Chapter

Epigenetic Regulation Mechanisms in Viral Infections: A Special Focus on COVID-19

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Abstract

The outbreak of Coronavirus Disease-2019 (Covid-19), caused by a novel and highly pathogenic coronavirus (severe acute respiratory syndrome coronavirus-2, SARS-CoV-2), is a persisting global health concern. Research so far has successfully identified the molecular mechanisms of viral entry, alterations within the host cell upon infection, and the stimulation of an immune response to fight it. One of the most important cellular regulatory machineries within the host cell to be affected by the SARS-CoV-2 infection is epigenetic regulation, which modulates transcriptional activity by DNA sequence-independent factors such as DNA-methylation, RNA interference and histone modifications. Several studies in the literature have previously reported epigenetic alterations within the host due to infections of the Coronaviridae family viruses including SARS-CoV and MERS-CoV that antagonized immune system activation. Recent studies have also identified epigenetic dysregulation of host metabolism by SARS-CoV-2 infection, linking epigenetic mechanisms with the pathophysiology and illness severity of Covid-19. Therefore, this book chapter aims to provide a comprehensive overview of the epigenetic regulation mechanisms in viral infections with a special focus on SARS-CoV-2 infection.

Keywords: Coronavirus infection, Covid-19, epigenetic regulation, host repression, immune evasion, cytokine storm, susceptibility

1. Introduction

Coronavirus Disease-2019 (COVID-19), which is caused by a newly emerged, highly pathogenic coronavirus (severe acute respiratory syndrome coronavirus-2, SARS-CoV-2), has been one of the gravest global health concerns of the last century. Previous infections of *Coronaviridae* family, including MERS-CoV and SARS-CoV resulted in human diseases and were associated with the spread of MERS (Middle East respiratory syndrome) and SARS, respectively. SARS-CoV-2 is an enveloped, positive-sense RNA virus. It has a large genome, which consists of six major open reading frames encoding four structural proteins S (spike), E (envelope), M (membrane), N (nucleoprotein) and sixteen non-structural proteins (Nsp1–16).

Epigenetic mechanisms are vital for the regulation of transcriptional activity. Alterations within the epigenetic landscape affect gene expression via influencing chromatin accessibility rather than changing the underlying DNA sequence. Therefore, epigenetic modifications provide a reversible and flexible mechanism of directing cellular function in response to environmental stimuli. Viral infections

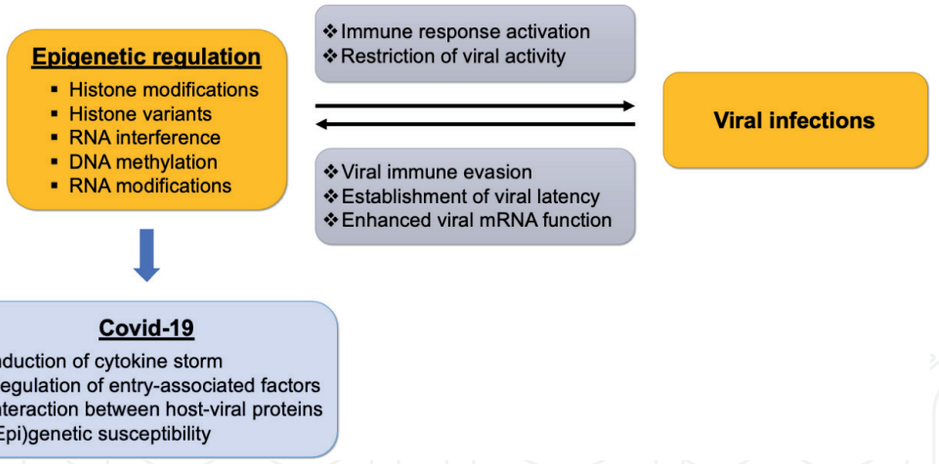

Figure 1.
Summary of the interplay between the host epigenetic regulation machinery and viral infections.

are important sources of such stimuli that cause drastic changes in the gene expression patterns of the host. While epigenetic reprogramming ensures transcriptional activation that is required for the induction of a proper immune response against viral infections, factors of the epigenetic regulation mechanisms are also hijacked by viruses to subvert the host antiviral defense machinery. This establishes a bidirectional relationship between the host cell and the virus, as depicted in **Figure 1**, controlling the viral life cycle and the dysregulation of the host gene expression [1].

