Metabolic–epigenetic crosstalk in macrophage activation

Epigenetic enzymes are emerging as crucial controllers of macrophages, innate immune cells that determine the outcome of many inflammatory diseases. Recent studies demonstrate that the activity of particular chromatin-modifying enzymes is regulated by the availability of specific metabolites like acetyl-coenzyme A, S-adenosylmethionine, α -ketoglutarate, nicotinamide adenine dinucleotide and polyamines. In this way chromatin-modifying enzymes could sense the macrophage's metabolic status and translate this into gene expression and phenotypic changes. Importantly, distinct macrophage activation subsets display particular metabolic pathways. IFN γ /lipopolysaccharide-activated macrophages (M_{IFN γ /LPS} or M1) display high glycolysis, which directly drives their inflammatory phenotype. In contrast, oxidative mitochondrial metabolism and enhanced polyamine production are hallmarks and requirements for IL-4-induced macrophage activation (M_{IL-4} or M2). Here we report how epigenetics could serve as a bridge between altered macrophage metabolism, macrophage activation and disease.

Keywords: chromatin-modifying enzymes • epigenetics • histone acetylation and methylation • inflammation • macrophages • metabolism

Macrophage polarization & implications in disease

Macrophages are key components of the innate immune system and are involved in the first line of defense against infectious microorganisms. In response to environmental triggers, macrophages can undergo distinct polarization cues. Toll-like receptor ligands like lipopolysaccharide (LPS) and Th1 cytokines including IFN γ elicit proinflammatory macrophage activation [1]. These so-called M_{IFN γ /LPS} or M1 macrophages display high iNOS-mediated NO production and produce high levels of proinflammatory cytokines (e.g., TNF, IL-1 β and IL-12) and reactive oxygen species (ROS) [1]. On the other hand, the Th2 cytokines IL-4 and IL-13 induce M_{IL-4/IL-13} or M2 macrophages which are associated with anti-helminth responses, allergic reactions, wound healing and tissue repair [1]. IL-4/IL-13-induced macrophages are char-

acterized by enhanced arginase activity, IL-10 secretion and the expression of specific surface markers like MMR, MGL1/2 and E-cadherin [2,3]. Despite the beneficial effects of proinflammatory macrophages for host defense, excessive responses are associated with tissue damage and chronic inflammatory diseases including atherosclerosis, rheumatoid arthritis and systemic lupus erythematosus [4,5]. Accurate and specific regulation of macrophage activation is therefore crucial for maintaining tissue homeostasis.

Epigenetics & its importance in macrophage function

Macrophage activation and function is regulated by alterations in gene expression [6] and epigenetic mechanisms now arise as key controllers of macrophage activity [7,8]. Epigenetic regulation modifies gene activity without altering the DNA sequence itself (Figure 1). The chromatin conformation controls DNA

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Figure 1. Epigenetic enzymes and their metabolic cofactors, substrates and inhibitors. (A) DNA methylation induces repressed gene expression and involves the transfer of a methyl group from SAM to cytosine by DNMTs, whereas DNA demethylation is governed by the action by TET proteins. TET proteins require α -ketoglutarate, iron (Fe [II]) and oxygen as cofactors while succinate and fumarate are inhibitors of TET-protein activity. (B) HATs transfer acetyl from acetyl-CoA to lysine residues on histones, thereby facilitating an open chromatin formation. HDAC activity involves removal of acetyl groups and creates a closed chromatin state. Sirtuin HDACs require NAD^+ for their HDAC activity while lactate is a HDAC inhibitor. (C) Histone methylation can both induce transcriptional activity and repression and is mediated by the action of HMTs, which utilize SAM as methyl donor, and HDMs, which erase methyl marks from histones. Alike to DNMTs, HMTs are α -ketoglutarate-, iron- and oxygen-dependent while succinate, fumarate and ROS are inhibitors of HDM activity. NAD^+ : Nicotinamide adenine dinucleotide; ROS: Reactive oxygen species; SAM: S-adenosyl methionine; TET: Ten-eleven translocation.

accessibility and therefore transcription factor binding, gene expression and phenotype. Heterochromatin is tightly condensed chromatin with poorly accessible DNA and is associated with gene silencing. Conversely, DNA is readily accessible in the open chromatin conformation allowing transcription factor binding and gene expression. The most extensively studied examples of epigenetic regulation are DNA methylation

and modulation of chromatin accessibility via histone modifications.

