Genetic and epigenetic regulation of natural resistance to HIV-1 infection. New approaches to unveil the HESN secret

Journal:	*Expert Review of Clinical Immunology*
Manuscript ID	Draft
Manuscript Type:	Review
Keywords:	Epigenetic, HESN, HIV-1 resistance, miRNA, Epigenetics, omics approaches, polymorphisms

Genetic and epigenetic regulation of natural resistance to HIV-1 infection.

New approaches to unveil the HESN secret

Abstract

Introduction

Since the identification of HIV, several studies reported the unusual case of small groups of subjects showing natural resistance to HIV-infection. These subjects are referred to as HIV-1–exposed seronegative (HESN) individuals and include people located in different regions of the world, with diverse ethnic backgrounds and routes of exposure. The mechanism/s responsible for protection from infection in HESN individuals are basically indefinite and most likely are multifactorial.

Areas covered

Host factors, including genetic background as well as natural and acquired immunity have all been associated to this phenomenon. Recently, epigenetic factors have been investigated as possible determinants of reduced susceptibility to HIV-infection. With the advent of the OMICS era, the availability of techniques such as GWAS, RNAseq, and exome-sequencing in both bulk cell populations and single cells will likely lead to great strides in the understanding of the HESN mystery.

Expert opinion

The employment of increasingly sophisticated techniques is allowing the gathering of an enormous amount of new data. The integration of such information will provide important hints that could lead to the identification of viral and host correlates of protection against HIV

infection, allowing the development of more effective and focused preventative and therapeutic regimens.

Keywords: Epigenetic, HESN, HIV-1 resistance, miRNA, OMICS approaches, polymorphisms.

Article highlights:

- A characteristic feature of every infectious disease is that not all exposed individuals become infected.

- The relative contribution of genetic polymorphisms to the phenomenon of natural resistance to HIV-1 infection has been ascertain for some genes and new pathways are currently under investigation (Antigen presentation, Vitamin D, Interferon λ genetic variants among others)

- Epigenetic is a new emerging research area whose contribution to progression of HIV-1 infection has been recently investigated. Only one report has so far explored the role of epigenetic in natural resistance to HIV-1 infection in highly-exposed seronegative (HESN) individuals. Further analyses in this direction are therefore mandatory.

- MicroRNAs (miRNAs) represents a post-transcriptional regulation mechanism responsible for changes in gene expression. In the field of HIV-1 infection, miRNA target genes include both viral and host gene transcripts, exerting a direct or indirect control of viral replication respectively.

- MiRNA profiling in cellular subsets from individuals displaying different susceptibility to HIV-infection is crucial to elucidate their role in controlling resistance to HIV-1 infection and HIV-1 replication competence

- The setting up of more and more sensitive OMIC techniques such as GWAS, RNAseq, miRseq, proteomic, metabolomic in bulk cell populations as well as single cell subtypes will result in the generation of a plethora of data that could explain the observed different susceptibility to HIV-1 infection/progression to disease.

- The integration of data obtained with these novel sophisticated technologies will allow the identification of new variants/pathways responsible for the atypical HESN phenotype. Such potential targets could be exploited in the fine-tuning of vaccine/drugs for HIV-infection.

Introduction

Human Immunodeficiency Virus (HIV) is a highly fatal lentivirus that is responsible for a millions of deaths all over the world. By destroying the host's immune system, HIV renders the infected subject vulnerable to a huge range of opportunistic microorganisms. However, population susceptibility to HIV as well as the clinical spectrum regarding disease progression and the development of progressive cellular immunodeficiency varies enormously in the population. Despite the extraordinary progresses achieved in our knowledge of the biology of this virus in the last decades, why and how individuals vary in their susceptibility to HIV-infection remains mostly unsolved. Disclosing the secret of natural defence against HIV is essential in the struggle to develop effective vaccines or microbicides to eradicate the pandemic. That is why over the last 30 years, many scientists have focused their studies on HIV-exposed seronegative (HESN) individuals, who regardless of recurrent contact with the virus through different routes do not become infected. The identification of a small percentage of homosexual men with a HESN phenotype dates back to 1989 [1]. Such unexpected phenomenon was initially explained by the presence of HIV-specific T helper cells in these fortunate cohorts, thus endorsing the hypothesis that these subjects had come into contact with the virus and that their antiviral immune response was able to protect them [2].

HESN cohorts include the following: heterosexual discordant couples, men having sex with men, sex workers, haemophiliacs, perinatally exposed infants, intravenous drug users, and health care workers with accidental percutaneous contact with infected biological samples. The prevalence of HESN individuals, their incidence within cohorts, and the risks of infection differ considerably in these groups [3], as well as the biological mechanism/s which hinder their infection. Based on the results so far obtained by researchers worldwide, we can assume that natural resistance to HIV-1 is an extremely complex multifactorial phenomenon,

whose roots sink into the genetic background of the host. These genetic peculiarities result in the activation of a protective immune response allowing HESN individuals to control the initial HIV infection and/or the spreading of infection, once the virus has entered the body.

Below, we will review the different determinants so far associated with the HESN phenotype with respect to three central topics in the field (fig. 1):

(1) Which genetic factors determine whether an individual will be naturally protected from HIV?

(2) Do epigenetic factors play a role in natural resistance to HIV?

(3) How can recent technological advances improve our knowledge of the HESN phenotype?

As each of these issue comprises several elements, we will take into consideration only those ones which have not been previously discussed in our preceding reviews [4–6] and have recently emerged in the scientific literature.

THE GENETIC BASES OF RESISTANCE TO HIV INFECTION

Although HIV-1 infection cannot be considered a classical genetic disease, the assumption that host genetic determinants regulate susceptibility to HIV-1 infection, leads to the theory that protective elements must be lacking in subjects who are HIV-1 infected and, on the contrary, must be prominent in individuals who remain uninfected. Therefore, the genetic studies performed in this field usually compare the frequency of likely protective genetic variants in cohorts of HESNs versus HIV-infected patients. Previous studies to detect and outline host genetic variants responsible for complete or partial resistance to HIV-infection or disease progression have focused on host genes involved in the HIV-1 replication cycle (CCR5, CCR2b, chemokines), immune surveillance (major histocompatibility complex class

I, ERAP2) or restriction factors (TLR3, TIM3, MX2, APOBEC3 family, defensins, CD209, TRIM family) [7–14]. All of these genes have been recently reviewed by our research team [4,5]. In spite of the huge and reliable data acquired in this field, what is manifest is that none of the genetic variables described so far is able by itself to control viral infection and disease progression. Even the forefather of all the genetic correlates of protection, the null allele for CCR5 known as CCR5Δ32, which encodes a truncated protein that cannot be expressed on the cell surface and function as a HIV co-receptor [15], provides a substantial but not complete protection from infection [16,17]. More genetic variables, thus, must co-operate with each other to confer protection. That is why the investigation of new genetic variants possibly conferring resistance to HIV-infection is fundamental to decode the multifaceted puzzle responsible for the HESN phenotype. Such studies are valuable, as they provide important clues to identifying the viral and host targets for developing alternative and possibly more efficacious therapies.