Herein, we unfold the complex regulatory pathways of epigenetic mechanisms affecting the host cell and the virus. Particularly, we discuss the epigenetic basis of viral entry and cytokine storm induction in relation to SARS-CoV-2 infection, as well as epigenetic susceptibility to Covid-19 from a molecular point of view.

2. Epigenetic regulation mechanisms

Epigenetics was first introduced to the scientific community as a term to describe the molecular mechanisms that cause heritable phenotypic changes, which are independent of the genetic material [2]. Since then, regulation of DNA accessibility through chromatin condensation has been identified as the main mechanism of epigenetic regulation, implicating them in several cellular processes like cell cycle, cellular proliferation, transcriptional memory, and DNA damage repair [3]. The level of chromatin compaction in a given genomic locus determines its transcriptional activity as genes within the loosely packaged euchromatin regions are actively transcribed and the highly condensed heterochromatin regions are transcriptionally silent [4]. The interplay between euchromatin and heterochromatin enables the establishment of differential gene expression patterns and is essentially regulated by epigenetic mechanisms involving DNA methylation, non-coding RNAs and RNA interference (RNAi), DNA replication-independent incorporation of histone variants and histone post-translational modifications (**Figure 2**).

In eukaryotic cells, chromatin condensation is achieved by packaging the DNA into chromatin by wrapping the naked DNA onto octamers of core histones H2A, H2B, H3, and H4 [5]. Deposition of the linker histone H1 leads to the formation of higher-order chromatin and is associated with transcriptional silencing [6]. Histones can be covalently modified by the post-translational addition of a variety of functional groups including but not limited to methyl-, acetyl-, phosphoryl-, ubiquitin and ADP-ribose that altogether constitute an epigenetic signature of transcriptional activity [7]. Histone acetylation is generally associated with an

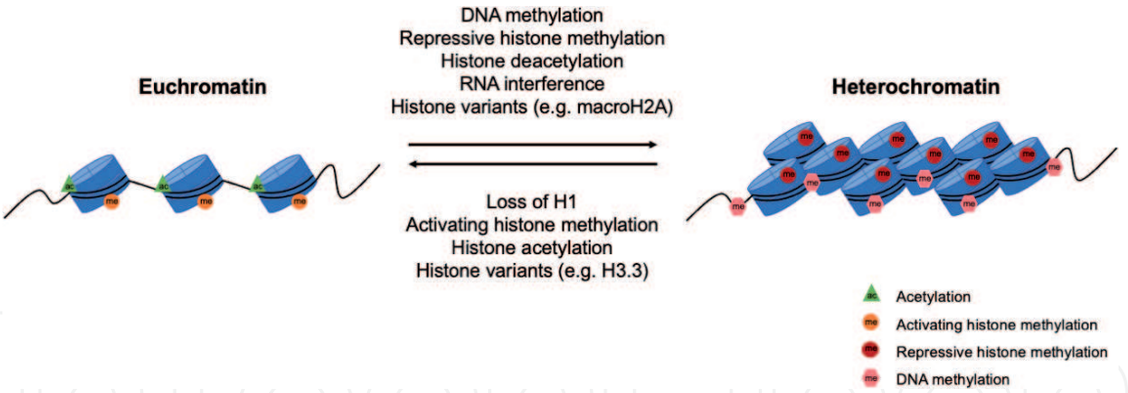

Figure 2.
The conformational transition between euchromatin and heterochromatin is mediated by epigenetic mechanisms.

open chromatin conformation and active gene expression. H3K9ac, H4K16ac and H3K27ac are the most abundant histone acetylations found at the promoters and enhancers of actively transcribed genes [8, 9]. On the other hand, the impact of histone methylations on gene expression strictly depends on their degree and location. For instance, H3K4me1–2-3 and H3K9me are marks of active transcription sites, while H3K9me2–3 and H3K27me2–3 are found in heterochromatin and are indicators of repressed gene state [10].