DNA methylation induces gene silencing

DNA methylation involves the addition of a methyl group to cytosine and can context-dependently lead to repressed gene expression (Figure 1A). This methyl group is transferred from the dietary derived metabo-

lite S-adenosylmethionine (SAM). DNA methylation is catalyzed by DNA methyltransferases, whereas the ten-eleven translocation (TET) family of proteins mediates DNA demethylation [9].

Histone acetylation correlates with transcriptional activation

Multiple post-translational modifications on histone tails, including acetylation and methylation, are known to modulate chromatin accessibility and in this manner the transcriptional machinery. Acetylation of lysine (K) residues 9, 14 or 27 on histone 3 (H3K9, H3K14, H3K27) is associated with transcriptional activation, whereas deacetylation of these lysine residues correlates with transcriptional repression [10]. The histone acetylation status is achieved through the opposing activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs). Selective recognition of histone acetylation patterns is accomplished by the ‘bromodomain and extra-terminal domain’ proteins. As discussed later in this report, both HATs and HDACs require particular metabolic intermediates to fulfill their function efficiently (Figure 1B).

Histone methylation can both induce transcriptional activation & repression

Histone methylation occurs at the positively charged amino acids: arginine, histidine and lysine. The location of the methyl marks and degree of methylation (mono-, di- or trimethylation) determines the transcriptional consequences [11]. Di- or trimethylation of H3K4, H3K36, H3K79 facilitates an open chromatin state for active transcription, whereas trimethylation of H3K27 and di- and trimethylation of H3K9 are linked to condensed chromatin and transcriptional repression. Like DNA methyltransferases, histone methyltransferases use SAM as methyl donor for histone methylation. Also histone demethylase activity is linked to metabolism via various factors as detailed later.

Epigenetic enzymes in macrophage function

Epigenetic enzymes are important regulators of macrophage function and determine disease outcome [12–19]. For instance, HDAC3 erases acetylation marks in regulatory regions of IL-4-induced genes, thereby repressing M2 and promoting M1 macrophage activation. HDAC3 deletion elicits an anti-inflammatory macrophage phenotype and improves atherosclerosis outcome [13,20], while Jmjd3 modulates both LPS-induced inflammatory responses and IL-4-induced gene expression [8,18–19,21–22].

Targeting epigenetic enzymes in order to modulate macrophage function is emerging as a potential therapeutic tool in treatment of macrophage-related diseases such as rheumatoid arthritis and systemic lupus ery-

thematosus [23,24]. So far, most work in macrophages focused on the regulation and modulation of histone-modifying enzymes themselves [12–13,21,25–27]. Yet, epigenetic enzymes require metabolites like acetyl-CoA, SAM, α -ketoglutarate and nicotinamide adenine dinucleotide (NAD⁺) to support their chromatin-modifying activity [28]. Recent findings in other cell types indicate that metabolite availability is a very important determinant of epigenetic enzyme activity and the resulting epigenetic outcome [29,30]. Here we report the putative links between the macrophage’s cellular metabolism, epigenetic enzyme activity and macrophage function.