Herein, we focus our attention on three pathways that have been proven to restrain and/or interfere at different levels with HIV life cycle in HESN: antigen presentation, Vitamin-D (VitD) and Type III interferon pathways (Table 1).

Antigen presentation pathway genes

Host genomic analyses of HIV-infection has reliably identified human leukocyte antigen (HLA) genes as key factors of HIV susceptibility and progression [18]. Data obtained even from small cohorts with different ethnic origin, exposed to different viral strains strongly confirmed such findings. Herein we report some selected studies suggestive of such association. In 2015, McLaren *et al.* described HLA-B as the major host genetic determinant of HIV viral load and disease progression [19,20]. Likewise, -35 HLA-C variant has been demonstrated to influence set-point viral load, and individuals with higher HLA-C expression

display a better control of HIV disease progression compared to subjects with lower expression [21]. In a study on African serodiscordant couples the HLA variants (B∗53:01, B∗14:01, and B∗27:03) and HLA-concordance between partners were demonstrated to explain the 13% and 6% of the variance in viral load, respectively [22]. In a study by Rallon and co-workers the presence of the HLA class II allele DQB1*03:02 was shown to be a correlate of immune protection against HIV infection, while the presence of the HLA class I allele A*02:01 was associated with being infected with HIV in a cohort of 29 HESN individuals in stable heterosexual relationships [23]. Additionally, the HLA-B*57 group allele can be protective against mother-to-child-transmission (MTCT), while HLA-B*35 groups alleles are consistently associated with HIV-1 MTCT [24]. More recently eight unconventional MHC molecules (HLA-E, HLA-F, CD1a-e, and MR1) recognized by non-classical MHC I restricted T cells, were demonstrated to be involved in protective immune responses to HIV [24,25]. Due to their intervention in primary protection -often in non-lymphoid tissues in which pathogen entry and/or replication occurs- these molecules represent novel advantages over classical T-cell targets in the setting up of anti-viral therapeutic or vaccinal approaches.

Within the antigen presentation pathway, other gene polymorphisms have been consistently correlated to HIV-susceptibility. ERAP2 is an aminopeptidase anchored to the endoplasmic reticulum, which is able to trim precursor peptides generated in the cytosol by the immunoproteasome, allowing them to perfectly lodge in the MHC class I groove and be presented to CD8-specific T lymphocytes. This enzyme is therefore indirectly responsible for the elicitation of a qualitatively different immune response [26]. So far two different ERAP2 isoforms have been described for this gene: the full-length form named HapA is functional; conversely the short form generated by alternative splicing (HapB) is degraded by nonsense mediated decay (NMD) [27]. The role of these two genetic variants in antigen presentation and natural resistance to HIV-infection in different HESN cohorts has been fully

demonstrated in previous studies [8,11,13] and reviewed in [4]. However, recent acquisition in the field have contributed to further explain the mechanism of action carried out by this enzyme. First, following inflammatory stimuli HapA of ERAP2 may be released in the secretome of immunocompetent cells and the addition of exogenous recombinant human (rh)ERAP2-FL in *in vitro* cellular culture results in protection from HIV-1 infection. Notably, this feature is even more evident in HomoB subjects who do not genetically produce it, presumably, following rhERAP2 internalization and re-localization into the ER, where it compensates for the natural absence of the protein [28]. The mechanism by which exogenous ERAP2 interferes with HIV-1 infection/replication seems to depend on CD8+ T cell activation and on the alteration of their maturation in favour of CD8+ effector memory re-expressing CD45RA (TEMRA). However, as control of HIV infection by rhERAP2 addition was observed also in PBMC cultures depleted of $CD8^+$ T cells, the ERAP2 antiviral effect must also stem for the activation of effector mechanisms independent from $CD8^+$ T cells. Notably, Ye and co-workers, among other genes, identified two new uncharacterized ERAP2 isoforms produced in response to influenza infection by analysing RNA-seq profiles of monocyte-derived dendritic cells described [29]. As physiologically ERAP2 homodimerizes and heterodimerizes with ERAP1 [30] to perform the final peptide trimming step, the author speculates that the translation of the flu-specific ERAP2 isoforms, that miss the catalytic domain, could exert a dominant negative effect towards either ERAP2 or ERAP1. This would interfere with the physiological antigen processing, creating a more immunogenic MHC peptide repertoire that could confer a fitness advantage in response to virus. As the dominant negative effect was reported even for HapB following *in vitro* HIV infection, it will be a priority to verify if such new variants are produced following HIV-infection and if their level of expression can be responsible for inter-subject differences in the risk of acquiring HIV-infection.

Variants in Vitamin D pathway genes

Vitamin D (VitD) is an essential controller of microbial host defence via the activation of genes and pathways that upregulate natural and acquired immunity. VitD exerts its biological functions by binding to the Vitamin D receptor (VDR); as the VDR is expressed by a variety of different cell types, this process modulates multiple cellular pathways. The role of VitD in HIV-infection is debated and not univocal. According to some authors, VitD deficiency contributes to the pathogenesis of HIV infection in terms of reduced peripheral blood CD4+ T-cell levels and quicker disease progression in HIV-infected patients [31,32]. Likewise, higher mRNA levels of Vitamin D and VDR were observed in subjects who naturally resist infection and were positively correlated with the mRNA levels of anti-HIV molecules, including Elafin, TRIM5, Cathelicidin, HAD-4 and RNase7, APOBEC3G and PI3, that have been previously associated with the HESN phenotype [33,34]. Higher serum concentrations of Vitamin D have been associated with polymorphisms in genes involved in the VitD pathway in quite a few studies performed on different HESN cohorts [33–37]. For example, the rs1544410 G allele of VDR was associated with delayed development of AIDS and increased resistance to HIV infection, which appears to be dependent on enhanced response to VitD [38,39].

However, other results are in disagreement with the idea that VitD could play a protective role against HIV infection. Thus, Sanchez de la Torre and co-workers demonstrated that VDR haplotypes established by the rs11568820, rs4516035, rs10735810, rs1544410, and rs17878969 polymorphisms influence the risk of HIV-1 acquisition [40]. In particular, the protective haplotype resulted in a reduced efficiency of the VitD signalling, suggesting that hindering VitD pathway may results in protection from infection.