The main constituents of nucleosomes are canonical histones and share only a certain level of similarity with their corresponding "replacement" variants. These differences in amino acid sequence affects gene expression both by causing conformational alterations of chromatin and disruption of existing interactions between histones and their chaperons, while establishing new ones (reviewed in [11]). Histone variants are involved in several chromatin-related processes such as transcriptional regulation (H3.3 and macroH2A), DNA damage signaling (H2A.X), nucleosome positioning (H2A.Z) and the formation of centromeres (CENP-A).

RNA interference (RNAi) is another mechanism of epigenetic regulation that facilitates heterochromatin formation and transcriptional silencing by the action of non-coding RNAs. X chromosome inactivation is a significant example of RNAi mediated transcriptional repression, which results in the random heterochromatinization and silencing of one of the X chromosomes in females to provide dosage compensation [12]. X-inactivation is initiated by the long non-coding RNA Xist (X-inactive specific transcript) and via the recruitment of histone modifiers and corepressor complexes, an inactive gene state throughout the X chromosome is achieved [13]. Furthermore, small non-coding RNAs including micro-RNAs (miRNAs), small interfering-RNAs (siRNAs) and Piwi-interacting RNAs (piRNAs) are important contributors of transcriptional regulation mediated by RNAi [14–17].

DNA methylation is a reversible post-translational modification of DNA that cause repression of gene expression when present at CpG islands in promoter regions. CpG denotes cytosine residues followed by a guanine nucleotide, where the methyl-group is covalently attached to the 5th carbon of cytosine, giving rise to 5-methylcytosine (5mC). DNA methylation is catalyzed by DNMT enzymes in mammals. 5mC marks that the *de novo* DNA methyltransferases DNMT3A and DNMT3B set during embryonic development are inherited in every cell division semi-conservatively [18] and the maintenance DNA methyltransferase DNMT1 methylates the newly synthesized, hemi-methylated strand [19]. Heterochromatin exhibits high levels of 5mC that correlates with lower levels of gene expression led by transcriptional silencing [20, 21].

Recent studies identified a number of RNA modifications that play a wide range of regulatory roles in various cellular processes and embryonic development.

The list of RNA modifications comprises of N7-methylguanosine (m7G), 2′-O-methylation (Nm), 5-methyl cytosine (m5C), N1-methyladenosine (m1A), N6-methyladenosine (m6A), and 3-methylcytidine (m3C). All these epigenetic modifications of RNA constitute a complex regulatory network over several aspects of mRNA metabolism such as translation efficiency, mRNA splicing and nuclear export [22].

3. Epigenetic regulation during viral infections

As intracellular parasites that lack critical cellular components for replication, protein synthesis, metabolism, and energy production, viruses are incapable of self-maintenance. Therefore, they strictly rely on the cellular machineries of the host cell for their propagation, including the host's epigenetic factors [23]. Viruses hijack the epigenetic regulation mechanisms for multiple reasons. Firstly, several molecular processes such as viral genome replication, transcription of viral proteins and the packaging of new viral particles may simultaneously take place within a single host cell. The existence and coordination of distinct viral genome states allowing these molecular processes are often orchestrated by the machineries of epigenetic regulation [1]. Secondly, as key regulators of gene expression, epigenetic factors are required for the transcription of viral proteins. Especially for viruses encompassing large genomes such as Herpesviruses, epigenetic regulation ensures that only the relevant set of genes are expressed in accordance with the stage of infection [24]. Lastly, the genetic material of DNA viruses is either found in a eukaryotic chromatin-like state in the viral particle or gets packaged within the host cell, making it a target for epigenetic regulation. For instance, the DNA of polyomaviruses (such as Simian Virus 40 – SV40) exists as chromatin throughout their life cycle and is regulated by histone modifications, RNAi, and nucleosome positioning [25, 26]. Similarly, the linear DNA of adenoviruses is packaged by viral proteins that are similar to histones, namely protein VII, which then get replaced by histones upon viral entry into the host [27].

There are three main outcomes of viral exploitation of epigenetic mechanisms, which are the restriction of viral replication by the host immunity and its evasion, regulation of viral latency and the enhancing of viral mRNA function.

