chromatin-modifying factors, including histone modifiers, to regulate the chromatin state at these genes. This regulation of the chromatin state ultimately affects the transcription of the differentiation genes, promoting their activation or silencing, which is crucial for the proper development and differentiation of DCs.

Irf8 serves as a decisive factor in the development of the DC lineage. It initiates the differentiation of HST and MPP into DCs, and its deficiency inhibits the transition of MDP into CDP (Becker et al. 2012; Chauvistré and Seré 2020; Kurotaki et al. 2019; Lee et al. 2017). Xu et al. identified a novel lncRNA termed lncIrf8, which is transcribed from the downstream +32 kb enhancer of the Irf8 locus and exhibits specific expression in pDCs, while it remains unexpressed in MPPs, CDPs, cDC1s, and cDC2s. lncIrf8 binds to the Irf8 promoter and demonstrates distinct epigenetic characteristics in pDCs compared to cDC1s. Elimination of the lncIrf8 promoter impairs the development of both pDCs and cDC1s, while leaving cDC2s unaffected. However, activating the lncIrf8 promoter notably enhances cDC1s development. In cDC1s, the +32 kb enhancer negatively regulates the interferon regulatory factor 8 (IRF8)-repressive protein complex, thereby restricting the auto-activation and expression of Irf8. Conversely, in pDCs, there is relatively less binding between the IRF8-repressive protein complex and the +32 kb enhancer, resulting in heightened transcription of Irf8 and lncIrf8 (Xu et al. 2023).

Histone modification modulates differentiation and maturation of DCs

In recent years, scientists have gradually unraveled the roles of various epigenetic modifier enzymes in DC lineage development and activation by generating mice or cells with specific deletions in distinct epigenetic modifications (Søndergaard et al. 2015). For example, Zhang and colleagues discovered the heightened level of HDAC3 in pDCs, and its deficiency significantly impairs pDC development. Mechanistically, the lack of HDAC3 results in a considerable decline in the gene transcriptions related to pDC differentiation, while genes linked to cDC differentiation are notably upregulated, consequently leading to a significant reduction in CDP's ability to differentiate into pDCs. This is due to the significant increase in H3K27ac, mediated by HDAC3 knockout, at critical genes for pDC differentiation such as Zfp366, Zbtb46, and Batf3, thereby regulating gene expression levels and the development and differentiation of the DC lineage (Zhang et al. 2023b). Moreover, during monocyte-to-DC differentiation, HDAC4 recruitment to the Arg1 promoter region is enhanced, leading to reduced H3 and STAT6 acetylation. This reduction promotes STAT6 binding to the Arg1 promoter and activation of Arg1 transcription, further enhancing the expression of Arg1 and facilitating DC differentiation (Yang et al. 2015). PCGF6 serves as a member of the Polycomb group involved in epigenetic regulation. PCGF6 expression is observed in resting-state DCs and is downregulated following DC activation. Furthermore, HDACs mediate STAT3 deacetylation, also contributing to monocyte-to-DC differentiation (Sun et al. 2009). Boukhaled et al. identified that PCGF6 interacts with the H3K4me3 demethylase JARID1c, jointly negatively regulating H3K4me3 modification in DCs, thereby impacting the chromatin accessibility of critical genes crucial for DC activation (Boukhaled et al. 2016). These findings suggest that HDACs and histone demethylases, among others, exert control over the fate transition of DCs by modulating chromatin modifications at key gene loci or modifying their TFs during DC maturation and differentiation.

RNA m^{6}A methylation regulates differentiation and maturation of DCs

In recent years, research into the involvement of RNA m^{6}A methylation in regulating DC development and activation has emerged. Yin et al. systematically profiled 16 different HSCs, progenitors,

and mature blood cells in the murine hematopoietic system, including MPP, CMP, GMP, MDP, and DC. They observed a higher m^{6}A modification level in long-term HSCs, which subsequently declined as they differentiated into myeloid and erythroid lineages, while the lymphoid cell population exhibited elevated RNA m^{6}A modifications (Yin et al. 2022). This observation suggests that RNA m^{6}A methylation negatively regulates the differentiation of HSCs into myeloid cells and DCs. However, during the maturation process within the DC lineage, Wang and colleagues discovered that METTL3-mediated m^{6}A modifications on transcripts such as Cd40, Cd80, TlrR4, and Tirap enhanced their recognition by YTHDF1, promoting their translation in DCs. This facilitated DC maturation and activation, thereby strengthening cytokine production induced by TLR4/NF-κB signaling (Wang et al. 2019). This indicates a positive regulatory impact of RNA m^{6}A methylation in the maturation and activation of DCs. Further research by Bai et al. revealed that the loss of the RNA m^{6}A reader protein YTHDF1 led to heightened recruitment of mDCs, elevated MHCII, as well as enhanced secretion of IL-12 (Bai et al. 2022). Consequently, this promoted infiltration of CD4$^+$ and CD8$^+$ T cells, boosting IFN-γ secretion, and thereby contributing to alleviating disease. Thus, RNA m^{6}A methylation plays a role in modulating DCs during immune responses.