Distinct cellular metabolic patterns exist in macrophage phenotypes

Different macrophage polarization subsets show altered cellular metabolism [31,32]. M1 activation induces a switch to an anaerobic glycolysis, resulting in increased glucose uptake and lactate production [31,32]. Enhanced glycolysis fuels M1 macrophages with fast energy and increased biosynthesis of building blocks that are required for the rapid eradication of pathogens [31]. Moreover, M1 polarization induces the pentose phosphate pathway, which produces nicotinamide adenine dinucleotide phosphate (NADPH) for NADPH oxidase-dependent ROS production [33]. M2 macrophages show elevated mitochondrial oxidative respiration and fatty acid oxidation, providing sustained energy for their long-lasting tissue repair and wound healing functions [31].

Arginine metabolism is differentially regulated in macrophage subsets

Differential arginine metabolism is one of the most distinctive factors between M1 and M2 macrophages [31]. Enhanced iNOS activity in M1 macrophages converts arginine into citrulline and NO, supporting their antimicrobial activity. In contrast, M2 macrophages display high arginase-1 activity, which indirectly leads to enhanced production of the polyamines putrescine, spermidine and spermine [34]. Functionally, polyamines are best described for their cell proliferation promoting effect but they regulate diverse other cellular functions in a variety of cells at the level of chromatin remodeling (as discussed later), gene transcription, mRNA stability and translation [35].

Several tricarboxylic acid intermediates accumulate in M1 macrophages

Lately, the appearance of a fragmented tricarboxylic acid (TCA) cycle was described in M1 macrophages, involving two breakpoints [36]. First, the expression of isocitrate dehydrogenase, which catalyzes the conver-

sion of citrate to α -ketoglutarate, is downregulated in M1 macrophages. This leads to the accumulation of citrate, required for fatty acid synthesis and antimicrobial itaconic acid formation. The other disruption takes place after succinate, which can induce HIF-1 α -mediated IL-1 β production [37]. Interestingly, succinate is a competitive inhibitor of α -ketoglutarate-dependent histone demethylases as discussed later. Thus, the accumulation of metabolic TCA intermediates in M1 macrophages is linked to proinflammatory function and possibly affects the epigenetic machinery in M1 macrophages.

Differential iron handling in M1 & M2 macrophages

Iron handling is another metabolic discriminator of M1 and M2 macrophages [32]. Macrophages are crucial for systemic iron homeostasis by iron recycling through erythrophagocytosis and recovering it for processes like erythropoiesis [38]. M1 macrophages demonstrate decreased iron export, thereby favoring iron storage. Since iron is an essential element for virtually all living organisms, decreased iron availability in tissues as a consequence of iron sequestration in macrophages restricts the growth and pathogenicity of extracellular microbes [38]. In contrast, M2 macrophages prefer ferroportin-mediated release and recycling of iron [32,39]. As α -ketoglutarate-dependent demethylases require iron as a cofactor, differential iron management in distinct macrophage subsets could possibly also affect their epigenetic methylation state.

Thus, macrophage polarization leads to shifts in various cellular metabolic pathways. Since metabolites regulate histone modifying enzymes [40], it is tempting to suggest that an altered metabolism affects the macrophage's epigenetic landscape and function.

Metabolites modulate epigenetics Acetyl-coenzyme A fuels histone acetylation

HATs transfer acetyl groups from acetyl-CoA to lysine residues on histones. Histone acetylation depends on the nuclear pool of acetyl-CoA, whereas mitochondrial acetyl-CoA is used for bioenergetic purposes (Figure 2A) [41]. In mammals, citrate is converted into acetyl-CoA by ACL and constitutes the major source for nuclear and cytoplasmic acetyl-CoA [41]. In addition, acetyl-CoA can be generated from acetate and pyruvate by ACS and PDC, respectively [42,43]. Inhibition of these acetyl-CoA-generating enzymes reduces global histone acetylation in cancer cells, underlying their importance in the epigenetic regulation [41–43].