3.1 Epigenetic mechanisms of host repression and viral immune evasion

Epigenetic mechanisms can alter gene expression patterns in the cell in response to environmental stimuli, enabling them to quickly adapt to external changes. As important external stimuli that induce cellular response, viral infections are often confronted by epigenetic alterations within the host cell to repress viral replication and gene expression [28]. Upon entry into the host cell, viral DNA rapidly gets packaged and heterochromatinized, inhibiting viral gene transcription. The epigenetic restriction of viral activity is considered as an innate immune response, which further participates in inducing adaptive immunity and apoptosis in the infected cells [23]. On the other hand, viruses have also developed epigenetic strategies to counteract and evade the cellular antiviral response both by suppressing host immunity and by creating a suitable environment for viral replication [28].

Viral DNA is distinguished and targeted for epigenetic repression by two main mechanisms involving pro-myelocytic leukemia nuclear bodies (PML-NBs) and interferon-inducible protein 16 (IFI16). PML-NBs consist of PML proteins and several epigenetics factors such as transcriptional co-repressors and histone

chaperons, which constitute a regulatory hub for gene expression. In alpha-herpes virus HSV-1, histone variant H3.3 carrying repressive histone modifications (e.g. H3K9me3) is incorporated into the viral DNA via PML-NB-associated histone chaperons HIRA, Daxx and ATRX [29, 30]. In hepatitis B virus, Smc5/6 proteins, together with PML-NBs, provide viral inhibition. In overcoming host repression, both herpesviruses and the hepatitis B virus target PML-NBs for degradation and dispersion of the effector proteins. In herpesviruses, the viral protein VP16 interacts with host proteins HCF-1 and Oct-1 to recruit histone demethylases LSD1 and JMJD2 for the removal of previously established repressive H3K9me3 marks [31, 32]. Next, activating H3K4me3 marks are deposited by histone methyltransferases Set1 and MLL1 to allow the transcription of viral immediate early protein ICP0 [33]. As an E3 ubiquitin ligase, ICP0 targets PML-NB for ubiquitylation and degradation, which subsequently releases Daxx and ATRX from the vicinity of viral DNA [34]. Likewise, pp71 protein in beta-herpesvirus HCMV, BNRF1 protein in Epstein–Barr virus and HBx protein in hepatitis B virus exert similar functions in disassembling the PML-NBs and avoiding the repressive mechanisms of the host [35–37].

Foreign DNA is recognized by several factors in the host cell, which trigger the induction of innate immunity and the secretion of cytokines and chemokines. IFI16 acts as an innate immune DNA sensor for viral DNA and induces inflammasome activation [38]. In addition to its key role in stimulating interferon-β secretion, IFI16 contributes to the restriction of viral propagation via deposition of repressive histone marks to the viral DNA and displacing transcription factors from viral gene promoters [39, 40]. Similar to the viral evasion of PML-NB-mediated host repression, IFI16 can be degraded by the ICP0 protein in HSV-1 and its repressive activity can be blocked by HCMV proteins [41, 42]. Furthermore, IFI16 itself is subjected to epigenetic regulation, in which its acetylation by p300 may provide another layer of modulating transcriptional activity [43].

Another viral mechanism that provides escape from recognition and elimination by the host immune system makes use of viral miRNAs that share sequence homology with cellular mRNAs and miRNAs. By specifically targeting and silencing transcripts for host proteins that might function as inhibitors of viral replication, such as regulators of antiviral immunity, viruses can avoid host repression [44]. Viral miRNAs have also been attributed additional roles in regulating viral protein expression and controlling viral replication [45]. The biogenesis of viral miRNAs relies solely on the cellular machineries of the host; whereby the host RNA polymerases, ribonucleases and endonucleases act in cohort to transcribe and process the viral miRNA precursors into mature viral miRNAs [46]. Viral miRNAs are detected in several types of viruses, including but not limited to the frequent human infectors such as Epstein Barr virus, herpes B virus, human cytomegalovirus, human immunodeficiency virus 1, herpes simplex virus 1 and 2, Kaposi sarcoma-associated herpesvirus and simian virus 40. Currently, there are more than 300 viral miRNA precursors and more than 500 mature viral miRNAs available in the miRBase collection [47].