ncRNA modulates differentiation and maturation of DCs

In addition to the aforementioned lncIrf8 and lnc-dc, numerous LncRNAs also participate in regulating the differentiation and maturation of DCs. For instance, LncRNA-MIR155HG can modulate the immune function of DCs by impacting HSC differentiation (Niu et al. 2020). Moreover, LncRNA-HOTAIRM1 inhibits monocytic differentiation into DCs by targeting the miR-3960/HOXA1 pathway (Xin et al. 2017). HOXA1 serves as a differentiation inhibitory molecule for DCs. The interaction between LncRNA-HOTAIRM1 and miR-3960 promotes HOXA1 expression, leading to the upregulation of monocytic markers CD14 and B7H2, ultimately maintaining the monocytic phenotype and suppressing their differentiation into DCs. Thus, LncRNA-HOTAIRM1, miR-3960, and HOXA1 form a competitive endogenous RNA network, exerting regulatory roles during DC lineage development (Xin et al. 2017). The migration of leukocytes is controlled by interactions between chemokines and their receptors, determining the characteristics and consequences of immune responses driven by DCs (Ardouin et al. 2016; Dress et al. 2018; Worbs et al. 2017). pDCs mature in response to microbial products or inflammatory signals, subsequently upregulating CCR7. CCL21 and CCL19 act as ligands for CCR7, regulating the drainage of DCs to lymph nodes to induce adaptive immunity (Förster et al. 2008; Ohl et al. 2004; Ulvmar et al. 2014). Abnormal DCs transport and aggregation are associated with the pathogenesis of diverse inflammatory conditions (Han et al. 2015). Research indicates that the chemokine receptor CCR7 expressed by DCs negatively regulates DC migration by inhibiting m^{6}A modifications on lnc-Dpf3 within DCs, leading to increased lnc-Dpf3 expression and thereby suppressing the occurrence and progression of inflammatory diseases (Liu et al. 2019a).

Discussion on the roles of epigenetic modifications on differentiation and maturation of DCs

The regulatory networks and mechanisms governing the plasticity of epigenetic control in the development and phenotypic characteristics of DC subsets have not been as clearly elucidated as in macrophage studies. Many questions remain unanswered in this regard. For instance, how signals from disease and the local microenvironment are transmitted to the epigenetic modifiers and chromatin during the differentiation phases of subsets like MPP, MDP, cDCs, or pDCs, especially during pathogen infections. Furthermore, how chromatin and epigenetic information reciprocally regulate their differentiation, migration, and activation in DCs. Another consideration is whether the differences in the epigenetic landscape of DCs directly reflect the phenotype, function, and activation status of DC subsets.

Epigenetic modifications modulate lineage development and activation of ILCs

In terms of function and development, ILCs resemble T cells but lack adaptive antigen receptors. ILCs primarily consist of ILC1s, ILC2s, ILC3s, and natural killer (NK) cells. Through the expression of various integrins, chemokine receptors, and cytokine receptors, ILCs rapidly sense environmental changes, enabling them to swiftly secrete potent cytokines to combat infections and tissue remodeling (Artis and Spits 2015; Brubaker et al. 2015). The lineage development, differentiation, and maturation of ILCs are also regulated, which depends on specific TFs and epigenetic mechanisms, with the expression of particular TFs also relying on the involvement of multiple epigenetic modifications (Cong et al. 2021; Domínguez-Andrés et al. 2023; Zhang and Cao 2021).

Epigenetic modifications regulate lineage development and activation of ILC1s, ILC2s, and ILC3s

Epigenetic modification influences lineage development and activation of ILC1s, ILC2s, and ILC3s by mediating the expression and function of key TFs. The ILCs lineage is determined by ID2, a TF that counters the specific gene transcription in T and B cells. Typically, the Id2 gene remains suppressed, awaiting future activation (Guo et al. 2015; Michieletto et al. 2023). Michieletto et al. conducted a comprehensive analysis of mature ILCs' three-dimensional (3D) genome structure, chromatin accessibility, and gene expression, revealing the mechanism by which ILC2s specifically activate through dynamic reshaping of the Id2 gene locus's 3D structure during early development (Michieletto et al. 2023). Their study found that the local 3D structure of the genome is selectively reconnected at sites relevant to ILCs function, facilitating the lineage development and functional differentiation of ILCs. Moreover, multiple interactions between Id2 gene locus and distal cis-regulatory elements bound by ILC2s-associated TFs GATA3 and RORα shape a unique local 3D structure, thereby promoting the development of ILC2s and allergic airway inflammation. Additionally, Mowel et al. discovered that lncRNA-Rroid in ILC1s interacts with the promoter sequence of the adjacent Id2. It is the gene locus of lncRNA-Rroid, rather than the molecule itself, that responds to IL-15 by enhancing chromatin accessibility and facilitating STAT5 deposition in the Id2 promoter, thereby governing the differentiation and function of ILC1s. Moreover, lncRNA-Rroid is also indispensable for the early development and homeostasis of ILC2s and ILC3s (Mowel et al. 2017).

ncRNA modulates the lineage development and activation of ILC1s, ILC2s, and ILC3s. Numerous ncRNAs participate in regulating ILCs lineage development. For instance, Liu et al. found high expression of lncRNA-Kdm2b expression in ILC3s. Lacking lncRNA-Kdm2b in the hematopoietic system results in reduced numbers and effector functions of ILC3s. This is because lncRNA-Kdm2b promotes the proliferation of ILC3s through

