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To target or not to target? The role of DNA and histone methylation in bacterial infections.

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Abstract

Epigenetics describes chemical modifications of the genome that do not alter DNA sequence but participate in the regulation of gene expression and cellular processes such as proliferation, division and differentiation of eukaryotic cell. Disruption of the epigenome pattern in a human cell is associated with different diseases, including infectious diseases. During infection pathogens induce epigenetic modifications in the host cell. This can occur by controlling expression of genes involved in immune response. That enables bacterial survival and replication within the host and evasion of the immune response. Methylation is an example of epigenetic modification that occurs on DNA and histones. Reasoning that DNA and histone methylation of human host cells plays a crucial role during pathogenesis, these modifications are promising targets for the development of alternative treatment strategies in infectious diseases. Here, we discuss the role of DNA and histone methyltransferases in human host cell upon bacterial infections. We further hypothesize that compounds targeting methyltransferases

are tools to study epigenetics in in the context of host-pathogen interactions and can open new avenues for the treatment of bacterial infections.

Keywords: bacterial infection, DNA and histone methylation, chemical targeting

Introduction

Bacterial infections affect DNA and histone methylation pattern in the host as a strategy for bacteria to improve pathogenesis and escape the immune response to infection. This review focuses on DNA and histone methylation in human cells in response to bacterial infections. In particular, it underlines the impact of the bacterial infection through methylation of the host genome on its immune response. Finally, we discuss that epidrugs that target these aberrant DNA and histone methylation patterns in host cells can constitute an alternative therapy to fight infectious diseases.

DNA and histone methylation in mammals

The DNA double helix molecule encodes the genetic information for the function, growth, development and reproduction of an organism [1]. A human genome consist of more than 25,000 genes and a diploid genome is around 6,400 mega base pairs (Mbp) in size packed into a nucleus of a cell of about 10 microns in diameter [2, 3]. To fit into the nuclei, the DNA is highly compacted. The first level of this compaction consists of the nucleosomes, a sequence of 147 bp of DNA wrapped around a histone octamer (eight histone proteins consisting of two copies of the dimer H2A- H2B and two copies of the dimer H3- H4) [4] (**Fig. 1**). Histone proteins are abundant in positively charged arginine and lysine amino acids that interacts with negatively charged DNA. This tight interaction enables dense packaging of the DNA molecule into the cell nuclei. The nucleosome is the basic unit of chromatin that is dynamic and may

form open (euchromatin) and condensed structure (heterochromatin) [5]. Transcription factors may bind to promoter regions and enhancers in euchromatin but not in the heterochromatin state, in which transcription is inactive.

Epigenetics describes modifications in the genome that control gene expression by modulating chromatin compaction without altering the DNA sequence. The main epigenetic modifiers are *writers*, *readers* and *erasers*, proteins that add, bind to and remove the epigenetic modifications [6]. In mammals, widely studied chemical modifications are methylation of DNA and methylation, acetylation, ubiquitination, phosphorylation and crotonylation of histones. The main function of epigenetic modification is regulation of gene expression. An epigenetic modification impacts chromatin structure and the accessibility of the transcription factors and enhancers to bind to DNA as shown by novel state-of-the-art sequencing techniques [7, 8]. Moreover, epigenetic modifications, also named marks, play an important role in the formation of protein complexes and machineries during replication and transcription [9].

DNA methylation is implicated in chromatin remodeling [10] and its effect on gene transcription depends on the DNA region that is methylated. DNA methylation most commonly correlates with repression of transcription when occurs in promoters [11], while activation of tissue-specific gene transcription is observed for example in gene bodies [12]. Histone methylation occurs on arginine (R) and lysine (K) residues, mostly on histone tails [13]. The effect of histone methylation on transcription depends on the residue and the methylation state mono-, di-, trimethylation for lysines (abbreviated as Kme1, Kme2, Kme3) and mono- and symmetric or asymmetric dimethylation for arginines (Rme1, Rme2s, Rme2a) [14, 15]. Selected methylation marks are presented in **Table 1** with examples of the corresponding methyltransferases and the effect of the methylation on transcription (**Table 1**). Depending on

which part of the genome the modification occurs and the level of methylation, it can induce gene repression or activation as cited in **Table 1**.

Table 1. DNA and histone modifications and methyltransferases in mammals

Modification	Methyltransferase	Transcription regulation	References
DNA 5mCpG	DNMT1, DNMT3A, DNMT3B	repression/activation	[16-18]
H3K79me1/2/3	DOT1L	activation	[19]
H3K36me2/3	NSD1, NSD2	activation/repression	[20]
H3K36me3	SETD2	activation	[21]
H3K27me1/2/3	G9a/GLP, EZH1, EZH2	repression	[22, 23]
H3K9me1	PRDM3, PRDM16, G9a/GLP	activation	[24, 25]
H3K9me2/3	G9a/GLP, SUV39H1, SUV39H2, SETDB1, SETDB2	repression	[25-28]
H3K4me1/2/3	SET1A, SET1B, MLL1, MLL2, MLL3, MLL4, MLL5, ASH1L, SMYD3	activation	[29-32]
H3R17me1/2	CARM1	activation	[33]
H3R2me1	PRMT1, PRMT3, PRMT5, PRMT6, PRMT7, CARM1	activation	[34]
H3R2me2a	PRMT1, PRMT3, PRMT6	repression	[35-37]
H3R2me2s	PRMT5, PRMT7	activation	[38]
H4K20me1	SET8	activation	[39]
H4K20me3	SUV420H1, SUV420H2	repression	[40-42]

Mechanism of DNA and histone methylation

The methylation mechanism is based on the transfer of a methyl group from the donor (the co-factor *S*-adenosyl-L-methionine, SAM) to the substrate (the cytosine in DNA or arginine

or lysine of histones) with release of *S*-adenosyl-L-homocysteine (SAH) as a byproduct [43] (**Fig. 1**). The reaction is catalyzed by the DNA methyltransferases DNMT or the histone methyltransferases (HMTs), the *writer* proteins. The histone lysine or arginine residues' methylation reaction is catalyzed by lysine methyltransferases (KMTs) or protein arginine methyltransferases (PRMTs), respectively [13, 44]. The majority of the KMTs bear the SET domain and therefore are grouped into SET-domain protein family. The SET-domain is a conserved sequence motif that forms a catalytic active site for lysine methylation [45]. The exception is the DOT1L family that does not have SET-domain but another conservative sequence motif [46]. Whereas, PRMTs are distinguished by a conserved catalytic core region of about 310 amino acids [47].