Dietary methyl feeds histone & DNA methylation

SAM is a sulfur-containing nucleoside and is the primary methyl-donor for DNA and histone methyltransferases. SAM is generated from ATP and methionine by MAT (Figure 2B) [44]. Synthesis of methionine depends exclusively on dietary methyl groups from substrates such as folate, choline and vitamin B. Therefore, changes in dietary intake of these factors can have profound effects on histone and DNA methylation, gene expression and human health. For example, folate deficiency in early pregnancy is associated with an increased risk for developing neural tube defects, suggested to be the outcome of abnormal methylation due to low dietary intake of folate [45].

Tricarboxylic acid-cycle intermediates control DNA & histone demethylation

Jumonji C (JmjC) domain-containing proteins are the largest group of histone demethylases. The JmjC domain utilizes TCA-intermediate α -ketoglutarate, iron (Fe [II]) and oxygen as cofactors to erase methyl groups [44]. In addition to histone demethylation, α -ketoglutarate also participates in the removal of methyl groups from DNA by TET proteins [46]. Succinate and fumarate, other TCA intermediates, share structural similarity with α -ketoglutarate and act as competitive inhibitors for α -ketoglutarate-dependent enzymes, including JmjC and TET proteins [47]. In line with this, accumulation of succinate and fumarate leads to an increase in genome-wide DNA and histone methylation, associated with tumorigenesis [47]. Thus, availability of TCA-cycle intermediates controls demethylase activity and thereby also the DNA and histone methylation levels in cells.

Nicotinamide adenine dinucleotide regulates cellular metabolism

NAD⁺ is required for the enzymatic activity of HDAC family of sirtuins (SIRT6) [28]. NAD⁺ is a key factor in metabolism and serves as an electron acceptor during several oxidative reactions in glycolysis and the TCA cycle, thereby forming NADH [28]. Since NAD⁺ participates in multiple pathways, consequences of an altered NAD⁺/NADH balance are not likely to be limited to SIRT activity.

Epigenetics as a possible bridge between altered metabolite levels & macrophage phenotype

There is a well-described relationship between metabolite abundance and the activity of histone-modifying enzymes [28]. While histone modifications clearly mediate macrophage function [48], evidence that directly

Figure 2. Interactions between metabolites and histone acetylation and histone methylation. (A) HAT activity depends on nuclear acetyl-CoA, generated by ACS, ACL or PDC. Sirtuins are NAD⁺-dependent HDACs. Other HDACs are NAD⁺ independent and their activity can be inhibited by lactate. Furthermore, ROS and increased glucose levels can deplete NAD⁺ and in this manner indirectly inhibit sirtuins. **(B)** HMTs donate methyl groups from diet-derived SAM to histones. Food-derived methyl donors are used for the generation of methionine, which in turn is converted to SAM by MAT. HDMs are α -ketoglutarate-, oxygen- and iron-dependent and their activity can be inhibited by ROS and TCA cycle intermediates succinate and fumarate. NAD⁺: Nicotinamide adenine dinucleotide; NADPH: Nicotinamide adenine dinucleotide phosphate; ROS: Reactive oxygen species; SAM: S-adenosyl methionine; TCA: Tricarboxylic acid.

links metabolism to epigenetics in macrophages remains scarce.

Putative effects of altered macrophage metabolism on histone methylation & macrophage function

Several metabolic characteristics of M1 macrophages, including increased ROS, NO and succinate and decreased oxygen levels, are known to inhibit demethylase activity [47,49–50]. Succinate inhibits α -ketoglutarate-dependent demethylases in human embryonic kidney cells, leading to alterations in DNA and histone methylation [47]. In macrophages, increased ROS and NO and reduced oxygen levels inhibit JmJc demethylase activity. The resulting increase in H3K9me3 on promoter regions for genes of the chemokine *CCL2* and the chemokine receptors *CCR1* and *CCR5* dampens mRNA expression of these genes [50]. As proposed before, LPS-induced Jmjd3 is likely to exert its proinflammatory functions in a demethylase-independent manner [18]. A possible explanation for this could be the presence of demethylases-inhibiting metabolic intermediates in M1 macrophages. In contrast to M1 macrophages, metabolism in M2 macrophages is not characterized by an increase of demethylase-inhibiting intermediates. Consistently, IL-4-induced Jmjd3 activity decreases H3K27 di- and trimethylation on specific M2 gene promoters (*Chi3l3*, *Retnla* and *Arg1*) [51]. Therefore, enhanced production of demethylase-inhibiting intermediates in M1, but a lack of this production in M2 macrophages could influence the epigenetic landscape and macrophage function.