Lastly, viral infections can induce global alterations in histone modifications or the chromatin composition of the host, resulting in distinct epigenetic landscapes. For instance, E1A protein in adenoviruses interacts with lysine acetyltransferases p300/CBP to preferentially block histone acetylation and to repress a set of genes that would normally inhibit infection [48]. Likewise, protein VII can act as a histone mimic due to its structural resemblance to histones and change the host chromatin composition. It also binds to high-mobility group proteins (HMGBs) and tethers them to chromatin, inhibiting their release that typically acts as a danger signal to activate immune system in response to inflammatory stimulus [49].

3.2 Epigenetic regulation of viral latency

Viral invasions often fail to achieve successful propagation and production of infectious progeny due to several reasons such as host repression, deficiency in host resources and failure to replicate the viral genome properly [50]. In contrast to the lytic infections that produce and release infectious progeny via host cell lysis, latent infections result in the stable maintenance of viral genome within the host cell without expression of viral antigens and production of viral particles. When viruses infect non-permissive cells, they repurpose the epigenetic mechanisms of host repression to enter a dormant state, which would allow establishment of long-term infections while avoiding the host adaptive immune response [51]. The majority of viruses that can achieve latency belongs to the families of herpesviruses and retroviruses. While herpesviruses accomplish latency by means of epigenetic repression, retroviruses reverse transcribe their RNA genome into DNA and integrate it to the host genome for viral persistence.

Latent infections are reversible, as it is possible to reactivate viral replication and switch to lytic infection under permissive conditions. The decision between a lytic and a latent infection requires the expression of distinct sets of genes, indicating epigenetic regulation [1]. During the establishment of latency, viral gene expression is tightly controlled in a temporal manner, in which the latency genes are first turned on and then partially turned off to limit the production of viral antigens while the lytic gene foci are heterochromatinized for transcriptional repression [23]. The silencing of lytic gene expression in latent infections is mainly orchestrated by the action of transcriptional corepressor complex Co-REST and the Polycomb complex [52–54]. Consequently, the viral genome is enriched in repressive histone marks such as H3K27me3 and H3K9me3, which are excluded from the latency related genes [50]. Likewise, activating histone methylations (e.g. H3K4me3) are found at the transcript start sites and the regulatory regions of latency genes [55]. Interestingly, viral genomes can harbor bivalent chromatin states consisting of both activating and repressive histone marks that enable transition between latent and lytic phases [56]. Formation of higher-order chromatin structures via chromatin organizing factor CTCF is implicated in the regulation of latency as well [57]. In addition to the host-driven mechanisms, viral proteins BNRF1, HCF1 and VP16 participate in the recruitment of histone chaperons and histone deacetylases to prevent lytic gene expression [58, 59].

In order to be stably maintained within the host cell through several rounds of cell division, the viral genome forms minichromosomes (episomes) and segregates along the host chromosomes following replication [1]. For this purpose, the viral episome is tethered to the host metaphase chromatin via viral proteins, replicated by the host replication machinery and the newly synthesized episomes are equally divided between the daughter cells prior to the completion of cell division [50]. The cellular targets of viral episome tethering includes AT-rich DNA, histones and other chromatin associated factors [60–62]. The formation of episomes also serves to protect viral genome integrity via formation of "endless" i.e., circular genomes [50].

3.3 Enhanced viral mRNA function

Viral RNAs are heavily modified by the covalent addition of functional groups that are similar to cellular mRNAs; however, some of these modifications are found in significantly higher levels in viruses than eukaryotes. Recent studies attributed important roles for RNA modifications in promoting viral replication, through enhanced stability of viral transcripts, increased efficiency of translation and escaping immune recognition [1]. N_6-methyladenosine (m^6A) constitutes a major source of RNA