The functional mechanism associated with these polymorphisms is debated. However, VDR rs1544410 has been associated with a reduced production and stability of VDR mRNA,

whereas the VDR rs10735810 T SNP leads to a lower VDR protein transactivation, hampering normal VDR function; the VDR promoter rs4516035, finally, could be biologically relevant to the immune system regulation by lowering the polarization toward Th2 response [41,42].

A downregulation of the VitD signalling could play a role in resistance to HIV-1 infection for more than one reason. Indeed, VitD production is coupled with an increased HIV-1 CCR5 co-receptor expression, a reduced production of chemokines and an augmented Th2 response. Even more importantly, VitD activates the HIV-1 long terminal repeat *in vitro* in a ligand-dependent manner [43].

More recently, the VDR rs2228570 polymorphism was associated with a delayed AIDS progression in antiretroviral therapy naïve HIV-Infected patients [44]. The authors speculate that this polymorphism might reduce VDR expression and weaken its function, which in turn would down regulate HIV-replication inhibition and stimulate a recover of CD4+ T cells [44].

Another study by Aguilar-Jimenez *et al.* investigated the connection between variants and haplotypes in genes of VitD and antiviral pathways with resistance/susceptibility (R/S) to HIV-1 infection in 3 different HESN cohorts [37]. Notably, >50% of the HESNs from each cohort had a resistance multi-locus genotype (interacting variants) suggesting that interactions involving genes of VitD and antiviral pathways are quite common despite allelic heterogeneity, possibly derived from differences in genetic history of the enrolled populations. Further replications in larger HESN cohorts and functional analyses to define casual associations and to elucidate the influence of VitD in resistance/progression of HIV-infection are definitely required. However, these studies highlight a key role for VitD in modulating the immune system and in turn the host antiviral response that could be exploited in the setting up of immunomodulatory therapies to reinforce ART intervention.

Interferon Lambda genetic variants

Type III interferons, also known as interferon lambdas (IFNλs), have been included into the IFN family in 2003 [45] and are considered the first responders to pathogens that enter the body through epithelial surfaces [46,47]. Humans have four IFNL genes, IFNL1 (formerly known as IL29), IFNL2 (IL28A), IFNL3 (IL28B), and IFNL4. Similarly to Type I interferons, they can induce the expression of interferon stimulated genes (ISG), but the expression of their receptor is narrowed to some cellular types only, including liver, epithelial and myeloid cells [48]. Remarkably, genetic variants in IFNL genes in humans are associated with outcomes to viral infections including HCV, HBV, rhinovirus, influenza virus, lymphocytic choriomeningitis virus (LCMV), respiratory syncytial virus (RSV), rotavirus, and West Nile virus (WNV) [49,50]. In particular, several GWASs revealed a significant association between some SNPs (rs12979860, rs8099917) upstream of *IFNL3* gene with spontaneous and therapy-dependent HCV clearance [51,52]. The functional SNP rs368234815 was subsequently located within a new gene forgoing IFNL3 on chromosome 19q13.13 designated IFNL4 [53,p.3]. This polymorphism includes a SNP (TT) plus an insertion that produces a frameshift in the coding region (ΔG/TT), producing a natural knockout of IFNL4 [53,p.3].

The role of these polymorphisms in the susceptibility/progression of HIV-infection has been investigated; results are interesting but controversial [54–59]. One of the earlier reports verified the association of rs12979860 in relation to both progression and protection from HIV-infection. Differences in genotype distribution were not observed either in the HESN or in the LTNP cohorts, suggesting that different mechanisms operate *in vivo* in the control of HCV and HIV infections [54]. The results of this study were further confirmed in other reports performed on two different LTNP cohorts of Afro-American origin [55,60].

Conversely, two studies successively performed on Caucasian cohorts reported an association between rs368234815 and rs12979860 and, respectively, resistance to/progression of HIV-infection [56,58]. In particular, Real *et al.* reported a strong association between the HCV protective rs368234815 allele (TT) and a decreased likelihood of being HIV-1 infected in male intravenous drug users; this association was not modified by the CCR5 genotype [58]. The association between the knockout allele of IFNL4 and innate resistance to HIV-1 infection was further reinforced by recent data obtained in two well-characterized cohorts of HESN subjects from Spain and Italy that were sexually HIV-exposed. These results would therefore suggest that genetic susceptibility to HCV and HIV-1 infection may shares common molecular pathways.

The biological reason for the loss of a potent antiviral protein should result in protection from viral infections is puzzling. Several studies suggest that INFL4 acts as a potent antagonist or a desensitizing factor of IFNA *in vivo* [53,61–63]. In particular, Obajemu *et al.* reported that, following viral stimulation, IFN-λ4 induces a particularly prompt and potent production of antiviral ISGs, but stimulates the generation of negative regulators of the IFN response, including USP18 and SOCS1, as well [61]. Therefore, they speculate that the earlier antiviral response endorsed by Type III IFNs can be harmful if it is unproductive and weakens the activity of other IFNs [64], or if it inhibits the acquired immune response [65]. Prompt antiviral release of IFN-λ4 might thus be useful for infections needing an extremely fast, although transitory, immune response, but it is a hazard in infections requiring a more protracted defence such as HCV or HIV. Notably, these studies showed a recessive model of inheritance for the minor alleles in the case of parenteral transmission [58], but a dominant model in the case of sexual transmission and HCV-infection [51,59,66,67]. These discrepancies further underline the need to replicate such researches on larger cohorts with different exposure route as different HIV-1 infection risk could correlate with the need of different IFNL4 levels.

In summary, it is evident that IFNL SNPs control HIV infection and replication, regardless of transmission route. It also seems that the advantageous alleles of IFNL SNPs exert protective roles in Caucasians but not in African Americans. Further investigations with the functional IFN-λ4-generating SNP rs368234815 rather than rs12979860 will disclose any proper correlation.

EPIGENETICS

Epigenetics plays a central role during viral infections, including those ones provoked by HIV [68,69]. Viruses exploit and hijack host's cells epigenetic tuning in order to regulate their life cycle; this results in establishment of, or emergence from, latency [70–73]; chromatin modification [74–76]; and transcription of both host and viral genes [77–79]. Furthermore, cancer-related viruses deeply affect cell's epigenetic regulation, re-directing host gene expression toward oncogenic transformations [80]. Indeed, it is known that HIV does affect the epigenetics of infected cells in order to regulate its own life cycle and to impair the immune response [68]. HIV directly interacts with a number of epigenetic factors and/or deregulates their expression [68].