In mammals, DNA methylation occurs mostly on position 5 of cytosine (5meC) at CpG sites that are regrouped in CpG islands (CpG-rich regions) (**Fig.1**). There are three different DNA methyltransferases: DNMT3A, DNMT3B, and DNMT1. DNMT3A and DNMT3B catalyze *de novo* DNA methylation and are active during early stages of the development in embryogenesis and gametogenesis [43]. Whereas, DNMT1 maintains methylation marks during cell division.

The methylation reaction is reversed by enzymes called demethylases, the *erasers* [44, 48]. The DNA demethylation process undergoes several oxidation steps catalyzed by the TET enzymes (Ten-Eleven-Translocation dioxygenases) [49, 50]. Whereas, histone methylation is reversed by histone demethylases. The proteins called *readers* recognize and bind to the methyl groups (on DNA or histones) [51, 52]. Many corresponding *reader* proteins are described to recruit protein complexes during transcription and triggering signaling cascades involved in gene regulation [53-55]. The cooperation of writers, erasers and readers ameliorates the dynamics and complexity of the process of gene expression.

Epigenetics plays an important role in regulation of cellular processes and cell functions. Consequently, disruption of the methylation pattern is found in many pathologies including cancer, neurodegenerative diseases, aging and infectious diseases [56].

In the following part we will focus on the role of methylation in bacterial infectious diseases.

Role of host DNA and histone methylation in infectious diseases

In bacteria, DNA methylation marks include N⁶-methyladenine, N⁴-methylcytosine and 5-methylcytosine (for review [57]), the most common modification being N⁶-methyladenine involved in the defense against pathogens, mostly bacteriophages [58]. Bacteria distinguish exogenic DNA from its own by restriction enzymes (endonucleases) that recognize specific unmethylated sequences of foreign nucleic acids. DNA adenine methyltransferase (DAM) and its homologs are present in various bacteria [58]. DAM methyltransferase is also present in some of the bacteriophages, for example *Escherichia* phage P1 (Bacteriophage P1) [59]. Although, there is no histone methylation in bacteria there are histone-like proteins that can be post-translationally modified [60]. Here we focus on DNA and histone methylation induced by the bacteria in human cells.

Epigenetic modifications including DNA and histone methylation are implicated in various mechanisms that increase pathogenicity, virulence and support immune response evasion during infection [61]. Bacteria can modify the host epigenome either by secretion of effector proteins that mimic host methyltransferases or by hijacking and regulating functions of host methyltransferases through production of microbial molecules, induction of the changes in the microenvironment and metabolic switch (**Fig.2**). The epigenetic regulation of the host

genes is clinically relevant to understand mechanisms of infection and opens to novel targets for treating bacterial infections.

How bacterial infection modulates the epigenome?

Bacteria modulate the host epigenome by (1) either directly inducing methylation changes in the host epigenome through effector proteins that catalyze the methylation or (2) by hijacking the host methyltransferases (**Fig.2**). The two mechanisms are described through examples.

1. Bacterial effector proteins induce epigenetic methylation changes in the host

As a result of evolutionary mimicry, bacteria secrete effector proteins that resemble methyltransferases [61, 62]. Nucleomodulins are bacterial effector proteins that are secreted into the host cell upon infection and translocate into the nucleus through nuclear localization signal (NLS). In the nucleus, nucleomodulins modify epigenetic pattern of the host genome, alter microenvironment and activate or inhibit signaling pathways implicated in the regulation of gene expression [61]. For instance, bacteria can target the NF- κ B signaling pathway with observed decrease in pro-inflammatory cytokines (e.g. IL-6 TNF- α) resulting in repression of genes associated with immune response [63]. Therefore, bacteria evade the immune response, escape clearance from the cell and persist within the host cell to proliferate and survive during infection through epigenetic reprogramming of host-pathogen interactions [64]. Nucleomodulins are secreted by a variety of bacteria, both Gram-positive and Gram-negative, both extracellular and intracellular pathogens, including *Legionella pneumophila*, *Burkholderia thailandensis*, *Chlamydia trachomatis*, *Chlamydothila pneumoniae*, *Bacillus anthracis*,

Mycobacterium tuberculosis, *Mycoplasma hyorhinis*, *Klebsiella pneumoniae*, *Salmonella enterica* and archaea *Methanosarcina mazei*. Bacterial methyltransferases target DNA regions of different genes, histones (H1, H3, H4) and non-histone proteins. **Table 2** gathers selected effector proteins that modulate the host epigenome and regulate gene expression in favour of an efficient pathogenesis, therefore playing an important role during infection.