modifications, which is deposited by METTL3 and recognized by the YTH domain of YTHDC1, YTHDC2, YTHDF1, YTHDF2 and YTHDF3 proteins [63]. m^6A has been shown to promote viral gene expression and replication, as well as to enhance immune evasion [64–66]. Mutations that alter m^6A deposition sites and thereby reduce m^6A levels result in a substantial decrease in viral pathogenicity, suggesting a novel strategy that could be used in engineering vaccines based on attenuated viruses [65]. 5-methylcytidine (m^5C) is another abundant RNA modification. It is catalyzed mainly by NSUN2 and its loss causes decreased translation efficiency of HIV-1 transcripts [67]. N4-acetylcytidine (ac^4C) is set by NAT10 and is found both in viral and cellular RNAs. Previous reports have established a link between ac^4C and improved stability and translation efficiency of viral transcripts and indicated that its loss at even 3′-untranslated regions of viral mRNAs leads to reduced levels of viral transcription and protein synthesis [23]. 2′O-methylation is a distinct type of RNA modification, in the sense that it can be deposited by the nucleolar protein FTSJ3 on either one of the three types of ribonucleotides (A, U and G) and on cytidine residues possibly by an unknown mechanism. Viruses that lack 2′O-methylation due to depletion of FTSJ3 activity trigger the cytoplasmic viral RNA sensor MDA5, implicating 2′O-methylation as a viral mechanism of escaping recognition by the host immune system [68].

4. Epigenetic regulation in relation to Covid19

4.1 Role of epigenetic mechanisms in the induction of cytokine storm

As detailed in the previous section, epigenetic regulation plays a significant role during viral infections. Viruses of the *Coronaviridae* family that previously caused MERS (Middle East respiratory syndrome, MERS-CoV) and SARS (SARS-CoV) have previously been shown to dysregulate the host immune system by inducing epigenetic changes that antagonize antigen presentation or activate interferon-stimulated genes (ISGs) [69, 70]. These viruses have also been implicated in blocking pathogen recognition and immune system signaling [71]. Due to this tight link with the host immune response, patients suffering from infections of coronaviruses, including SARS-CoV-2, are characterized by an abnormal induction of acute inflammation, namely cytokine storm. The excessive secretion of proinflammatory cytokines and recruitment of immune cells at the site of infection often leads to tissue damage and organ failure, which are hallmarks of Covid-19-related deaths [72].

The transcriptional regulation of cytokine production is under tight control of epigenetic mechanisms. Promoters of interferons (IFNs), tumor necrosis factors (TNFs) and ISGs that are drastically upregulated in Covid-19 patients are enriched by histone marks of open chromatin in activated macrophages and dendritic cells [71, 73, 74]. In addition to the common histone modifications, Covid-19 patients exhibit elevated levels of arginine citrullination on histone H3 [75]. Citrullination, which is a marker of a specific type of immune response to infection, namely neutrophil extracellular traps (NETs), is associated with chromatin decondensation and transcriptional activation [76]. Induction of NETosis is hypothesized to lead to sustained inflammation during SARS-CoV-2 infection and the subsequent cell death due to cytokine storm [77].

4.2 Regulation of SARS-CoV-2 entry-associated factors by epigenetic mechanisms

The novel coronavirus SARS-CoV-2 interacts with and requires the action of multiple host proteins for viral entry. Spike (S) protein, which is anchored into the

viral envelope, binds to angiotensin converting enzyme 2 (ACE2) on the host cell surface [78]. ACE2 is a membrane protein found in a wide variety of cell types. The interaction between ACE2 and the receptor binding domain (RBD) within the S1 subunit of the spike protein initiates entry, while S2 subunit triggers direct membrane fusion or endocytosis upon cleavage and activation by host proteases FURIN and TMPRSS2 [79]. Two members of the cathepsin family, namely CTSB and CTSL are also involved in the viral glycoprotein processing and the fusion between viral and endosomal membranes [80].

Among all SARS-CoV-2 entry-associated host factors, ACE2 is the best characterized protein in terms of epigenetic regulation. ACE2 is located on the X-chromosome, which typically gets heterochromatinized and undergoes X-inactivation in females to achieve dosage compensation. In line with this, higher ACE2 expression was observed in males than in females, accompanied by marks of open chromatin [81]. The heterozygosity of ACE2 alleles, hence the lower levels of ACE2 expression in females is considered as a significant advantage in counteracting SARS-CoV-2 infection [82]. However, X-inactivation is often incomplete, and a significant proportion of X-linked genes, including ACE2, escape silencing [81]. Therefore, ACE2 seems to show a rather heterogeneous sex bias [83].