activating the TF ZFP292, thereby sustaining ILC3s maintenance. Mechanistically, lncRNA-Kdm2b recruits some CRCs to the Zfp292 promoter to drive its transcription. The lack of ZFP292 disrupts ILC3 maintenance, increasing susceptibility to bacterial infections (Liu et al. 2017). Furthermore, the circular RNA circTmem241 exhibits high expression in ILC3 and its progenitor cells (Liu et al. 2022). Its depletion impairs the function of ILC3 and inhibits antibacterial immunity. Within ILC precursors (ILCPs), circTmem241 interacts with the NONO protein, recruiting the histone methyltransferase ASH1l to the Elk3 promoter. At the Elk3 promoter, ASH1l facilitates H3K4me3 and H3K36me3, thereby heightening chromatin accessibility and initiating Elk3 transcription. Conditional gene editing experiments in ILCPs mice indicated a substantial disruption in the differentiation capability of ILC3s and increased susceptibility to bacterial infections upon the loss of circTmem241, Nono, or Ash1l. Conversely, overexpression of Elk3 in mice with ILCPs-specific deficiencies in circTmem241, Nono, or Ash1 defects restored the differentiation ability of ILC3s and enhanced their resistance to infections. This suggests that the circTmem241-Nono-Ash1l-Elk3 axis emerges as pivotal in steering ILCPs toward mature ILC3s, highlighting the axis' critical regulatory potential in therapeutic strategies targeting infectious diseases (Liu et al. 2022). Moreover, circular RNAs also interact with RNA m^6A modifications to regulate ILC3 development. Liu et al. discovered the high expression of the circular RNA circZbtb20 in ILC3. The lack of circZbtb20 diminishes ILC3 numbers and increases susceptibility to *Citrobacter rodentium* infection. Mechanistically, the 1200-1605 region of circZbtb20 interacts directly with ALKBH5. Subsequently, ALKBH5 removes the m^6A on Nr4a1 mRNA, which enhances the stability of Nr4a1 mRNA. Subsequently, NR4A1 activates the Notch2 signal to maintain ILC3 homeostasis. Meanwhile, mice with the lack of Alkbh5 or NR4A1 disrupt ILC3s lineage development and intestinal immune homeostasis, rendering them more susceptible to *Citrobacter rodentium* infection (Liu et al. 2021). This further corroborates the role of circZbtb20-Alkbh5-Nr4a1 axis in regulating the development and maturation of ILC3.

RNA m^6A methylation modulates the lineage development and activation of ILC1s, ILC2s, and ILC3s. Additionally, RNA m^6A methylation participates in regulating ILCs lineage development. Zhang et al. observed that the absence of METTL3 had minimal impact on the homeostasis of ILC or the cytokine-induced responses of ILC1 or ILC3. However, it significantly reduced the proliferation, migration, and effector cytokine production of ILC2, leading to compromised immune function. Mechanistic studies revealed that METTL3 facilitated the high methylation of Gata3 mRNA in ILC2s, thereby enhancing Gata3 mRNA stability to promote ILC2 activation (Zhang et al. 2023c). GATA3 stands as an essential TF for the development of all ILCs (Yagi et al. 2014).

Histone modification regulates the lineage development and activation of ILC1s, ILC2s, and ILC3s. Histone acetylation and methylation have also been found to participate in regulating the activation of ILCs. During the multipotent HSC stage, HDAC3 promotes the normal differentiation of immune cells by maintaining chromatin structure and genomic stability. The absence of HDAC3 at this stage prevents lymphoid progenitors from efficient DNA replication, leading to cell cycle S-phase arrest and ultimately diminishing the development and differentiation of ILCs (Summers et al. 2013). Toki and colleagues discovered

that trichostatin A, an inhibitor of HDACs, reduced allergen-induced ILC2s activation and the early innate immune response to inhaled protease-containing airborne allergens (Toki et al. 2016). The bromodomain and extra-terminal (BET) bromodomain is an evolutionarily conserved protein domain capable of recognizing and binding acetylated lysine residues on histones. Its inhibitor, iBET151, effectively hinders human ILC2 activation and suppresses type 2 immune responses (Kerscher et al. 2019). Antignano et al. identified the role of lysine methyltransferase G9a in regulating ILC2s development and function (Antignano et al. 2016). They found that hematopoietic cell-specific G9a deficiency led to a drastic reduction in peripheral ILC2s. Mechanistic studies revealed that H3K9me2 mediated by G9a is necessary for silencing ILC3s-related genes in ILC2s and inhibiting the development of ILC3 lineage. Simultaneously, G9a is crucial for promoting the expression of ILC2s-related genes in mature ILC2s. Additionally, studies reported that ILCs from Gfi1-deficient mice exhibited reduced ILC2 frequencies and dysregulated expression of ILC3-related genes (Spooner et al. 2013), a phenotype similar to G9a-deficient ILC2s. Furthermore, Gfi1 has been shown to directly interact with G9a (Duan et al. 2005), suggesting that the G9a-Gfi1 interaction may play a crucial role in the epigenetic regulation of ILC development.

Epigenetic modifications modulate lineage development and activation of NK cell

Holmes et al. elucidated the transcriptional and epigenetic networks governing human NK cell differentiation, identifying Bcl11b as a central regulatory factor in several steps of NK cell differentiation (Holmes et al. 2021). BCL11B maintains a transcriptional program that enhances NK cell receptor expression, effector functions, and proliferation in response to viral infections (Holmes et al. 2021). The level of DNA methylation within Fcgra3a promoter negatively correlates with CD16a levels during NK cell maturation (Victor et al. 2018). ID2 also plays a critical role in NK cell development. Nandakumar et al. observed severely impaired NK cell development in mice lacking the histone deubiquitinase MYSM1, which led to suppressed Id2 expression. This deficiency occurred because MYSM1 interacts with NFIL3, facilitating their recruitment to the Id2 gene locus. This interaction shifts the chromatin of the Id2 gene region from a repressed to an activated state, crucially promoting NK cell development (Nandakumar et al. 2013). This suggests that MYSM1 is a pivotal epigenetic regulator of NK cell development, controlling the chromatin status of the Id2 gene region, which is crucial for NK cell development, and transcriptional regulation of Id2. Moreover, miRNAs have emerged as essential regulators in NK cell development. Reduced levels of miR-181 inhibit the differentiation of hematopoietic progenitor cells into mature NK cells, whereas its overexpression increases NK cell differentiation. Additionally, miR-181 expression in NK progenitors increases as they progress through differentiation stages. Mechanistic studies indicate that miR-181 influences NK cell differentiation by down-regulating the Notch signaling pathway through its target, the NF-κB essential modulator (nemo)-like kinase (Cichocki et al. 2011). Notch signaling appears to be indispensable during NK cell maturation (Schenk et al. 2016).