Table 2. Examples of bacterial effector proteins that methylate host substrates

Pathogen	Bacterial effector protein	Mammalian methylation target	Impact on the host	Reference
<i>L. pneumophila</i>	RomA/Leg AS4	H3K14, non-histone proteins	Repress genes involved in innate immune response (e.g. <i>IL6</i> , <i>TNFA</i>)	[63, 65, 66]
<i>B. thailandensis</i>	BtSET	H3K4 methylation in rDNA	Induction of rDNA transcription and promotion of bacterial intracellular replication	[66]
<i>C. trachomatis</i>	Nue	H2B, H3 and H4	Chromatin remodeling	[67]
<i>B. anthracis</i>	BaSET	H1	Repression of the NF- κ B pathway	[68]
<i>M. tuberculosis</i>	Rv1988	H3R42	Repression of genes involved in host defense	[69]
<i>M. hyorhinis</i>	CG- and GATC-specific DNMT	CG- and GATC-specific DNA	Activation of oncogenes and promotion of cell proliferation	[70]
<i>K. pneumoniae</i>	HsdM	DNA methylation <i>in vitro</i>	Unknown	[71]
<i>S. enterica</i> Typhi	upregulation of KDM6B	Decrease of H3K27me3	activation of <i>DAAMI</i> , <i>PPARδ</i> , <i>CSNK1D</i>	[72]
<i>M. mazei</i>	Gö1-SET	MC1- α at lysine 37	Unknown	[73]

Human gastrointestinal tract is an environmental niche for both commensal and pathogenic bacteria [74]. A Gram-negative bacteria *Salmonella enterica* serotype Typhi (*S. typhi*) is a pathogen found in human gut microbiota when infected food is ingested. Upon infection, the *S. typhi* modifies the epigenome of human gut cells to evade immune response and support chronic infection [72, 75]. As shown in **Table 2**, *S. typhi* reprograms host-pathogen

interactions through the functional *Salmonella Pathogenicity Island I (SPII) loci* that leads to upregulation of histone demethylase KDM6B and abolishment of H3K27me3 in human colonic epithelial cells and *in vivo* [72]. Consequently, decrease in H3K27me3 activates expression of genes through WNT signaling pathway upregulating *DAAMI*, *PPAR δ* , *CSNK1D*, involved in cytoskeletal reorganization, fatty acid oxidation and casein phosphorylation. The epigenetic changes induced by *S. typhi* shifts polarization of macrophages into M2 macrophages to evade immune response and survive within the host cell. These changes are cell-type-specific [75].

2. Bacteria hijack methyltransferases of the host

In parallel, certain components of the bacteria and produced molecules can also affect the action of the host methyltransferase and induce methylation changes that favor the bacteria infection. These components are either recognized by host receptors that trigger signaling pathways or are delivered to the host cells.

2.1. Bacterial structures and molecules are recognized by the receptors on the host cells

During infection, bacteria secrete several molecules and structures, among them virulence factors such as toxins, that can induce epigenetic modifications in the host (**Fig.3**). These are recognized by the host cell through receptor recognition. The receptor-dependent host-pathogen recognition activates intracellular signaling in the infected cell that results in hijacking of the host methyltransferase and ultimately triggers modifications of the host epigenome.

Toxin from *Bacteroides fragilis* (called Enterotoxigenic *Bacteroides fragilis* (ETBF)-secreted *Bacteroides fragilis* toxin (BFT)), is linked to the development and progression of colorectal cancer caused by epigenetic changes in the host [76]. Based on the transcriptomic and methylome data combined with chromatin accessibility assays, the authors

observed dynamic changes in chromatin accessibility and DNA hypomethylation of promoter regions of several transcription factors, genes involved in bacterial pathogenesis (e.g. *CEACAM6*, *MUC2*) and development and progression of colorectal cancer (e.g. *FOSL1*, *JUN* and *JDP*) [76]. Infection by *Helicobacter pylori* (*H. pylori*) is linked to development and progression of gastric cancer. *H. pylori*-infected cells show hypermethylation in the promoter regions of various transcription factors, including upstream stimulatory factors 1 (*USF1*) and *USF2*, involved in gene regulation related to immune response, cell cycle and cell proliferation, consequently leading to downregulation of *USF1* and *USF2* [77-79]. The treatment with 5'-azacytidine (5azaC), inhibitor of DNMT, abrogated hypomethylation and re-established expression of *USF1* and *USF2* *in vivo* [80]. Moreover, *H. pylori* inhibits telomerase activity by hypermethylation of the promoter of Telomerase Reverse Transcriptase (*TERT*) in *TERT* human gastric epithelial cells and murine gastric mucosa in infected mice. The expression of *TERT* is recovered upon treatment with 5azaC *in vivo* [49].

Some bacterial structures and molecules are pathogen-associated molecular patterns (PAMPs) that are recognized by receptors, e.g., toll-like receptors (TLR) and NOD-like receptors (NLR) on the host cell [81]. The PAMPs-host cell receptor recognition can activate a cascade of signaling pathways, release of cytokines and metabolites that change the microenvironment of the infected cell, shifts metabolism, induces oxidative stress and redirects cell to necrosis through changes in the methylation pattern of the host cell. Ultimately, these changes suppress immune response to infection by evading clearance of the bacteria from the cell and allow bacterial replication and survival within the host [81]. Pneumolysin is a virulence factor released by *Streptococcus pneumoniae* recognized by the Toll-like receptor 4 (TLR4) on the host cell and triggers immune response via nuclear factor- κ B (NF- κ B) signaling pathway through epigenetic regulation [82]. Cole *et al* studied the epigenome of the human monocyte derived macrophages (MDM) exposed to pneumolysin during *S. pneumoniae* infection. The

study evaluated the relative abundance of histone marks in MDM cells challenged with *S. pneumoniae* expressing pneumolysin compared to *S. pneumoniae* mutants not expressing pneumolysin showing an increase in the relative abundance of histone marks H3K4me1, H4K16ac and a decrease in H3K9me2 and H3K79me2 in MDM challenged with pneumolysin. These changes in histone marks confirm the manipulation of the host epigenome by bacteria.