Metabolic intermediates in M1 macrophages might inhibit histone deacetylases

NAD⁺ is required as an important redox factor by enzymes involved in various metabolic pathways such as glycolysis, the TCA cycle and fatty acid β -oxidation [28]. Disruption of these pathways can alter the NAD⁺/NADH ratio and limit NAD⁺ availability for NAD⁺-dependent SIRT activity. Although SIRT were originally identified as histone deacetylases, most literature regarding SIRT in inflammation focuses on the deacetylation of nonhistone substrates (e.g., transcription factors such as NF- κ B and HIF-1 α) [52]. Nevertheless, Sirt6-mediated deacetylation of H3K9 was found to epigenetically inhibit NF- κ B signaling in mouse embryonic fibroblasts, thereby suppressing proinflammatory gene expression [53]. Increased glucose, accompanied by elevated ROS production depletes cellular NAD⁺ and reduces SIRT activity [54]. In addition to SIRTs, NAD-independent HDACs modulate macrophage function [13,15,17]. Interestingly, lactate is an end product of anaerobic glycolysis and has been

shown to be a weak HDAC inhibitor [55]. Moreover, lactate polarizes tumor-associated macrophages into an M2-like phenotype via HIF-1 α [56] and potentially also via epigenetic reprogramming. Thus, in parallel with demethylase activity, factors associated with M1 macrophage metabolism might modulate histone acetylation levels in macrophages and alter their immunological repertoire.

Effects of polyamines on chromatin & macrophage polarization

A selective set of M2 markers like E-cadherin, MMR, Ym and Fizz1 require polyamines for their IL-4-induced expression [3,34]. Conversely, the polyamine spermine dampens the LPS-induced secretion of proinflammatory cytokines in mouse and human macrophages [57–59]. In agreement, polyamine-depleted macrophages show an increased inflammatory response [3]. Thus, while polyamines are needed for the IL-4-induced expression of several M2 markers, they inhibit the LPS-mediated expression of proinflammatory genes in M1 macrophages. Since polyamines can affect the chromatin conformation in various manners, it is tempting to speculate that the effects of polyamines on macrophage function are at least partially mediated via epigenetic mechanisms. For example, increased polyamine levels can upregulate and activate HAT enzymes, resulting in enhanced gene expression as reviewed recently by Pasini *et al.* [60]. Moreover, both the polyamine synthesis pathway and, DNA and histone methylation require SAM as a methionine backbone or methyl donor, respectively. In this way, enhanced polyamine synthesis in IL-4-treated macrophages may lead to DNA hypomethylation and ultimately M2 gene induction. Yet, this does not explain why some M2-associated genes rely on polyamines during their induction by IL-4, while others are polyamine independent. Conceivably, the promoter and enhancers of polyamine-independent M2 genes are readily accessible, while the regulatory regions of polyamine-dependent genes need more chromatin remodeling to allow the initiation of transcription. Moreover it remains elusive how polyamines inhibit the expression of proinflammatory mediators. In contrast to the described transcription-promoting effects of polyamines, other researchers produced evidence that enhanced polyamine levels rather promote chromatin condensation and gene repression [60]. These observations would support the polyamine-mediated suppression of inflammatory cues in macrophages. Since the transcription promoting and repressing mechanisms of polyamines may differ in macrophage subsets, this area certainly warrants future studies.