An extremely low number of epigenetic studies have been performed on HESN so far. This is rather surprising, as HESN are an excellent established model of natural resistance against HIV. Su and colleagues focused their efforts on interferon regulatory factor (IRF)-1 both as an immunoregulatory factor and as a driver regulator of HIV replication [81]. The subjects enrolled in the study were all Kenyan commercial sex-workers who had previously been immunologically and epidemiologically characterized. Notably previous data obtained in this cohort showed that polymorphisms of IRF-1 are associated with resistance against HIV [82]. IRF-1 expression and activity upon *ex-vivo* interferon (IFN)-γ stimulation of PBMCs

isolated from HIV HESN was further analyzed in these individuals. Interestingly, authors reported a stronger but transient up-regulation of IRF-1 in HESN, whereas a sustained over time IRF-1 up-regulation was seen in susceptible individuals [81]. Additionally, a higher level of IRF-1 expression in basal condition was observed in HESN. Although HESN showed a slightly higher level of histone H4 acetylation compared to HIV-susceptible individuals in basal condition, similar level of the transcriptional regulators STAT1 and NF-kB/p65 were reported to be bound onto *IRF-1* promoter [81]. Changes in IRF-1 expression were accurately mirrored by epigenetic changes in histone H4 acetylation at the *IRF-1* promoter region, which also correlated with the level of STAT1, NF-kB/p65 and histone de-acetylase (HDAC)-2 recruited at *IRF-1* promoter [81]. When analyzing IRF-1 binding to promoter region of target genes, such as *IL-4* and *IL-12*, and their expression level, no differences were observed between the two groups [81]. The transient IRF-1 upregulation observed in HESN resulted in a degree of cytokine production and immune activation that was similar to that of HIV-susceptible individuals. However, as the authors state, the rapid down regulation of IRF-1 expression suggests that this could curb HIV replication, since IRF-1 could potentially bind HIV-1 LTR [81]. In fact, direct IRF-1 binding to viral LTRs has been correlated to transactivation of HIV transcription [83]. Similar results were produced by transiently silencing IRF-1 expression by siRNA in healthy donor CD4+ T cells and monocytes [84]. Such IRF-1 artificial down-regulation was tuned to be modest, mirroring the physiological level observed in the Kenyan cohort. The authors suggested that IRF-1 expression down-regulation could be crucial to prevent a successful immune activation [84]. A fine tuning of IRF-1 expression in early phases of infection has a dual purpose: it is sufficient to induce a strong immune response but not to enhance viral LTR transactivation [84].

As previously mentioned, HIV is known to affect and manipulate a plethora of host's epigenetic factors. HESN individuals have not been tested yet for the vast majority of these, according to the literature. Here we gathered some of the epigenetic factors affected by HIV that, in our opinion, should be analyzed in HESN.

In general, it is known that viral LTRs of HIV proviruses display typical epigenetic signature, encompassing DNA methylation and histone modification. In fact, integrated HIV proviruses always have a couple of nucleosomes positioned onto the 5' LTR. Such nucleosomes (usually referred as nuc-0 and nuc-1) are remodeled by post-transcriptional modification and thus viral expression is modulated by epigenetic processes as well [85,86]. Disruption of nuc-1 coincides with transcriptional activity of viral sequences [76]. Viral DNA itself is hyper-methylated at the CpG islands [72,87,88], and nucleosomes within LTRs are indeed hyper-methylated and contain hypo-acetylated histonic regions [70,89,90]. This is considered a mark of suppression of viral expression and it is associated with latency [72,73,86,89,91].

A total of 94 CpG islands, all of which are potential target of DNA methylation, were identified in the HIV DNA sequence; 11 of these islands are localized in the LTRs [92,93]. The level of methylation of HIV proviruses fluctuates overtime [92]. A peculiar methylation pattern of CpG islands was not identified when AIDS progressors, long-term non-progressors (LTNP) and elite controllers (EC) were compared [92]. Conversely, Palacios and colleagues observed a stronger hyper-methylation of HIV LTRs in LTNP/EC compared to ART-treated progressors. In this study, patients were not followed overtime, but samples were rather analyzed at a single time point [93]. No correlation were found between the LTR methylation level and the viral load [93].

Histone de-acetylases (HDACs) are typically accumulated on HIV LTRs. Consequently, histones are thoroughly de-acetylated and transcription is suppressed [94–96]. HDACs inhibition (i.e. by trichostatin A – TSA) and histone acetylation modify chromatin and make

it accessible; this results in an increase in viral gene expression and a sharp rebound of viral replication [96,97]. HDACs combined with ART were utilized to reactivate HIV within so-called “kick and kill” strategies in the attempt of purging latent proviruses in reservoirs [97,98]. Cellular factors, such as YY1, LSF, CBF-1 and NF-kB p50 are reported to promote HIV latency by recruiting HDACs on HIV LTRs [94,95,99,100].

While histone acetylation is univocally considered a promoter of gene expression, histone methylation produces a complex and variegated scenario [101]. According to which residue is methylated (mainly lysine, but arginine and histidine as well), which grade of methylation (mono-, bi- or tri-methylation on lysine) and on which histone (H1 to H4), the balance can be shifted toward a positive or a negative gene expression modulation [101]. The most accurately studied methylation are generally on histone 3 (H3) lysine 4 (K4), H3K9, H3K27 and H4K20. In the HIV field, Chéné and colleagues showed that the methyl-transferases SUV39H1 and HP1γ, and the consequent tri-methylation of H3K9 (H3K9me3) play a major role in HIV latency. Silencing of HP1γ was enough to reactivate viral expression [102–105]. EZH2, a component of polycomb repressive complex (PCR)-2 silencing machinery, is the methyl-transferases responsible for H3K27me3, which is a common epigenetic signature of latent proviruses, up to 40% [104]. Conversely, SMYD3 tri-methylates H3K4 (H3K4me3) is considered a typical epigenetic activation mark, commonly found in cancer and viral cancerogenic infections, such as HTLV-1 [106–108]. Hence, during HIV latency, H3K4me3 signature is almost absent within proviral sequences.

Many studies have been performed to dissect which are the epigenetic factors hijacked by the virus. Britton and colleagues analyzed by mass spectrometry all the histone post-translational modifications induced by HIV infection [109]. Their results clearly show how the cellular epigenetic machinery is profoundly modulated in infected cells and many enzymes are involved. Briefly, the main modulated factors are: histone lysine methyl-transferases

(EZH1, some of the SETD and SUV39 family), histone demethylases (some of the JMJD and KDM family), histone arginine methyl-transferases (many of the PRMT family), histone acetyl-transferases (MYST1, MYST2), and histone deacetylases and sirtuins (HDAC5, SIRT2, SIRT3, SIRT4) [109]. Some of the epigenetic changes upon HIV infection actually results from cell activation rather than an active infection. In fact, some of the above-mentioned enzymes are modulated even in the case of UV-inactivated HIV infection [109].