The main component of Gram-negative bacterial cell wall is lipopolysaccharide (LPS). It has been shown that LPS can stimulate epigenetic changes in many cell types, including macrophages of central nervous system, monocytes, keratinocytes, fibroblasts and endothelial cells. During infection, LPS binds to TLR4 on the host cell triggering a cascade of signaling pathways that activate transcription factors such as NF- κ B and interferon-regulatory factors (IRFs) implicated in the expression of genes coding for pro-inflammatory cytokines and chemokines such as cyclooxygenase-2 (COX-2), interleukin-6 (IL-6), IL-1 β , tumor necrosis factor- α (TNF- α), and IL-12. It also activates phosphatidylinositol 3- kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK) signaling pathways [83]. During activation of NF- κ B signaling pathway, the repressive mark H4K20me3 is decreased, which represses expression of TLR4 in macrophages important for recognition of LPS during infection-activated immune response [84]. Stimulation of macrophages with LPS activates TLR-mediated signaling and regulates expression of DNA and histone methyltransferases. Mouse macrophages of central nervous system stimulated with LPS induced expression of IRF1, IRF7, and IRF9 and upregulation of DNMT3L, histone methyltransferase SETDB2, and histone demethylases KDM4A through TLR4 activation [85]. This suggests that epigenetic regulation is involved during immune response to LPS stimulation in the mouse-derived macrophage.

Other examples of the link between LPS and the epigenetic modulators comes from bacteria *Porphyromonas gingivalis* involved in *P. gingivalis*-associated periodontitis.

Stimulation of human HaCaT keratinocytes with LPS from *P. gingivalis* resulted in the downregulation of DNMT1 and DNMT3a and H3K27me3 demethylase JMJD3 [86]. In fibroblasts derived from human periodontal ligament (HPDL), *P. gingivalis* LPS-stimulation increased DNMT1 expression with, in particular, DNA hypermethylation of RUNT-related transcription factor 2 (RUNX2), involved in cell cycle regulation. Treatment with DNMT inhibitor, 5-aza-2'-deoxycytidine (5azadC) reversed DNA hypermethylation and diminished DNMT1 expression.

Stimulation of macrophages with LPS from *E. coli* caused the increase in H3K4me3, which activated the NF- κ B signaling pathway mediated and expression of pro-inflammatory genes Nos2 and IL6 [87]. LPS triggered the expression of enzymes involved in methylation such as PRMT4 (CARM1), PRMT6, and SET7/ 9 in kidney cells. These methyltransferases deregulated global histone arginine and lysine methylation as well as DNA methylation and induced pro-inflammatory response [88].

2.2. Microvesicles deliver bacterial structures and molecules to the host

The bacterial structures and molecules can also be delivered into the infected cell via microvesicles (MV), small particles of around 50-100 nanometer in diameter shredded from the layers of outer surface of the bacterial cell. MV carries different components of the bacterial cell including components of the periplasm (LPS, peptidoglycan, phospholipids) as well as molecules found in the cytoplasm (nucleic acids, proteins, enzymes, metabolites and toxins) [89]. In *L. pneumophila* infection, bacterial MV are carriers of miRNAs that target host immune genes [90]. The extracellular particles play an important role in delivery and transport of different compounds and are implicated in cell signaling. For instance, Vdovikova *et al* showed that MV isolated from pathogenic bacteria *Vibrio cholerae* and non-pathogenic bacteria *E. coli* induced an increase in H3K4me3 at transcription start sites (TSS) region changing global gene

expression of 465 and 738 genes in colorectal cancer cells, respectively [91]. Only MVs from the pathogen *V. cholerae* activated genes associated with endothelial differentiation, contributing to cancer development and progression. Another study on the role of MV in infection investigated the effect of pathogen *P. aeruginosa* on lung macrophages isolated from patients diagnosed with cystic fibrosis (CF). Armstrong *et al* observed a decrease in DNA methylation of the *CFB* gene proposing an epigenetic mechanism of MHC suppression in infected macrophages of CF patients [92]. The DNA hypomethylation induced the suppression of eleven different MHC class II molecules that are important for antigen presentation during infection and activation of immune response. Simultaneously, the study showed a disrupted microenvironment in the infected macrophages, with an increase in pro-inflammatory molecules (IL1 β , IL8, CXCL1), anti-inflammatory genes (*IL6* and *IL10*), leukocytes (both T and B cells) and chemoattractants (CCL18 and CCL23). Moreover, MV from *P. aeruginosa* triggered alterations in DNA methylation affecting immune response in human lung macrophages. Consequently, bacterial MV induced DNA hypomethylation in enhancer, DNase hypersensitive regions and gene body regions including *NFKB1*, *CREB5*, *BCL2*, *IL1B* and *IL6*. These observations suggest the involvement of DNA methylation in regulation of NF- κ β pathway during innate immune response [93].

Another example of bacterial MVs affecting epigenetic pattern in the host are MVs from *Bacteroides thetaiotaomicron*. Murine bone marrow derived monocytes treated with MVs from *B. thetaiotaomicron* showed increased levels of increase levels of H3K4me1, together with IL-10 and decreased levels of TNF- α [94]. Further studies are summarized in [95, 96].

Bacterial infection induces epigenetic modifications implicated in trained immunity

The epigenetic modifications have an impact on the fate of the immune cells to become either effector or memory T cell. The changes in the methylation pattern induced by bacterial infection regulate genes involved in the development of trained immunity. For instance, an *in vivo* study of Laval *et al* showed that hematopoietic stem cells (HSCs) derived from mice exposed to LPS from *P. aeruginosa* induced long-term epigenetic alteration of chromatin accessibility in the C/EBP β pathway, induced by direct LPS-TLR4 recognition [97]. Moorlag *et al* showed that mice exposed to β -glucan showed an increase in H3K27ac, K3K4me3 in the *IL1* genes and a decrease in H3K9me3 at *IL1B* gene, when re-exposed to *Mycobacterium tuberculosis* (*M. tuberculosis*) [98]. Monocytes exposed to bacillus Calmette-Guérin (BCG) vaccine against tuberculosis are reprogrammed to trained immunity response via IL-1 β -induced epigenetic regulation in promoter regions of *TNFA*, *IL6* and *IL1B* caused by the increase of H3K4me3 and a decrease in H3K9me3 [99].