Discussion on epigenetic modifications regulation of the lineage development and activation of ILCs

As mentioned above, epigenetic modifications play an indispensable role in the development and plasticity of ILCs, responding to the local microenvironment shaped during homeostasis and

infection, as well as disease signals. Establishing a comprehensive TFs network and epigenetic modification landscape during ILC lineage development would aid in understanding and developing strategies for reprogramming progenitor cells or ILCs using epigenetic modifications and their associated enzymes. This could regulate the host's innate immune homeostasis.

The role of epigenetic modification in pathogen infection

Epigenetic modifications operate at various levels, including transcription, posttranscriptional modifications, and posttranslational modifications, to regulate innate immune signaling upon infection, thereby preventing infection and inflammatory damage. Upon pathogen invasion, IICs utilize PRRs to detect pathogens, rapidly transmitting the infection signals to the cell nucleus. This process shapes a specific epigenetic modification pattern in IICs and alters the expression of relevant epigenetic enzyme genes. In turn, specific epigenetic modification patterns confer IICs with a distinctive gene expression profile. Subsequently, by activating or suppressing PRRs and regulating the transcription of pro-inflammatory cytokines and antimicrobial peptides (AMPs), these patterns help balance and sustain the intensity of the innate immune response.

Epigenetic modifications modulate the PPRs of IICs to balance the innate immune response

PRRs are essential components of the IICs that perceive PAMPs and DAMPs. They primarily include TLRs, RIG-I-like receptors, and Nod-like receptors (NLRs). Activation of these PRRs induces the production of cytokines and IFNs, thereby initiating antimicrobial and antiviral responses in IICs. Gene expression of PRRs and their signaling molecules is subject to epigenetic regulation, encompassing processes ranging from the initiation of transcription mediated by DNA methylation, histone methylation and acetylation to chromatin remodeling. Additionally, RNA stability and translation rates are modulated by RNA-binding proteins (RBPs) and m^6A modification proteins.

Transcriptional regulation

The transcription initiation of PRRs and their signaling molecules represents a crucial checkpoint for IICs to resist pathogenic invasions. Studies have revealed that macrophages, during the early stages of bacterial infection, activate TLR4 by promoting the generation of acetyl-CoA from glucose (Lauterbach et al. 2019). This enhances histone acetylation, independent of HDACs and HATs. Subsequently, the signaling cascade through MyD88 and TRIF leads to the activation of ATP citrate lyase, further promoting the transcription of LPS-induced gene sets (Lauterbach et al. 2019) (Fig. 4A). This research underscores the potential of targeting the metabolic-histone acetylation modification axis to regulate innate immune responses against invading pathogens. The histone methyltransferase Ezh1, dependent on lysine methyltransferase activity, directly binds to the proximal promoter of Tollip (TLRs interacting protein), a negative regulator of TLR signaling, and maintains H3K27me3 to suppress Tollip transcription. Consequently, Ezh1 promotes the production of inflammatory cytokines triggered by TLRs by inhibiting the negative regulatory factor Tollip, contributing to the full activation of innate immune responses against invading pathogens (Liu et al. 2015). Furthermore, macrophage Ezh2, by suppressing SIRT1-mediated deacetylation, maintains H3K27ac in the promoter of lncRNA-Neat1 (Yuan et al. 2022) (Fig. 4B). The increased

chromatin accessibility facilitates p65-mediated transcription of lncRNA-Neat1, a critical mediator in the assembly and activation of NLRs-mediated inflammasomes. Simultaneously, p53 competes for binding to the lncRNA-Neat1 promoter region, recruiting the deacetylase SIRT1 for H3K27 deacetylation. This antagonizes Ezh2-induced transcription of lncRNA-Neat1 and downstream inflammasome activation. This suggests that Ezh2 and p53, through competitive interactions, maintain H3K27ac, thereby participating in the transcriptional activation of lncRNA-Neat1 and subsequently regulating NLRs activation (Yuan et al. 2022). The H3K4-specific histone methyltransferase WDR5 and H3K79 methyltransferase DOT1L, by mediating histone methylation, enhance the binding of IRF3 to the Nlrp3 promoter and promote Nlrp3 transcription in liver macrophages induced by STING, thereby enhancing cell pyroptosis and liver inflammation (Xiao et al. 2023) (Fig. 4B). In primary macrophages, KMT2B directly promotes the transcription of the Pigp gene by increasing H3K4me3 levels at its promoter (Austenaa et al. 2012). The product of Pigp is essential for proper membrane anchoring of CD14, an accessory receptor for TLR3-mediated signaling.

During the activation of macrophages by LPS, HDAC3 is recruited to ATF2 binding sites, activating Tlr4 transcription (Nguyen et al. 2020). Loss of HDAC3 in macrophages protects mice from lethal exposure to LPS (Chi et al. 2020; Nguyen et al. 2020). Additionally, HDAC3, independent of its classical nuclear histone deacetylation function, translocates to mitochondria during macrophage NLRP3 inflammasome activation (Fig. 4B). HDAC3 deacetylates the HADHA at the K303 site during fatty acid oxidation, reducing its catalytic activity. This, in turn, hampers macrophage fatty acid oxidation metabolism efficiency, ultimately promoting the maturation and secretion of IL-1β mediated by the NLRP3 inflammasome, exacerbating inflammatory responses, and inducing inflammatory damage to the organism (Chi et al. 2020).