However, HIV transactivator, Tat, is demonstrated to directly interact with and associate to a number of host cellular factors. Tat protein is fundamental for an efficient transcription of viral genes and its expression is therefore associated to the end of the latency phase. Thanks to this viral protein, nucleosomes within HIV provirus, nuc-1 in particular, are acetylated, numerous chromatin modificator and transcription elongation factors are recalled [98]. In fact, positive transcription elongation factor (P-TEF)-b is an essential co-factor for Tat and it is part of the “super-elongation complex” that is formed on proviral LTRs, resulting in a strong enhancement of viral transcription [98,110,111]. Moreover, Tat directly interacts with a number of chromatin modifying enzymes, such as the histone acetyl-transferases (HAT) p300, CBP, p300/CBP-associated factor (PCAF) and GCN5 [94,97,112]. When HAT enzymes are hijacked by Tat onto HIV LTR promoter regions, they promote and facilitate HIV mRNA transcription [109]. SWItch/Sucrose Non-Fermentable (SWI/SNF) has a crucial role in HIV transcription as well [76,113,114]. In the presence of Tat, SWI/SNF contained in the BAF complex is activated, strongly enhancing mRNA transcription [76,98]. Other bromodomain histone modifying proteins are bound by Tat, such as BRD4, some of the TRIM family members, TFIID, SP140. This become particularly relevant as a number of drugs targeting these proteins have been developed in this last decade [98].

MicroRNA role in resistance to HIV-1 infection

Although a strict definition of epigenetics entails DNA methylation and histone modifications, micro RNA molecules (miRNAs) act as epigenetic modulator [115] as they modulate gene expression without modifying DNA sequences [115]. Indeed, miRNAs and epigenetics are deeply and mutually interconnected. In fact, miRNA regulates mRNA level of epigenetic factors, and in turn their expression is modulated by epigenetic modifications [115]. MiRNAs are non-coding RNA, 19-23 nucleotides in length, involved in gene repression through imperfect base pairing to complementary sequences in the untranslated regions (3′UTR) of targeted transcripts. This leads to the subsequent degradation of mRNA [116].

Genes codifying for miRNA are scattered in all the genome within introns or in untranslated regions of protein-coding DNA, and are commonly transcribed by RNA pol II. This enzyme can produce primary RNA (pri-miRNA) which act as regulatory RNAs, form a typical hairpin loop structure and undergoes several canonical or alternative processing steps to become mature miRNAs [117–119]. The mature miRNA is then incorporated into RNA-induced silencing complex (RISC) which by binding to mRNA targets blocks their translation and/or cleavage, thus reducing the final protein output. About 30% of human protein-coding genes are under miRNA regulation [120]. Nevertheless, since miRNAs do not show a perfect match with mRNA target sequences, they can regulate approximatively 200 multiple targets [121]. For these reasons, miRNAs are involved in a wide range of biological processes, including embryogenesis, cell proliferation, apoptosis and play a role in several human conditions including autoimmune, inflammatory neurodegenerative, oncologic and viral diseases. In the field of HIV-infection, miRNAs can act as positive or negative regulators of HIV transcription. Indeed, according to the target they interact with, they can both suppress or activate HIV replication. One of the first study showing the relationship between miRNAs and host-virus interaction was presented by Triboulet and colleagues. Their results clearly show that the knockdown of the two main miRNA processing enzymes, *Dicer* and *Drosha,* promotes HIV-1 replication [122]. MiRNAs that target HIV-1 replication can be further divided

according to whether they directly target HIV-1 transcripts, or indirectly affect HIV-1 by targeting host factors involved in the HIV-1 life cycle (Table 2). Many studies have been performed to identify miRNAs that target the RNA of HIV-1 genes *env*, *pol*, *gag*, *vif*, and *tat*. Hariharan *et al.* using a target prediction software, showed that, within the HIV-1 genome, miR-29a and miR-29b target nef; miR-149 targets vpr; miR-378 targets env and, finally, miR-324-5p targets vif [123]. Huang *et al.* showed that miR-28, miR-125b, miR-150, miR-223, and miR-382-5p target the 3′UTR region of the HIV-1 mRNA in cells, thus reducing CD4+ T-cell activation during the resting period. For this reason, these miRNAs were designated as the anti-HIV miRNAs, that are involved in control of HIV-1 replication [124]. Further supporting these findings, these miRNAs were demonstrated to be abundant in IFNα and IFNβ treated cells and to contribute to the down regulation of the susceptibility of human MDM isolated from healthy controls to HIV-1 infection [125]. Witwer *et al.* showed that miR-125b and -150 strongly correlate with HIV-1 viral load in HIV-1- infected patients [126]. Amaral *et al.* have recently discovered that levels of miR-34c-5p expression, a miRNA responding to T-cell receptor stimulation in naïve T cells, decreases during the disruption of host immune responses by HIV-1 [127]. Houzet *et al.* demonstrated that fives miRNAs: miR-133b, miR-138-5p, miR-326, miR-149-5p and miR-92a-3p, strongly suppress HIV-1 replication [128]. The 3'UTR region of HIV-1 RNA genome has been identified as the target of miR-196b and miR-1290. Indeed, the inhibition of these two miRNAs can lead to the activation of latent HIV-1 in antiretroviral therapy (ART)-treated patients [129]. Furthermore, six miRNAs (miR-15a, miR-15b, miR-16, miR-20a, miR-93, and miR-106b), were found to directly target Tat HIV-1 protein and were associated with lower levels of Pur-α expression and reduced susceptibility to HIV-1 infection in monocytes [130].

As HIV-1 uses the host cell machinery to replicate itself, some miRNAs can indirectly modulate HIV-1 infection by targeting HIV-dependent factors. An evidence of the involvement of miRNAs on HIV-1 replication stems from a study showing that mir-17-92

induces the degradation of the histone acetyltransferase p300 and PCAF. This factor is essential for tat acetylation and up-regulates the transcription of the HIV-1 LTR [122].

Sung *et al.* proposed an anti-HIV-1 role for miR-198, which targets the cyclin T1 mRNA a chief component of the eukaryotic RNA polymerase II prolongation complex. They discovered that this miRNA reduces HIV-1 replication in HIV-1 infected human macrophages [131]. Moreover, low levels of miR-198 have been observed in CD4 resting cells. Other miRNAs, such as miR-27b, miR-29b, miR-223, target cyclin T1, and their down-regulation correlates with increased HIV-1 susceptibility *in vivo* [132]. More recently, Amaral *et al.* discovered that miR-34c-5p, a miRNA triggered by T-cell receptor stimulation in naïve T cells, decreases during the disruption of host immune responses by HIV-1 [127].

Other results show that mir-21 is downregulated in the initial phase of HIV-infection in monocytes. This miRNA inhibits the expression of interferon inducible protein 10 (IP-10) an important inflammatory cytokine that triggers immune dysfunction and disease progression during HIV-1 infection [133]. Finally, Bochnakian *et al.* showed that mir-128, an interferon stimulated gene, inhibits HIV-1 replication in Jurkat cell lines as well as in CD4+ T-cells and blood-derived monocytes by targeting TNPO3 for degradation, an essential factor in HIV-1 nuclear import and viral replication [133].