Conclusion & possible implications for health & disease

It is clear that targeting epigenetic enzymes in macrophages can be applied to improve their function and ameliorate disease outcome [8,13,21,25–27]. The use of pharmaceuticals that directly affect the activity of epigenetic enzymes is one way to the macrophage's epigenetic landscape and phenotype [21]. In this report, we propose that altering the availability of metabolites that are used by chromatin-modifying enzymes could be an alternate way to modulate macrophage epigenetics. Indeed, studies in other cell types indicate that the activity of epigenetic enzymes is determined by the levels of cofactors that they require to fulfill their function. For example, histone acetylation by HATs is impaired if the production of acetyl-CoA by PDC or ACS is diminished [42,43]. As such, alterations in cellular metabolism and metabolite levels could be translated into macrophage phenotype, with epigenetic enzymes as the interpreters.

So modifying metabolism, possibly combined with epigenetic targeting, could be a novel way to alter histone modifications and thereby to manage macrophage function and disease.

Diet can affect metabolite and cofactor availability for epigenetic enzymes, and thus potentially also macrophage activation and disease development. For example, SAM that is used for DNA and histone methylation is derived from dietary methyl intake, with folate, vitamin B and choline being the most important methyl suppliers. Accordingly, dietary folate intake affects epigenetics in both pregnancy and adulthood [61].

In addition, pharmaceutical interventions that directly alter cellular metabolism and affect metabolite availability for epigenetic enzymes could be a promising tool to modulate epigenetics and macrophage phenotype. For example, inhibiting glycolysis or stimulating mitochondrial metabolism could reduce the formation of the HDAC inhibitor lactate. It is noteworthy that targeting tumor cell glycolysis is a widely studied approach for therapeutic intervention [62]. How and if such metabolic interventions

could also be applied to improve macrophage function and whether their putative effects are partially mediated via epigenetic reprogramming needs to be researched.

Future perspective

Before the modulation of epigenetic enzymes through metabolic interventions can be used to improve macrophage function and disease outcome, numerous questions have to be solved. First, it has to be elucidated which particular enzymes are important for the generation of metabolites that are used by epigenetic enzymes as cofactors. Indeed, while it is known that ACS is important for macrophage function [63], it remains unstudied whether it indeed affects epigenetics. Changes in the other acetyl-CoA-generating enzymes PDC and ACL were linked to epigenetic alterations, but their role in macrophages remains speculative. If such enzymes are indeed expressed and regulated in macrophages, it needs to be demonstrated that they indeed alter nuclear metabolite levels. Even more importantly, one has to demonstrate that metabolite availability is indeed limiting and determines epigenetics in macrophages as shown earlier in other cell types [37,41]. Provided that the proposed metabolic–epigenetic crosstalk indeed regulates macrophage function, strategies need to be developed to target this axis specifically in macrophages. Since most of the described metabolites and enzymes are involved in general cellular housekeeping functions, mechanisms need to be identified that are solely important in particular macrophage activation cues. Although several issues need to be elucidated, directing metabolism to modulate the epigenetic landscape and macrophage phenotype could be a promising therapeutic strategy for the management macrophage-related diseases such as atherosclerosis, rheumatoid arthritis and systemic lupus erythematosus.

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Executive summary

- Epigenetic enzymes are important modulators of macrophage function and influence disease outcome.
- Epigenetics is tightly connected with the cellular metabolism since numerous metabolites are required as substrates or cofactors for epigenetic enzymes.
- Macrophage activation leads to shifts in diverse cellular metabolic pathways.
- The metabolites succinate, lactate, reactive oxygen species and NO regulate macrophage function.
- Epigenetic enzymes could translate metabolic changes into macrophage phenotype.
- Metabolic interventions, possibly via the modulation of the epigenetic landscape, could be a novel therapeutic target for the treatment of macrophage-associated inflammatory diseases.

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