Moreover, murine Natural killer (NK) cells stimulated with LPS for 13 days and re-exposed to LPS on day 14 differentiated into memory NK cells *in vivo*. This was mediated by an increment in H3K4me1 within enhancers at the *ifng* locus and increase in INF- γ in murine memory-like NK cells [100]. The treatment with sinefungin, a non-specific inhibitor of methyltransferase, reversed methylation of H3K4me1 and disabled differentiation of NK cells into memory cells [100, 101]. Thus, histone methylation is involved in the immune response to bacterial infection.

The abovementioned observations emphasize the importance of understanding the mechanisms of epigenetic modifications in infection, as it could open to the discovery of novel strategies in treatment of infectious diseases. Thus, chemical inhibitors of these modifications

could be useful tools to investigate the epigenetic modifications in host cells upon bacterial infections and, depending on their properties, become the starting point for the development of novel drugs.

Perspectives of chemically targeting Epigenetics in bacterial infections

As described above, bacteria induce DNA and histone methylation modifications upon infection [102]. These chemical modifications of DNA and histone are reversible and chemical probes and drugs have been designed to inhibit these modifications in cancer cells. However, the chemical targeting of the methylation induced by bacteria infection in host cells is still little studied. Due to extensive and improper use of antibiotics and consecutive increase in antibiotic resistance, there is an emerging need for alternative therapies to detain growing antimicrobial resistance (AMR) [103]. Thus, it is of importance to explore alternative therapeutic strategies. The epigenetic alterations induced in the host cells during host-pathogen interactions could be a potential novel target. Specific chemical inhibitors could contribute to elucidate the role of these epigenetic changes and their potential as antibiotics target. **Figure 4** depicts a proposed strategy of chemical targeting of DNMTs and HMTs in bacterial infections (**Fig.4**).

Epigenetic modifications are reversible and thus constitute a potential target. While very little has been explored for bacterial infection, there are several Food and Drug Administration (FDA)-approved epidrugs in the field of cancer [104]. Two drugs are DNA hypomethylation agents, Vidaza® (Azacytidine, 5azaC) and Dacogen® (Decitabine, 5azadC). Both have been approved for the treatment of Myelodysplastic Syndrome (MDS), chronic myelomonocytic leukemia (CMML) and acute myeloid leukemia (AML) [16, 105, 106]. Tazverik® (tazemetostat) targets histone methyltransferase EZH2 (responsible for H3K27me2/3) and has

been approved for the treatment of epithelioid sarcoma and follicular lymphoma and solid tumours [107-110]. The DOT1L inhibitor named Pinometostat (Epz-5676) was tested in Phase I/ II clinical trials for treatment of MLLr leukaemia (clinical trial reference number NCT03701295 and NCT03724084 at <https://clinicaltrials.gov>), however withdrawn due to poor efficacy. Two PRMT5 inhibitors (GSK3326595 and JNJ-64619178) inhibit the growth of melanoma tumors in murine model and are currently being investigated as the treatment of solid tumors, Non-Hodgkin's lymphoma and breast cancer [111-113]. The latter are bisubstrate inhibitors that consist of a chemical moiety mimicking the SAM and one mimicking the substrate linked together with a linker aiming to increase the specificity of the inhibitors [114].

Only one clinical trial study investigating the effect of 5azaC in treatment of pneumonia (clinical trial reference number NCT03941496 at <https://clinicaltrials.gov>).

Reasoning that upon infection aberrant methylation triggers expression of pro-inflammatory chemokines and cytokines, these aberrant patterns are potential target to reverse inflammation. Indeed, chemical inhibition of methylation diminished release of pro-inflammatory molecules during infection [100]. Zambuzi *et al* showed a decrease in inflammatory cytokines (IL-1 β , TNF- α , and IFN- γ) upon treatment with decitabine of mononuclear cells infected with *M. tuberculosis* [115], increasing bacterial phagocytosis and abrogating control of infection. Moreover, treatment with decitabine induced macrophage polarization towards M2 macrophage phenotype. Interestingly, treatment with hypomethylating agent increased bacterial phagocytosis and abrogated control of infection in monocytes [115]. These data support the role of bacterial infections in regulation of inflammation and further studies are needed.

Pathogens also induce changes in the cell environment and immunometabolism that affect the function and enzymatic activity of some methyltransferases. The manipulation of the cell environment may indirectly change the epigenome of the host.

The complexity of the effect of the methylation on cell functions and its role in infection emphasized the importance of studying the epigenetic regulation in host-pathogen interaction.

Challenges in targeting methyltransferases

The use of epigenetic inhibitors could benefit to the understanding of the molecular mechanism in epigenetic changes upon infection and their development could constitute the starting point for new treatment of infectious diseases. There are several open challenges. The specificity is one. On one hand, all methyltransferases share the same co-factor, the SAM and the catalytic domains are structurally similar (as for example the SET domain of most KMTs) [45]. Interestingly, bacterial effectors can induce directly methylation patterns that are not common in host cells, as H3K14 methylation by *L. pneumophila* [63]. Thus, it is important to design molecules that target specifically bacterial effector proteins without inhibiting host methyltransferases. This could be achieved with bisubstrate inhibitors [114]. On the other hand, all epigenetic methyltransferases are involved in global effects on methylation and are part of protein complexes, interacting with other proteins and epigenetic modifications. Furthermore, targeting epigenetic modifiers should be performed carefully and with consideration of side effects. A recent study of Marcos-Villar *et al* showed that treatment of lung epithelial cells infected with influenza virus with Pinometostat resulted in repression of genes involved in NF- κ B signaling pathway (such as RUBICON and TRIM25), consequently leading to diminished immune response important during infection [116]. These results support further evaluation of the effect of compounds targeting methyltransferases on the expression of genes involved in innate and adaptive immune response in infectious diseases

These are some of the challenges that need to be considered to design efficient inhibitors targeting the epigenetic regulation in bacterial infections.