Posttranscriptional regulation

The protein expression of various signaling molecules of PRRs is extensively subject to transcriptional post-regulation, with a critical contribution from RBPs and m^6A modification proteins. Luo et al. discovered that in LPS-induced sepsis, METTL3 facilitates m^6A modification of Tlr4 mRNA in neutrophils, enhancing Tlr4 mRNA translation rate and inhibiting its degradation. This leads to elevated levels of TLR4 protein, ultimately promoting TLR4 signaling activation in neutrophils, exacerbating the outbreak of inflammation, and subsequently increasing mortality rates (Luo et al. 2023). RBP DDX5 interacts with METTL3 and METTL14 to form an m^6A writing complex. This complex adds m^6A to the transcripts of Tlr2 and Tlr4, promoting their decay through RNA degradation mediated by YTHDF2. As a result, the expression of TLR2/4 is reduced, balancing the inflammatory response induced by bacterial infection (Xu et al. 2024) (Fig. 5). In our previous studies, we found that YTHDF1, by recognizing the key factor m^6A modification in TRLs and NLRs signaling, participates in innate immune responses (Zong et al. 2021c). Traf6, a crucial regulatory factor in TLRs and subsequent NF-κB signaling, is recognized by RBP DDX60 through its HELICc domain, interacting with Traf6 mRNA. DDX60 also utilizes its HELICc domain to interact with the P/Q/N domain of YTHDF1, recruiting YTHDF1. Subsequently, YTHDF1 recognizes the m^6A of Traf6 mRNA through YTH domain, promoting Traf6 translation and its mediation of intestinal immune responses (Zong et al. 2021c) (Fig. 5). Additionally, both our lab and other researchers have
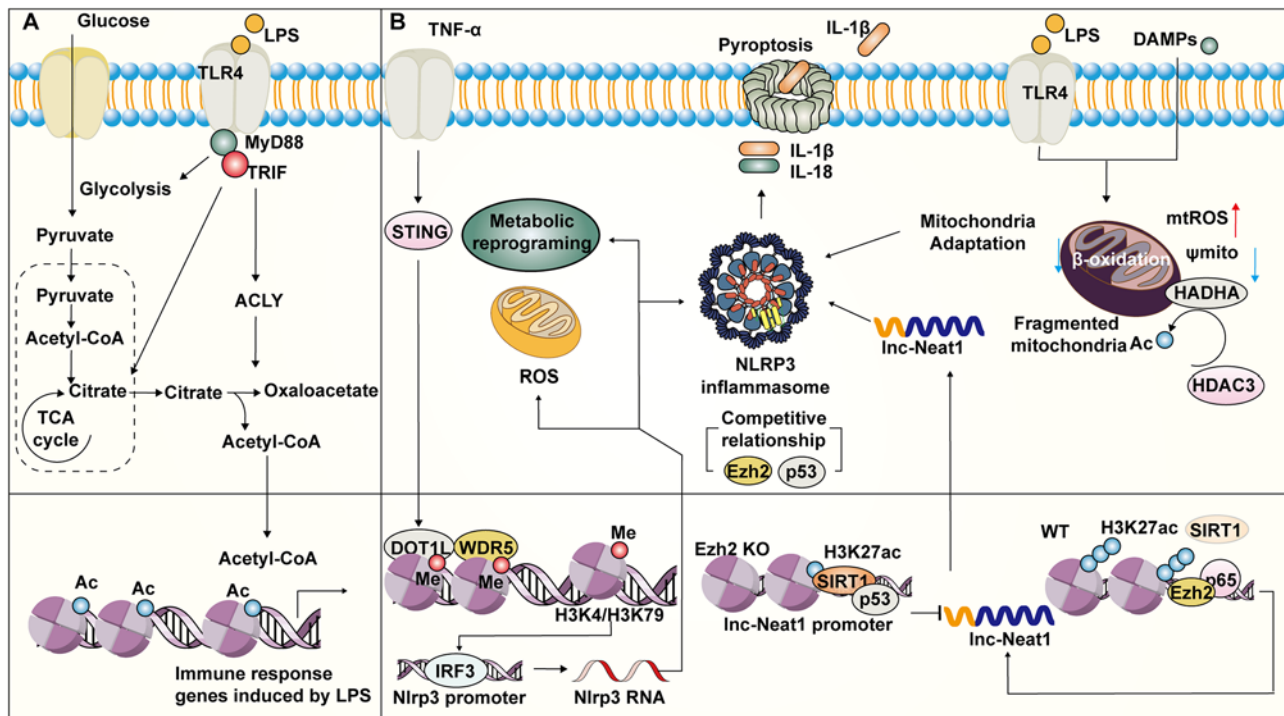
Figure 4. Epigenetic mechanisms mediating PRRs transcription signaling. A. Bacterial infection activates TLR4, leading to the MyD88/TRIF-dependent pathway that promotes glucose metabolism and the production of CoA. This, in turn, regulates histone acetylation modifications, enhancing the transcription of immune response genes. B. The interplay between the pattern recognition receptor NLRP3 and epigenetic modifications.
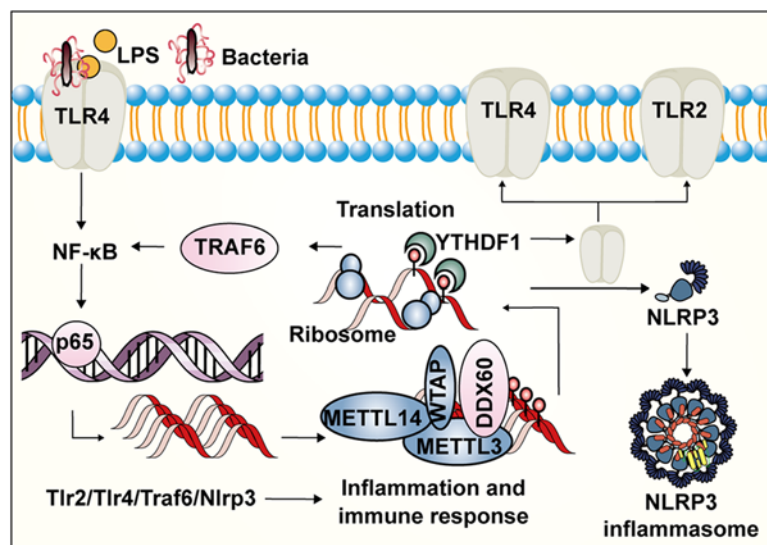