Given the key role played by some miRNAs in the regulation of HIV-replication is not surprising that the expression of some of these elements was significantly correlated to the phenotype of subjects showing a non-canonical relationship with HIV-infection/replication. Indeed, several anti-HIV miRNAs were shown to be related with HIV viral load and CD4 T cell concentration in LNTP and EC. For example, the levels of miR-223, miR-150, miR-146b, miR-16 and miR-191 were lower in T cells isolated from patients with faster disease progression [135]. Recently, decreased levels of mir-382 and mir-155 were shown to correlate with control of disease progression in LTNP [136]. The expression of miR-9, a

molecule that inhibits IL-2 production, was observed to be significantly increased in CD4+ T cells of LNTP patients, suggesting a role for this miRNA in reducing HIV-replication [137]. Moreover, Lin-Bo Yin compared LNTP with a high or a low (< 1000 copies/ml) viral load and observed that miR-19b is significantly down regulated in the latter group of patients. Studying the antiviral function of miR-19 they discovered that this miRNA is able to target PTEN (phosphatase and tensin homolog) in CD8+ T cells, a phosphatase that plays a key role in the regulation of cellular survival and proliferation. Notably, mir-19b overexpression also stimulated IFN-γ and granzyme B production and inhibited CD8+T cell apoptosis, suggesting that miR-19b could be an innovative target for immune intervention in HIV-infection [138].

Several authors also verified the hypothesis of a possible correlation between miRNA expression and the EC phenotype. Results showed that this could be a plausible mechanism justifying their resistance to virus-induced disease progression. Supporting this theory, Reynoso *et al.* described miR-31, miR-29b-3p, miR-33a-5p, and miR-3146a-5p levels to be significantly higher in plasma of EC compared to normal progressors. Moreover, serum miRNA profiling of HIV positive and negative individuals, relieved that miR-221, miR-27a, miR-27b, and miR-29b were up-regulated in elite controllers and HIV-1-negative individuals, and were downregulated in HIV-1-infected patients on ART [139]. Remarkably, an increase in mir29b expression had already been reported by Egaña-Gorroño et al in 2014 while comparing PBMCs from EC and viremic HIV-infected patients undergoing therapy [140].

As mentioned above the contribution of epigenetic factors on the definition of HESN phenotype has been only partially analysed. To our knowledge, we are the only group that profiled a miRNA outline in HESN subjects. In our study, we analysed miRNA profiles in plasma, PBMC and *in vitro* HIV-infected cell culture media of HESN, HIV+ individuals and HC. In particular, we performed a PCR array of 84 miRNAs involved in the regulation of

immune responses and/or in host-virus interaction. Results showed that there is an upregulation of several miRNAs (miR29a, miR-138, miR-150, miR-190, and miR-223 in unstimulated PBMC; miR-28, miR-29a, miR-29b, miR-29c, miR125b, miR-146, miR-150, miR-155, miR-190, and miR-382 in plasma; and miR-28, miR-29a, miR-125b, miR-150, and miR-223 in supernatants) in HESN and HIV+ individuals compared to HC, suggesting that exposure to HIV-1 can modify the miRNA profile even in the absence of a manifest infection. Among all these miRNAs, mir-29a and mir-223, were significantly augmented exclusively in *in vitro* HIV-1-infected PBMC of HESN, underling a protective role for these molecules in resistance to HIV-1 infection [141]. Supporting the idea that these miRNAs play a role in resistance to HIV-infection, the expression of mir-29 family members was found to be significantly increased in *in vitro* HIV-1-infected CD4+ T cells from HESN, presumably as a consequence of the IL-21 upregulation seen in the same cells [142]. Notably, as previously reported [138,139], a higher expression of miR29 is a distinctive feature of controlled viral replication in EC as well.

There is growing evidence that different miRNA profiles correlate with control of HIV-infection. However, further studies are needed in order to exploit them as clinical biomarkers for diagnosis and prediction of HIV progression and susceptibility.

We are living the epigenetic era and epigenetic drugs are now a reality and a fast-growing field [143–146]. In our opinion, virology and immunology should exploit these new approaches and tools, in order to translate such scenarios into new antiviral strategies and into new realm of possibilities, in addition to current therapies [147,148].

OMICS APPROACHES TO THE ENIGMA OF NATURAL RESISTANCE TO HIV-INFECTION

Recent research advances have allowed to quickly gathering a plethora of information, which are crucial to clarify the molecular mechanisms involved in diseases pathogenesis. In particular, genome-scale quantitative multi-omics approaches (transcriptomics, proteomics, metabolomics, single-cell omics etc.) allow researchers to simultaneously acquire an enormous set of data on several biomolecular pathways and to better understand unique molecular interactions between products of different origins, included viral and cellular ones. As the pertinent technology continues to progress, these studies have improved in complexity (e.g., to include host whole exome and whole genome sequencing) and in breadth, thus permitting to even better integrate multiple data. These methodologies have been applied to the study of HIV-infection/replication as well, providing important insights into cellular/viral processes such as gene expression regulation and metabolism.

GWAS (genome-wide association studies), which investigate common gene variant's role in HIV-infection, explain approximately 20% of viral load variation and disease progression, suggesting that other still unknown factors are involved in the control of this disease. Notably, innovative technologies in genome sequencing allow the identification of uncommon variants as well, both by sequencing of the entire exome (2% of the genome), or through the deep sequencing of the complete genome or transcriptome (RNA-seq). In 2018, a study published by Nissen *et al.* on whole exome sequencing (WES) on 7 LTNPs and 4 ECs led to the identification of 24 relevant variants localized in 20 different genes, mainly encoding innate immune sensors (LRRIF1P, IRAK2, TAB2, NOD2, SLX4) and proteins involved in HIV uptake and intracellular trafficking (FN1, FRK, PIK3C2B, PIK3R5, MAP1A, PIK3R6) [149]. However, no single unifying mechanism common to both ECs and LTNPs was identified, suggesting that slow disease progression in these two different phenotypes may depend on a diverse genetic background.

In 2019, a study by Barclay *et al.* used an OMIC approach (proteomics and RNAseq) to investigate the differences in protein and nucleic acid content of exosomes from uninfected and HIV-1-infected T-cells and monocytes [150]. Results showed that the cargo packaged into exosomes might be significantly altered in HIV-infected cells, presumably exerting a different functional effect on the recipient cells. In particular, exosomes from HIV-infected cells were enriched in histones, cyclin dependent kinases (CDKs), Src family kinases (T-cells) as well as long noncoding RNAs (monocytes); these molecules were almost lacking in uninfected cell exosomes. Based on these results, it would be extremely interesting to verify if exosomes isolated from HESN subjects are loaded with “protective” determinants that could confer a resistant phenotype even to the neighbouring cells.