Conclusions

In summary, this review emphasizes the important role of DNA and histone methyltransferases in infectious diseases. Bacteria induce changes in DNA and histone methylation pattern in the host by secretion of bacterial effector proteins or hijacking host methyltransferases through bacterial structures and molecules stimuli. Modifications in methylation affect signaling pathways, transcription factors, chromatin structure, host receptors and microRNAs. This results in altered gene expression involved in immune response, which enables evasion of immune response, bacterial survival and replication within the host cell (**Fig.4**). Ultimately, these epigenetic changes contribute to efficient infection. Current studies on compounds that target methyltransferases show clinical relevance in the treatment of diseases where epigenetic patterns are disrupted, for instance in cancer. The studies on epigenetics and inhibitors of methyltransferases in infection could bring a better understanding of the molecular mechanisms of infection. Specific chemical inhibitors could help address these questions. This could contribute to identification of novel treatment strategies that ultimately diminish the use of antibiotics and help to tackle growing antimicrobial resistance. The field is still little explored and more studies are necessary to study the potential and real effect of epidrugs on infection.

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Data availability statement

No data was collected for the review, only published and available data were used.

Comment

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Author contributions

MB conceptualization, writing, creating the figures and tables, PBA conceptualization, corrections and finalization of the manuscript.

Abbreviations

5-aza, 5-azacytidine

5-azadC, 5-aza-2'-deoxycytidine

5mC, 5-methylcytosine

DNA, deoxyribonucleic acid

DNMT, DNA methyltransferase

H3, histone 3

H4, histone 4

IL, interleukin

K, lysine

KMT, lysine methyltransferase

LPS, lipopolisaccharide

LTA, lipoteichoic acid

MBD, methyl-binding protein

me1, monomethylation

me2, dimethylation

me3, trimethylation

NLS, nucleus localization signal

NF- κ B, nuclear factor κ B

PAMP, pathogen associated molecular patterns

PGN, peptidoglycan

PRMT, arginine methyltransferase

R, arginine

SAM, *S*-adenosyl-L-methionine

SAH, *S*-adenosyl-L-homocysteine

TET, ten-eleven translocation dioxygenase

TLR, Toll-like receptor

TNF, tumor necrosis factor- α

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Figures and legend

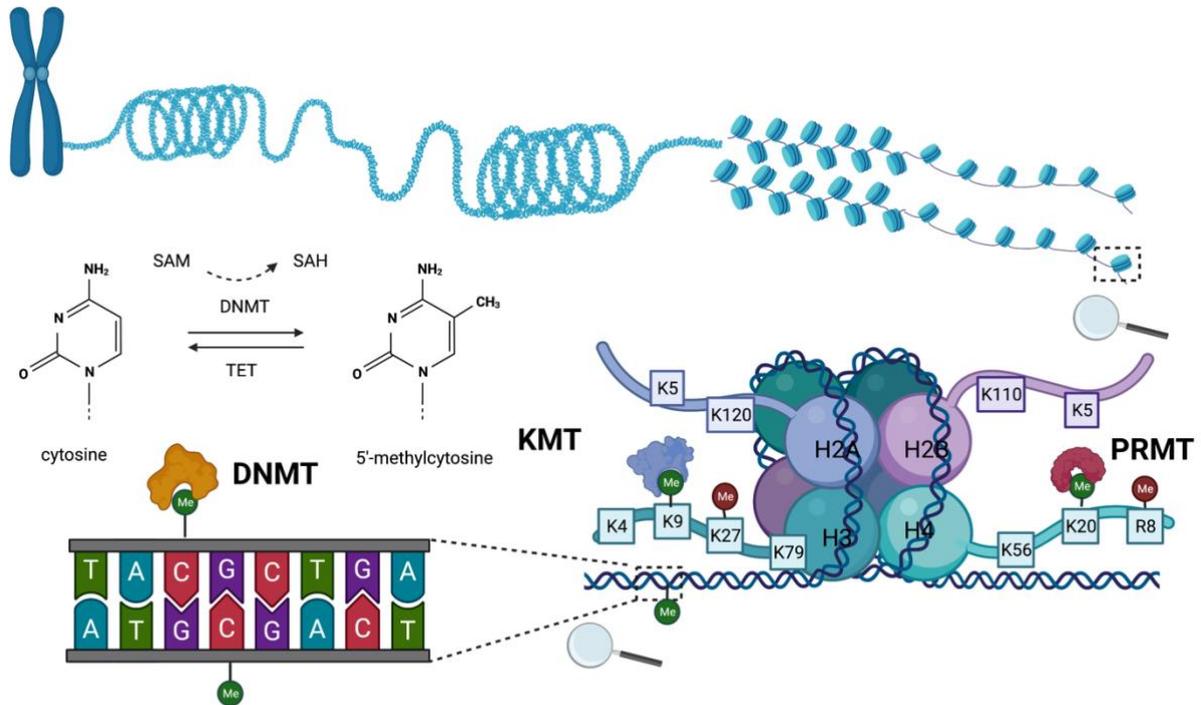


Fig.1 DNA and histone methylation in mammals. The DNA molecule is wrapped around histone octamers (eight histones consisting of dimers: H2A, H2B, H3, H4) to form the nucleosome, further compacted into chromatin fibres, the unit of chromatin that forms chromosomes. In mammals, DNA is mainly methylated at cytosine position 5 by the DNMTs (DNA methyltransferases). Whereas histones are methylated at lysine (K) and arginine (R) residues by KMTs (lysine methyltransferases) and PRMTs (protein arginine methyltransferases), respectively. The methylation reaction is catalyzed by the methyltransferases using SAM (*S*-adenosyl-L-methionine) as a cofactor and donor of methyl group and release of SAH (*S*-adenosyl-L-homocysteine) and reversed by the demethylases. Created with BioRender (biorender.com).