Several epigenetic factors such as DNA methyltransferase DNMT1, histone acetyltransferases p300 and HAT1, histone deacetylases HDAC2 and SIRT1, histone methyltransferase EZH2 and histone demethylase KDM5B have been reported as potential regulators of ACE2 expression [84, 85]. Accordingly, histone marks H3K27ac, H3K27me3, H3K4me1 and H3K4me3 were detected within the ACE2 locus. Furthermore, studies have shown that ACE2 is under tight regulation of DNA methylation. In all tissues tested, lung epithelial cells exhibited the lowest levels of DNA methylation in ACE2 promoter, which positively correlated with high expression [86]. It was also claimed that the CpG methylation pattern of ACE2 promoter is associated with age and gender, suggesting a possible explanation for increased mortality in elderly men during SARS-CoV-2 infection [84, 86].

Other SARS-CoV-2 entry-associated factors are subject to epigenetic regulation as well. A recent study identified a regulatory region upstream of FURIN gene that is heavily occupied by the histone acetyltransferase p300 in T cells [87]. Also, DNMT1-mediated hypermethylation of TMPRSS2 was associated with its down-regulation [88]. Moreover, loss of DNA methylation was implicated in increased levels of CTSL/CTSB in pancreatic adenocarcinoma, which could cause greater susceptibility to SARS-CoV-2 infection [89]. In accordance with this finding, silencing of CTSL/CTSB was shown to inhibit SARS-CoV-2 replication and virally induced apoptosis [90]. Additionally, significant hypomethylation of CTSL promoter was observed in chronic myeloid leukemia [91].

4.3 Interaction between the host epigenetic factors and viral proteins

Interactome analysis of SARS-CoV-2 proteins has provided experimental evidence of physical interaction between several viral proteins and human factors, implicating them in a variety of cellular processes such as epigenetic regulation of gene expression, RNA processing, DNA replication, trafficking and transport of proteins, mitochondrial function, cellular structure, and cell signaling pathways [92]. Viral envelope protein E interacts with bromodomain proteins BRD2 and BRD4 via its C-terminal end that mimics the N-terminal tail of histone H3. As specific binders and readers of histone acetylation, bromodomain-containing proteins are associated with transcriptional activity [93]. By disrupting BRD2/4 binding to histone H3, protein E can induce genomic alterations that affect host gene expression. Another inhibitory link with histone acetylation was established between

Nsp5 and HDAC2, which could potentially influence the host immune response against SARS-CoV-2. HDACs are commonly classified as transcriptional repressors since their main task is the removal of histone acetylation, a mark of active chromatin. However, HDAC2 plays an activating role during the transcriptional elongation of ISG expression via regulating BRD4 availability at newly activated promoters [94]. Similarly, Nsp8 was identified as a binding partner of histone lysine methyltransferase NSD2, which sets H3K36me3 at the gene bodies of actively transcribed genes [95]. H3K36me3 is suggested as an epigenetic mark of transcriptional memory in ISGs, indicating another layer of innate immune response regulation [96]. Viral proteins Nsp13 and Orf10 interact with ubiquitin specific peptidase USP13 and the components of the Cullin-RING E3 ubiquitin ligase complex, respectively. USP13 has previously been attributed significant immune response-related roles in interferon-induced signaling by STAT1 targeting and deubiquitination [97] and increased immune cell infiltration in several types of cancers [98]. Interestingly, USP13 antagonizes antiviral response via ubiquitination of STING, an important effector of innate immune signaling in response to viral infections [99]. Likewise, Cullin-RING E3 ubiquitin ligase complex members are often hijacked by viruses, inducing the proteasomal degradation of host restriction factors, and promoting viral replication [100]. Nsp13 also interacts with TLEs and TBK1/TBKBP1 proteins which are modulators of NF-kB-dependent inflammatory response and IFN signaling [101].

The list of interactions between SARS-CoV-2 and the epigenetic factors of the host cell that are based on experimental evidence has also been extended by in silico approaches that identified p53 as a binding partner of spike (S) protein [102] and several human miRNAs targeting SARS-CoV-2 transcripts [103]. Conversely, an interplay between SARS-CoV-2 miRNAs and the immune signaling pathways of the host was suggested, which could contribute to the prolonged latency of the virus leading to asymptomatic individuals.