Using OMICS analysis, Zhang and colleagues performed RNA and miRNA profiling (RNAseq and mirSeq) of PBMC from ART naive and early HIV infected patients (EHI). A total of 1365 circular RNAs (circRNAs), 30 miRNAs, and 2049 mRNAs were shown to be differentially expressed when PBMC of ART-naive and EHI patients were compared to HCs. Among miRNAs, mir-novel-chr21_21352, miR-101-3p, and miR-31-5pmiRNA were those whose expression was most differentially modulated in PBMCs from EHI. Notably, thanks to the inclusive approach of this technique circRNAs regulating HIV replication were for the first time associated to the control of HIV pathogenesis in EHI. It would be interesting to verify if the same circRNAs profile characterize HESN, thus providing novel potential targets for influencing HIV-infection/replication [151].

Single-cell omics is another recent technique that offers a new chance to explore HIV pathogenesis from an exclusive and extraordinary point of view. The field of single-cell omics has been boosted by improvements in numerous techniques including high-throughput single-cell separation methods and sophisticated analytical methods to investigate genomes, epigenomes, transcriptomes, and proteomes at single-cell resolution. In the field

of HIV-1 research some single-cell transcriptomic studies have been published [152,153]. Proteins, unlike nucleic acids, lack technologies for their amplification, thus the investigation of the proteome at single-cell level is rather challenging.

Metabolomic, a large-scale study of metabolites, within cells, biofluids, tissues or organisms, is another innovative and powerful technique. Indeed, metabolites and their concentrations directly reflect the underlying biochemical activity and state of cells/tissues. Thus, metabolomics best represents the molecular phenotype. This approach was recently used to define plasma metabolomics bio-signatures that may relate to HIV infection, rate of disease progression, and immunological response to therapy [154]. The study allowed to identify 25 distinctive metabolites, five of which discriminate between rapid progressors and immunological non-responders. Likewise, a study by Tarancon-Diez *et al.* investigated pathways and potential biomarkers associated with loss of virological control in ECs [155]. Results showed that, before the loss of control, ECs displayed a definite circulating metabolomic profile characterized by aerobic glycolytic metabolism, deregulated mitochondrial function, increased immunological activation and oxidative stress [155]. As there is a close relationship between oxidative stress status [156] and immune activation [4] in HIV infection/progression the application of these methodologies to the characterization of HESN cohorts would significantly increase our knowledge on their phenotype.

As summarized above, "-Omics" techniques can be used to acquire a "system-wide" understanding of several essential biomedical processes. A plethora of new data has been rapidly and inexpensively acquired at different molecular levels, even in the field of HIV-infection allowing to monitor the effectiveness of treatment and opening new approaches to drug development. A further step in the understanding of the molecular complexity of disease aetiology is represented by the integrative analyses of the so-called "Big Data" obtained across multi-level omics studies set in a synergistic fashion. Such integration is

undoubtedly more powerful than single level analysis but requires new ways to analyse the overwhelming amount of the data that are gathered [157].

The use of these omics approaches to better characterize individuals who naturally resist HIV-1 infection will definitely contribute to the clarification of the complex molecular interactions that generate this phenotype.

CONCLUSIONS

In the present review, we have summarized the state-of-the-art knowledge of the genetic and epigenetic basis of the diverse susceptibility to HIV-1 infection in HESN and of the different progression to AIDS in HIV-infected individuals. What is rather evident is that in spite of the undeniable advancements accomplished in recent years in this field, none of the correlates of protection so far identified can be regarded as the unique responsible for resistance to HIV infection. The HESN phenotype, thus, seems to result from the fortunate blend of multiple factors acting at different biological levels. While the genetic modulation of some key physiological pathways, such as antigen presentation, vitamin D and type IV interferons, is more and more firmly associated to the control of HIV infection/replication, the role of epigenetics in this field has been only marginally investigated. [84,141]. Considering that epigenetic modifications can be influenced by several factors including environment/lifestyle, further studies on HESN cohorts, living in different areas of the world and with a differential exposure to the virus are therefore mandatory to provide some important hints in the setting up of new antiviral strategies.

Interdisciplinary multi-Omics approaches, gathering Big data, are today available to detect host factors that control susceptibility to HIV-1 infection at different biological levels. GWAS, exome sequencing, transcriptomic, miRnomic, proteomic and metabolomic investigations allow to simultaneously analyse hundreds of thousands of molecules regulating host-virus interactions. The development of bioinformatic systems permitting the integrative analyses of all these data represents the future challenge to unveil the HESN secret.

EXPERT OPINION

Since the initial discovery of HIV epidemic, the identification of highly-exposed seronegative (HESN) individuals introduced into the scientific community the concept that natural resistance to HIV-1 infection though unusual, was possible. From then on, numerous HESN cohorts with different route of exposure and varied epidemiological background were identified worldwide, generating the expectation to identify and translate HESN correlates of protection into an effective therapy or neutralizing vaccine to prevent/control infection. Such expectancy has been partially satisfied, as documented by the cases of the “Berlin patient” in 2008 and “London patients” in 2019 [158]. Indeed, exploiting the notion that in the absence of CCR5 co-receptor expression CD4+ cells cannot be infected, these patients received a

bone marrow transplant to treat a haematological malignancy from selected donors who also carried the CCR5-Δ32 mutation and apparently were cured from HIV. Although it clearly not conceivable to use this approach in every HIV patients, such therapeutic successes suggest that host genetic variants interfering with viral life cycle have an important clinical value, indicating that the identification of suche variants in subjects who naturally resists HIV-infection is definitely worthwhile.

For the same reason, the investigation of molecular mechanisms that can alter the expression of genes involved in viral replication, represents an exciting and still unexplored research field. As a matter of fact, epigenetic studies on HESN cohorts are almost lacking probably for two main reasons. The first one is that such research area is relatively new. The molecules responsible for genetic and post transcriptional control of gene expression have been only recently identified and new ones are continuously recognized. Likely, while their role in the progression of infection is progressively becoming evident, the same factors will be analysed in relation to susceptibility to HIV-infection. The second and more general reason concerns the definition of HESN status itself. Actually, the resistant phenotype is attributed only upon repeated and documented exposure to the virus, classically over a period of years. Hence, analyses are usually performed when the biological mechanisms

that could be responsible for natural resistance are no longer active, except for the genetic profile of course. The risk is, therefore, to obtain false negative results in HESN when analysing correlates of protection that are dependent on gene expression. A possible strategy could be to replicate whatever finding, resulting from the dissection of the HESN phenotype, into other cohorts of subjects displaying an unconventional relationship with HIV. LTNP and EC provide such an opportunity. Indeed, most correlates of protection associated with a reduced susceptibility to HIV infection have been connected with control of viral replication in HIV infected individuals as well, and vice versa [6].