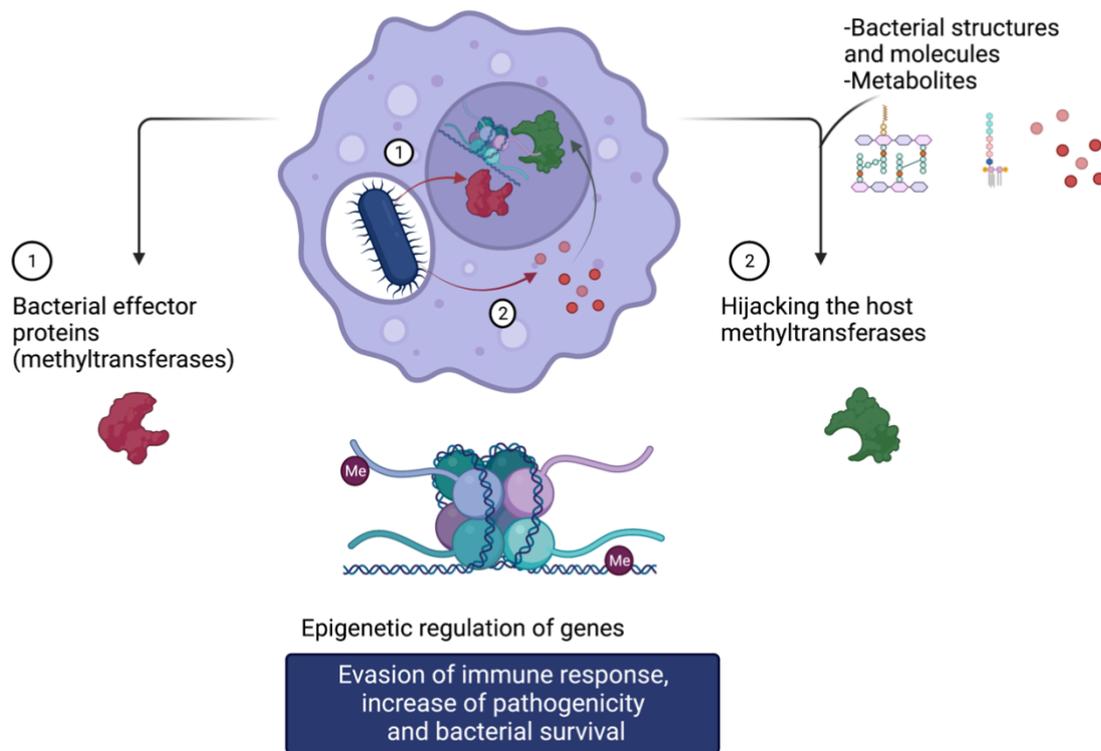


Fig.2 Bacteria infection induce epigenetic changes in the host. Bacteria modify the genome of the host through 1) release of bacterial effector proteins (such as methyltransferases-like proteins) that modify the host genome or 2) stimulation of the host cell environment through release of bacterial molecules or stimulation of signaling pathways by bacterial structures to hijack epigenetic modifiers including DNA and histone methyltransferases. Created with BioRender (biorender.com).