Figure 5. Epigenetic mechanisms mediating posttranscriptional regulation of PRRs. Notes: Bacterial infection activates the NF-κB-p65 signaling pathway through TLR4, leading to the transcription of Tlr4, Tlr2, Traf6, and nlrp3 genes. Subsequently, these Tlr4, Tlr2, Traf6, and nlrp3 RNAs are recognized by DDX60, which recruits METTL3 to promote their m^{6}A modification. Under the influence of YTHDF1, this modification enhances their translation in ribosomes. This process subsequently triggers a positive feedback loop that regulates the expression of TLR4 and NLRP3.

discovered that YTHDF1 directly recognizes the m^{6}A modification in macrophage Nlrp3 mRNA (Hao et al. 2022; Zong et al. 2021b) (Fig. 5). This promotes its translation rate in polysomes, leading to NLRP3 inflammasome activation and, consequently, facilitating intestinal bacterial infection. Mice lacking YTHDF1 are protected from various detrimental effects of bacterial infections (Hao et al. 2022; Zong et al. 2021b). Similarly, after viral infection, RBP DDX46 recruits ALKBH5, which, through the DEAD helicase domain of DDX46, removes the m^{6}A modification from transcripts associated with antiviral responses such as Mavs, Traf3, and Traf6 (Zheng et al. 2017). This inhibits their nuclear export,

preventing their translation in the ribosome and suppressing IFN production, ultimately suppressing the antiviral innate immune response (Zheng et al. 2017). In addition, miRNAs also participate in the regulation of posttranscriptional modifications of TLRs. For instance, in alveolar macrophages from patients with severe asthma, there is a significant reduction in the expression of TLR7, accompanied by a substantial increase in the expression of miR-150, miR-152, and miR-375. Further investigations have revealed that these three miRNAs collectively inhibit the expression of TLR7, leading to a reduction in IFN production and facilitating viral invasion (Diebold et al. 2004; Rupani et al. 2016).
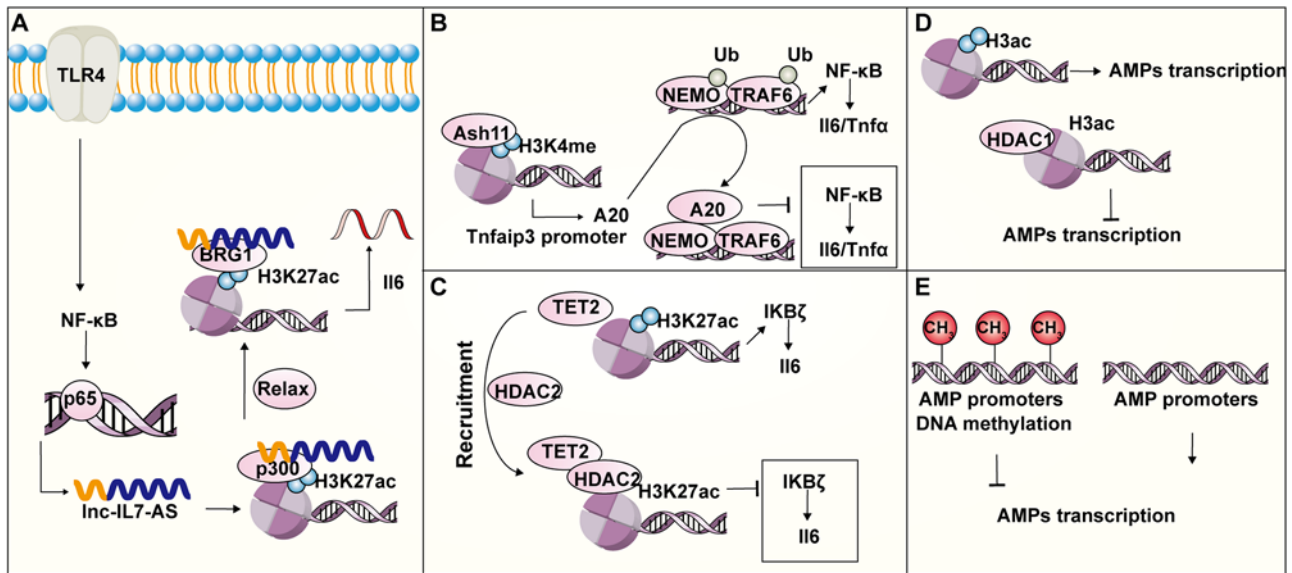
Figure 6. Epigenetic mechanisms mediating the transcription of innate immune factors. A. Lnc-IL7-AS and H3K27ac in the regulation of Il6 transcription. B. ASH1l-mediated H3K4me regulation of Il6 and Tnf-α transcription. C. TET2 and HDAC2 in regulation of Il6 transcription via H3K27ac. D. Histone acetylation levels on AMPs gene loci correlate positively with AMP transcription. E. DNA methylation levels at AMPs promoters correlate negatively with AMP transcription.