Finally, the OMICS era that we are experiencing, offers a unique chance to gather a huge amount of data, unconceivable until a few years ago. Such "Big data" can provide a three-dimensional overview of the HESN profile, through the investigation of all the biological levels that define an individual: from genomic to transcriptomic, from proteomic to metabolomics etc. The great future challenge for the scientists committed in the disclosure of the HESN secret, is to be able to manage and integrate such information to figure out which protective factors must be combined and triggered to resist/control HIV-infection.

FIGURE LEGEND

Figure 1. Schematic representation of the different determinants associated with the HESN phenotype discussed in this review. Such depicted correlates of protection from HIV-1 infection are grouped as genetic polymorphisms (yellow background), epigenetic mechanisms targeting the DNA or the histones (light blue), and miRNA (light green).

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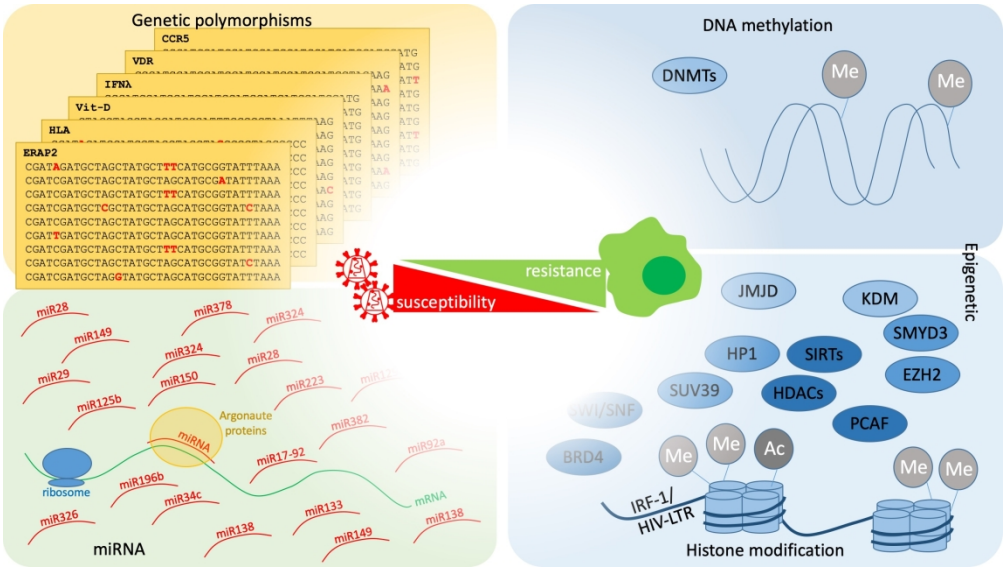

Schematic representation of the different determinants associated with the HESN phenotype discussed in this review. Such depicted correlates of protection from HIV-1 31
infection are grouped as genetic polymorphisms (yellow background), epigenetic mechanisms targeting the DNA or the histones (light blue), and miRNA (light green).

338x190mm (225 x 225 DPI)

Gene variants	Associated to	Mechanism of action(s)
HLA-B*27	Slow progression to AIDS	Presentation of HIV immunogenic epitope (19, 20)
HLA-B*35	Fast progression to AIDS	Reduced presentation of immunogenic epitope (20)
HLA-B*35	Associated to mother-to-child-transmission	Reduced presentation of immunogenic epitope (24)
HLA-B*57	Slow progression to AIDS	Presentation of HIV immunogenic epitope (20)
HLA-B*57	Protective against mother-to-child-transmission	Presentation of HIV immunogenic epitope (24)
HLA-B*57:01	Slow progression to AIDS	Presentation of HIV immunogenic epitope (22)
HLA-B*53:01	HIV set point	Presentation of HIV immunogenic epitope?(21)
HLA-B*27:03	HIV set point	Presentation of HIV immunogenic epitope?(22)
HLA - A*02:01	Being infected HIV	Unknown (23)
HLA-DQB1*03:02	Immune protection	Unknown (23)
HLA—A*23:01	HIV set point	Presentation of HIV immunogenic epitope?(22)
-35 HLA-C	Increased risk of infection	Reduced expression of HLA-C (21)
ERAP2 HapA	Resistance/Susceptibility	CD8 T cell activation (4,8,11,13, 28)
VDR rs1544410	Slow progression to AIDS	Reduced VDR mRNA (38, 39)
VDR rs10735810	Slow progression to AIDS	Lower VDR protein transactivation (40)
VDR rs4516035	Slow progression to AIDS	Reduces Th2 polarization (40, 41,42)
VDR rs2228570	Slow progression to AIDS	Induced CD4 T cell recovery (41, 44)
IFNL3 rs12979860	Resistance/ progression to AIDS	Induce production of antiviral ISG (55, 56, 58, 60)
IFNL4 rs368234815	Resistance/ Slow progression to AIDS	Induce production of antiviral ISG (56, 58, 61)

Table.1 Genes involved in resistance to HIV-1 infection and or in disease progression to AIDS

	Target(s)	miRNA	Effect(s) on HIV	Reference(s)
HIV-factors	**Nef**	miR-29a, miR-28b	Impair HIV-1 replication	(129, 145, 148)
	Env	miR-378	Impair HIV-1 replication	(129)
	Vif	miR-324-5p, miR-155	Impair HIV-1 replication	(129,142)
	Vpr	miR-149	Impair HIV-1 replication	(129)
	3'UTR mRNA	miR-28, miR-125b, miR-150, miR-223, an miR-382-5p, miR1290, miR-196b	Impair HIV-1 replication and control viral load	(130, 131, 132, 135, 141, 142, 148)
	3' LTR	miR-133b, miR-138-5p, miR-326, miR-149-5p, miR-92a-3p,	Impair HIV-1 replication	(134, 148)
	Tat	miR-15a, miR-15b, miR-16, miR-20a, miR-93, miR-106b	Impair HIV-1 replication	(136)
Host cell factors	**PCAF**	mir-17-92	Impair HIV-1 replication	(128)
	cyclin T1	miR-198, miR-27b, miR-29b, miR-223	Impair HIV-1 replication	(137, 138, 148)
	IP-10	mir-21	Impair disease progression	(139)
	TNPO3	miR-128	Impair HIV-1 replication and nuclear import	(140)
	IL-2	miR-9	Impair HIV-1 replication	(143)
	PTEN	miR-19	Impair HIV-1 replication	(144)

Table2. A list of miRNAs nvolved I HIV-1 replication