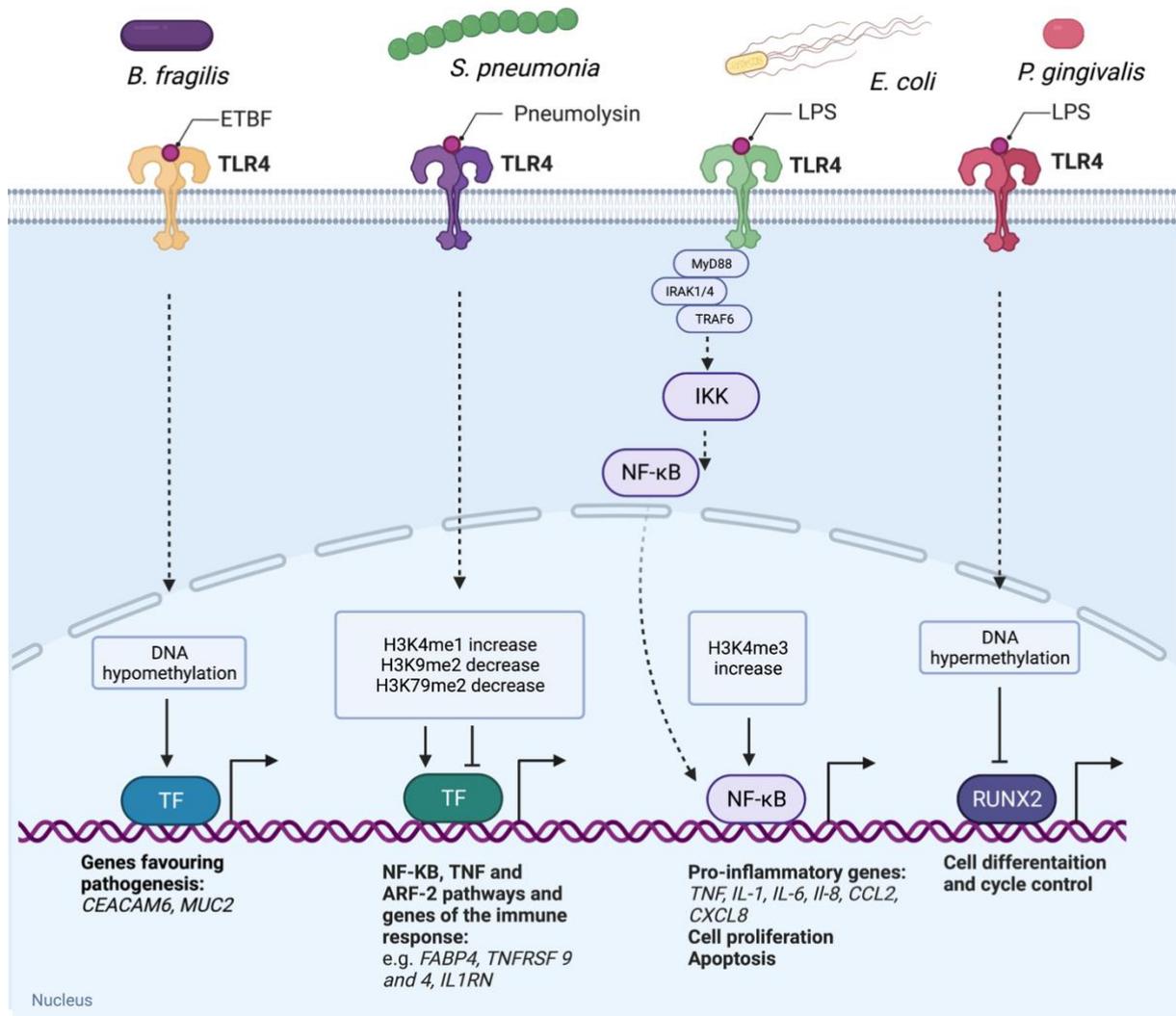


Fig.3 Bacteria regulate gene expression through epigenetic control via TLR signalling. Bacteria-host interaction activates TLR signalling that triggers gene expression signaling pathways. Epigenetic modifications (such as DNA and histone methylation) affect the binding of transcription factors (TF) to promoters. Bacteria release ligands that activate TLR4, for example *B. fragilis* (ETBF), *S. pneumoniae* (Pneumolysin), *E. coli* (LPS), *P. gingivalis* (LPS)) and introduce changes in the host epigenome. Depending on the DNA or histone mark, different transcription factors are affected, such as NF-κβ, RUNX2 and transcription factors favouring efficient pathogenesis (genes of immune response, pro-inflammatory cytokines and chemokines, adhesion molecules, cell proliferation, cell differentiation). Created with BioRender (biorender.com).

Epidrugs: targeting DNMTs and HMTs

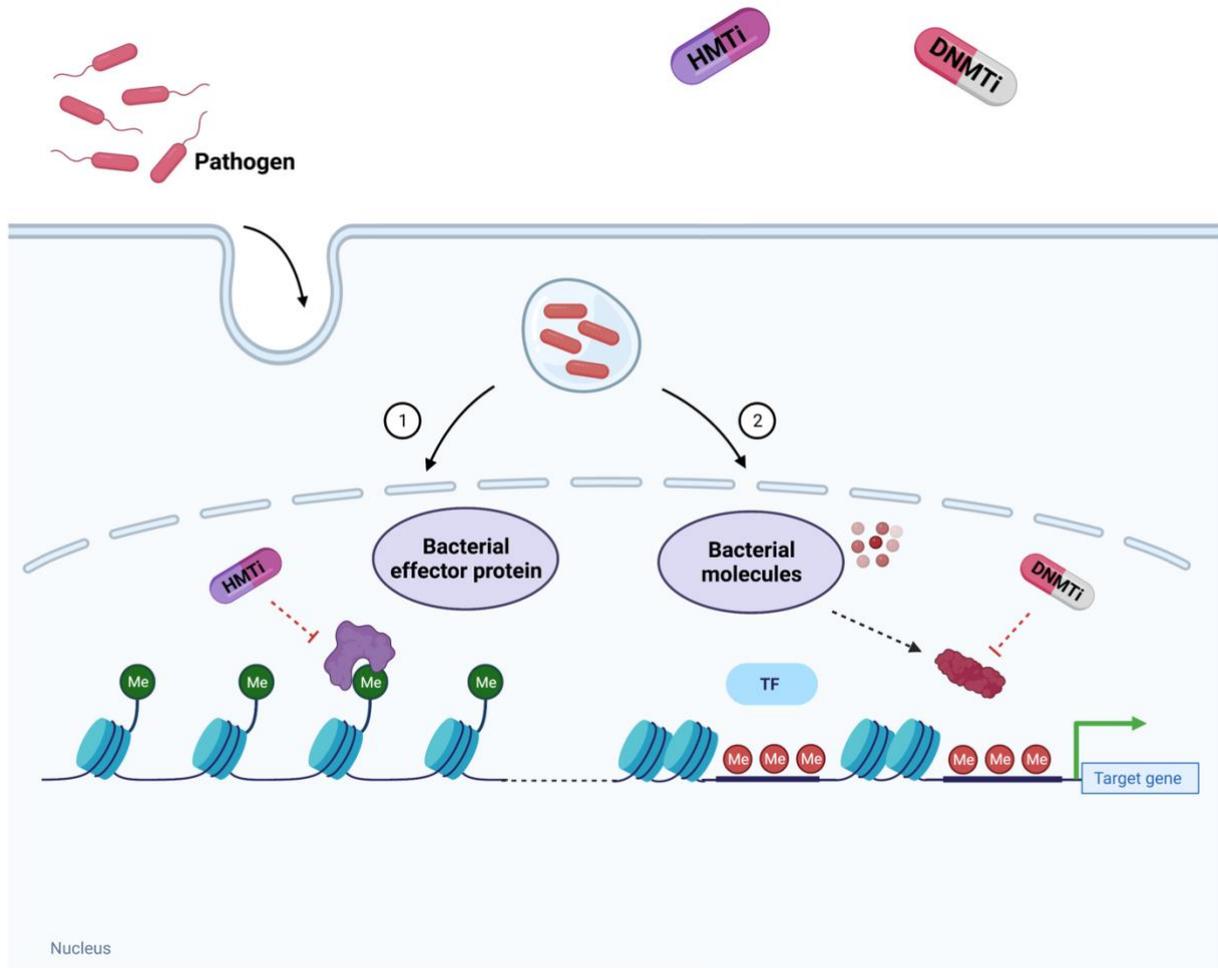


Fig.4. Potential of epidrugs in targeting methyltransferases in infection diseases. Targeting methyltransferases in infections could potentially inhibit the epigenetic modifications induced by bacteria in the host. Bacteria release effector proteins, bacterial methyltransferases, (1) or hijack host methyltransferases via release of bacterial molecules and structures (2) to modify the host epigenome. A novel compound targeting bacterial methyltransferase could constitute a novel therapeutic approach. However, this hypothesis needs to be explored. DNA methyltransferase inhibitor (DNMTi), histone methyltransferase inhibitor (HMTi), transcription factor (TF). Created with BioRender (biorender.com).