4.4 (Epi)genetic susceptibility to Covid-19

Certain risk groups have been associated with increased susceptibility and disease severity since the emergence of the SARS-CoV-2 outbreak. Age is one of the main risk factors for Covid-19, as evident by its high occurrence and mortality rates in elderly patients [104]. Epigenetic machineries often become defective during the process of aging as well, which results in increased genomic instability, altered gene expression profiles and loss of resilience [105]. These age-related epigenetic changes could hamper the activation of innate and adaptive immune responses, which could also be manipulated by viruses to evade host repression. Coronaviruses have previously been linked with accelerated rate of host immune system aging through epigenetic mechanisms such as DNA methylation and transcriptional silencing that impede with host antigen presentation and the expression of major histocompatibility complexes [70]. Moreover, age-dependent fluctuations in the levels of glycosylation and NAD+, which have epigenetic associations, are implicated in predisposition to SARS-CoV-2 infection [106, 107].

There is a growing body of evidence pointing towards the role of DNA methylation in Covid-19 severity. Analysis of genome-wide DNA methylation profiles of severe COVID-19 cases revealed increased methylation of IFN-related genes while inflammatory genes were hypomethylated [108]. Likewise, a genome-wide association study identified a total of 44 CpG sites, most of which were located to coding genes including the components of the inflammasome complex and the major histocompatibility complex HLA-C as potential markers of COVID-19 severity and respiratory failure [109]. Furthermore, in lupus patients, loss of DNA methylation

in ACE2 and interferon/cytokine-regulated genes, together with enhanced NF-kB expression were defined as contributors of severe COVID-19 [110]. Lastly, SARS-CoV-2 can demethylate and activate the expression of Syncytin-1 and Syncytin-2 genes of the host that are required for the creation of giant multinucleated cells, a process known as syncytium formation [111]. Syncytin genes are normally methylated and silenced during development, except for the mammalian placenta, where induction of multinucleated cells provides tissue impermeability in aid of immune tolerance between mother and child [112]. Syncytium formation followed by extensive cell death is suggested as an underlying cause of the detrimental effects of cytokine storm in COVID-19 patients [113].

5. Clinical implications and future perspectives

Until the successful development of the first Covid-19 vaccine in December 2020, one of the greatest challenges in fighting the disease was the lack of specific medication and vaccination. To this end, epigenetic mechanisms have been considered as promising targets for novel therapeutic approaches due to the important role of epigenetic regulation during viral infections including viruses of the *Coronaviridae* family. Several epigenetic modifier enzymes such as DNMTs, HATs, HDACs, HMTs and KDMs are proposed as candidate targets for the treatment of Covid-19. For instance, histone demethylase KDM5B could be targeted for the prevention of Covid-19 as its inhibition stimulates interferon production and provides resistance to viral infections [84]. Targeting epigenetic modifiers could open up a new revenue for the inhibitors against these enzymes, which are already in the market for therapeutic purposes, as potential antiviral agents to be used in drug repurposing attempts. In line with this, Decitabine, an inhibitor of DNA methylation (NCT04482621) and Dipyridamole, an inhibitor of NET formation (NCT04391179) are currently in clinical trials for Covid-19 therapy [28, 114]. Other clinical trials based on epigenetic markers aim to study microRNAs and DNA methylation patterns in relation to Covid-19 (NCT04403386 and NCT04411563) [28].

In conclusion, it is critical to characterize the molecular pathways that take part in SARS-CoV-2 infections to the best of our knowledge to have a better understanding of Covid-19 and to develop better therapies and vaccines for treatment. Epigenetic regulation machineries are involved in several virus-related cellular processes, suggesting epigenetic factors as promising targets for therapy. In this book chapter, we provided a comprehensive overview of epigenetic mechanisms in viral infections with a special focus on SARS-CoV-2 infection, which we believe will be useful for future studies.

Conflict of interest

The author declares no conflict of interest.

DOI: http://dx.doi.org/10.5772/intechopen.98866

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