Epigenetic modifications modulate the innate immune effector of IICs to balance the innate immune response

Pro-inflammatory cytokines

Epigenetic modifications play a crucial role in modulating the chromatin remodeling of IICs in response to innate immune responses. For instance, RelB induces facultative heterochromatin formation by directly interacting with G9a. Subsequently, heterochromatin protein and G9a form a complex at the Il-1β promoter, promoting Il-1β transcription (Chen et al. 2009). Prolonged stimulation of macrophages with LPS increases the expression of miR-221 and miR-222, which, in turn, suppresses Brg1, an ATPase subunit of the SWI/SNF. This alteration leads to changes in the level or composition of the SWI/SNF complex, thereby inhibiting the transcription of STAT2-dependent pro-inflammatory cytokine genes (Seeley et al. 2018). Similarly, antisense IL-7 is a recently discovered lncRNA in humans and mice. Mechanistic studies reveal that lncRNA-IL-7-AS interacts with p300, regulating the level of histone acetylation in the Il-6 gene promoter region. Simultaneously, the complex formed by lncRNA-IL-7-AS and p300 participates in the regulation of SWI/SNF-mediated chromatin remodeling at the Il-6 gene promoter, promoting Il-6 transcription (Liu et al. 2019b) (Fig. 6A).

Furthermore, histone methylation and acetylation are major factors in the transcription of pro-inflammatory cytokines in IICs. For example, Ash1l, through the SET domain's H3K4 methyltransferase activity, induces H3K4 methylation at the Tnfaip3 promoter, enhancing the expression of the deubiquitinase A20. Ash1l promotes TRAF6 deubiquitination mediated by A20, inhibiting the NF-κB pathway and subsequent production of Il-6 and Tnf-α, protecting mice from sepsis (Fig. 6B). The histone methyltransferase SETD4 rapidly translocates from the cytoplasm to the nucleus upon LPS stimulation, positively regulating Il-6 and Tnf-α transcription in macrophages by directly activating H3K4 methylation at the gene promoters, independent of upstream regulatory factors such as p38, ERK, JNK, p65, and IκBα (Zhong et al. 2019). Additionally, histone deacetylation modification is a mechanism

that inhibits the transcription of pro-inflammatory cytokines during the resolution of inflammation. For example, Tet2, independent of DNA methylation, inhibits Il-6 transcription by recruiting HDAC2. Compared to wild-type mice, Tet2-deficient mice are more susceptible to endotoxic shock and dextran sulfate sodium-induced colitis, leading to aggravated inflammation and IL-6 storm (Zhang et al. 2015) (Fig. 6C). In addition, during the polarization process of porcine macrophages stimulated by LPS, the expression of DNTM3b is reduced, leading to a downregulation of the methylation level of the Tnf-α gene promoter, thereby promoting its transcription (Zhang et al. 2020). Similarly, porcine reproductive and respiratory syndrome virus infection in porcine macrophages inhibits the expression of FTO, resulting in an increase in m6A methylation levels. This, in turn, enhances the expression of IL-13 through the functional modulation of m6A modifications (Gong et al. 2024).

In summary, these studies directly indicate that epigenetic modifications regulate the transcription of cytokines in IICs in response to pathogenic infections through chromatin remodeling, histone modifications, DNA and RNA methylation, and ncRNAs. However, it remains unclear whether the sustained development of IICs will reciprocally regulate epigenetic modifications, thus reversing the activation state of IICs and forming a feedback loop to activate and inhibit ILCs-mediated innate immune responses timely.

Antimicrobial peptides

AMPs are a class of cationic host defense peptides that not only possess direct bactericidal properties but also enhance the functions of various IICs through immunomodulation, thereby resisting pathogenic infections (Fu et al. 2023). Currently, research on the epigenetic regulation mechanisms of AMPs in IICs predominantly focuses on histone acetylation. For instance, HDACi has been confirmed as effective inducers of AMPs (Lyu et al. 2023; Rodríguez-Carlos et al. 2021) (Fig. 6D). A groundbreaking study by Garcia et al. established a connection between histone

acetylation, AMPs transcription, and intracellular bacterial infection. Infections by *Bacillus thuringiensis* in THP-1 macrophages resulted in the silencing of AMPs expression (Garcia-Garcia et al. 2009). Mechanistic investigations revealed that infection promoted the HDAC1 expression. Increased binding of HDAC1 to the AMPs gene promoter was observed, leading to a significant reduction in histone H3 acetylation in infected cells. This ultimately inhibited the open chromatin state and gene expression of AMPs. Overexpression of HDAC1 enhanced bacterial infectivity, while HDAC1 inhibition significantly reduced bacterial load (Garcia-Garcia et al. 2009). Further studies demonstrated that during bacterial infection, inhibition of HDAC promoted acetylation of the p65 K310 lysine residue by histone acetyltransferase p300, enhancing the expression of the hBD2 gene without a concomitant increase in inflammatory cytokines, thereby reinforcing antimicrobial immune modulation capabilities (Fischer et al. 2016).

Regarding the regulation of AMPs transcription by DNA methylation, research indicates that DNA methylation in the AMPs promoter region leads to transcriptional downregulation, increasing the host's susceptibility to bacterial infections (Chen et al. 2017a; Noh et al. 2018; Wang et al. 2021) (Fig. 6D). In our previous investigation into the relationship between RNA m6A methylation and AMPs expression, we discovered an interaction between the TF FOXO6 and METTL3. This interaction triggered the transcription of GPR161 and its subsequent regulation of AMPs transcription, contributing to the resistance against *Enterotoxigenic Escherichia coli*-induced inflammatory responses (Zong et al. 2021a). However, the precise mechanisms of DNA methylation and RNA methylation in regulating AMPs transcription or posttranscriptional modifications remain to be studied.

Conclusion and prospects

A functional immune system relies on the precise and swift regulation of IICs in response to ever-changing signals within their ecological niches. This capability hinges upon the diverse functionality and high adaptability of these cells. Disruption of the plasticity of IICs can trigger innate immune dysregulation and excessive inflammatory responses, ultimately leading to the onset of immune-related diseases in the host. Epigenetic modifications play a pivotal role in maintaining the functionality and plasticity of IICs, as well as in the innate immune responses associated with infections and chronic inflammation. Current research primarily focuses on elucidating how epigenetics undergo reprogramming and how this reprogrammed epigenome, in turn, establishes functionally specific gene expression patterns in innate immunity. The establishment of comprehensive single-cell epigenomic and transcriptomic profiles of IICs, particularly during *in vivo* innate immune responses, at the single-cell and single-molecule levels through single-cell transcriptomics and single-cell epigenomic sequencing technologies, is poised to decode the epigenetic blueprint of innate immunity comprehensively. Targeting the regulation of epigenetic modifications in IICs is considered a promising strategy. Utilizing epigenetic inhibitors to remove disease-associated epigenetic modifications that contribute to altered gene expression patterns in the host, may aid in re-establishing immune homeostasis, pathogen clearance, and mitigating tissue inflammation. However, epigenetic inhibitors typically exert systemic effects when used in the human body, making it challenging to specifically target key subsets of IICs. Moreover, some epigenetic inhibitors used in clinical trials demonstrate broad effects lacking specificity for gene loci, potentially reactivating non-beneficial or silenced genomic sequences. Epigenetic drugs often exhibit inherent biological activity, thus necessitating researchers to rely on medicinal chemistry design to enhance compound selectivity and specificity, ensuring their safety by avoiding toxicity. This aspect holds particular importance, particularly in the context of recurrent infections or ailments marked by sustained inflammation, demanding prolonged therapeutic strategies. Additionally, the combination of different epigenetic inhibitors might augment the efficacy of each drug. For instance, the combined use of DNMTi and HDACi increased M2 polarization in lung tissues, ameliorating acute lung injury caused by sepsis. This suggests that combination therapy might be the most beneficial approach for treating particular pathological conditions; however, further investigation is imperative to substantiate this premise.

Important issues to be addressed in the future

1) It utilizes recently developed cell lineage tracing tools (Bowling et al. 2020), investigating longitudinally and in multiple dimensions at the single-cell level, accurately characterizing the phenotypic plasticity and epigenome of IICs at each critical time point during lineage development and differentiation. This aims to identify the key TFs and epigenetic modifications defining the state of IICs and determine whether the plasticity of these cells could be extended through controlling epigenetic modifications to treat associated diseases.

2) The conservation of lncRNAs is relatively low, and their folding and structure heterogeneity presents significant challenges in related studies. Therefore, our current understanding of lncRNAs in regulating the fate and function of IICs still needs to be improved. Developing third-generation long-read RNA sequencing, ultra-high-resolution imaging techniques, and gene editing technologies offers new opportunities for studying lncRNAs. Leveraging machine learning to analyze vast datasets encompassing genomics, epigenomics, transcriptomics, proteomics, and phenomics of IICs can aid in identifying causal relationships and pathways. This approach may help discover crucial lncRNAs regulating IICs fate. With the continued advancement in the research of the biological functions and mechanisms of lncRNAs, targeted lncRNA therapies are poised to play a significant role in disease diagnosis, targeted treatment, and drug development.

3) How will integrative analysis of epigenomics and other omics data transform our understanding of the lineage development, differentiation, and activation of IICs? While comprehensive epigenomic and transcriptomic studies have been conducted at the macrophage subtype level, a method to demonstrate the total epigenetic modification rate in individual macrophages is yet to be established. If such an approach is developed, could it accurately predict macrophage development or polarization states? Moreover, can it explain the functional and phenotypic differences between early activated macrophage polarization for pathogen resistance and long-term activation leading to host damage? If this concept can be realized, doctors would only need to collect a few milliliters of a patient's blood, isolate macrophages using biochemical instrumentation, and detect their overall epigenetic modification rate through appropriate methods. This would enable doctors to determine the state of immune activation in patients, greatly assisting in the targeted treatment of patients and reducing the misdiagnosis of clinical symptoms.

4) Achieving specific epigenetic modifications targeting particular subsets of IICs is a critical concern. Additionally, the combined use of different epigenetic inhibitors has shown improved efficacy. Can similar epigenetic drugs induce synergistic effects by acting on various subsets of IICs? However, these issues are of clinical significance and require not only researchers to conduct specific targeted experiments and safety studies in model organisms such as mice to provide a theoretical basis, but also for doctors to utilize these reliable results to conduct clinical trials on a large scale by recruiting patients for individual testing and analysis. This approach is necessary to address the questions above.

5) Can highly specific epigenetic inhibitors retain biological activity while reducing toxicity?

6) Can combining immunotherapy with epigenetic therapy lead to more effective treatment strategies?

7) Exploring additional epigenetic mechanisms such as chromatin condensation, DNA (hydroxy)methylation, and gaining deeper insights into the roles of enhancers and 3D chromatin architecture. These novel epigenetic mechanisms may pave a new path toward the treatment of specific, previously untreatable diseases.

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