

Review Article

DNA Methylation and Its Role in the Development of Leukemia

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ABSTRACT

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Epigenetic changes play an essential role in cancer pathogenesis. It has been established by next-generation sequencing that more than 50% of the human cancers carry mutations in mechanisms involved in the organization of the chromatin and epigenetic regulations. DNA methylation is among the most common epigenetic changes in leukemia. In contrast to DNA mutations which are passively inherited from DNA replication, epimutations, for example, the hypermethylation and epigenetic silencing of tumor suppressor genes, must be actively maintained because of being reversible. Actually, the reversibility of epimutations by small-molecule inhibitors provides the basis for the use of such inhibitors in new cancer therapy strategies. However, DNA methylation mechanism and its role in leukemia are not fully understood; there are some serious concerns about the use of these drugs. In this study, we will review the mechanisms of DNA methylation and the genes that are methylated in leukemia. Moreover, new interesting findings of the epigenetic changes caused by adult T-cell leukemia/lymphoma have been fully discussed.

Introduction

The science of epigenetics is the study of inherited changes in phenotype or gene expression [1]. Mechanisms of epigenetic regulation in mammals contains DNA methylation, post-translational modification of histones, chromatin remodeling, micro-RNA and long noncoding RNAs [2]. Aforementioned mechanisms play a critical role in the regulation of the molecular processes such as transcription, replication, repair, and RNA processing. DNA methylation is commonly disrupted in diseases such as cancer [3]. Genes that are hypermethylated in cancers include those involved in the cell cycle (*PI4ARF*, *Rb*, *p15INK4a*, and *p16INK4a*), DNA repair-related genes (*BRCA1*, *MGMT*) and Apoptosis-related genes (*DAPK*, *TMS1*) [4]. Understanding the underlying mechanisms involved in the regulation of the epigenetic can be a great help in the diagnosis and treatment of several diseases. In general, DNA methylation tends to inhibit transcription [5]. In cancers, generally, tumor suppressor genes tend to be hypermethylated and oncogenes tend to be hypomethylated [6]. Many drugs have been designed based on changes in epigenetic mechanisms five of which are successful in obtaining Food and Drug Administration approval including Azacitidine (Vidaza), Decitabine (Dacogen), Belinostat (Beleodaq), Panobinostat (Farydak), Romidepsin (Istodax), and Vorinostat (Zolinza) [7]. Interestingly, all of them treat the diseases related to leukemia and show the importance of this therapeutic approach in leukemia treatment. In this article,

we will review recent findings on the role of DNA methylation in leukemia progression.

Methylation mechanism

DNA methylation has been found in the eukaryotic and prokaryotic genome being involved in various biological processes including gene silencing, X chromosome inactivation and imprinting [8]. Methylation occurs in dinucleotide cytosine with transmitting methyl group of s-adenosyl methionine to position 5-cytosine by enzyme DNA methyltransferase [9]. CpG islands are often located in the promoter and the first exon of genes [10]. In mammals, DNA methylation occurs almost exclusively in CG dinucleotides and is estimated to occur at ~70–80% of CG dinucleotides all over the genome [11]. Of the approximately 28 million CpGs in the human genome, 60% to 80% are methylated in somatic cells [12]. Methylation of CpG islands, specifically those islands colocalized with promoters or other regulatory regions, is usually related to gene repression [13]. Methylation in frequent regions such as centromeres is significant for chromosomal stability [14] and is also likely to suppress the expression of transposable elements thereby having a function in genome stability [15]. Mammals have 3 types of DNA methyltransferase (DNMT): DNMT1, DNMT3a, and DNMT3b. DNMT1 is the most greatly DNMT in cells and act is as the principal maintenance methyltransferase to methylate hemimethylated DNA after DNA transcription and preserves parental DNA methylation templates in daughter cells. In

contrast, DNMT3a and DNMT3b act as de novo methyltransferases to methylate entirely unmethylated CpG sites [16]. Identification of the exact role of DNMT3A in controlling the expression of the genes involved in hematopoiesis is an important issue in this background since the decreased or increased activity of this enzyme causes irreversible complications in myeloid precursors as well as the incidence of malignancy [17].

DNA methylation in leukemia

Myeloid leukemia

Myeloid leukemia includes acute, chronic and myelodysplastic syndromes [18]. Acute myeloid leukemia (AML) is one of the most common leukemias involving many countries. Chronic myeloid leukemia (CML), which is indicated by t (9; 22) (q34;q11)/ BCR-ABL and patient treated with imatinib, can survive for many years [19]. However, a number of patients are resistant to this drug, and this indicates the role of other gene changes in addition to t (9; 22) (q34;q11) [20]. BCR-ABL in these patients and myelodysplasia syndromes have dysplasia changes and a lot of patients ultimately get acute leukemia [21]. Here we have mentioned some of the genes that are being methylated in these disorders. Genetic defects and also hypermethylation can contribute to the initiation and maintenance of AML. Hypermethylation of tumor suppressor genes is a commonly deregulated mechanism in AML and CML [22].

Acute myeloid leukemia

The *E-cadherin* gene (*E-cad*) is located on chromosome 16q22.1 and is often named a “metastasis suppressor” gene because the

E-cadherin protein can suppress tumor cells invasion and metastasis [23]. *E-cadherin* expression is necessary for erythroblast and normoblast maturation. Cadherin gene hypermethylation has been detected in DNA of 78% of patients with leukemia, containing both acute and chronic types (AML, Acute lymphocytic leukemia (ALL), and chronic lymphoid leukemia (CLL) actually both alleles of the *E-cadherin* gene are often hypermethylated [24].

CXXC5 is located on 5q31.2, a region recurrently deleted in AML with del (5q) [25]. *CXXC5* mRNA was down-regulated in AML with *MLL* rearrangements, t (8;21) and *GATA2* mutations as a mechanism of *CXXC5* inactivation [26]. Patients with *CXXC5* expression under the medial level had a lower relapse rate and better overall survival, of course, regardless of cytogenetic risk groups and known molecular risk factors. Lower *CXXC5* expression was associated with up-regulation of cell cycling genes and co-down-regulation of involved genes in leukemogenesis (*WT1*, *GATA2*, *MLL*, *DNMT3B*, *RUNX1*). *CXXC5* inhibit leukemic cell proliferation and Wnt signaling and impress the p53-dependent DNA damage response [27]. Epigenetic modifications, such as hypermethylation DNA as well as transcriptional regulation by factors like *GATA2* and *WT1* might contribute to aberrant *CXXC5* expression in AML [28]. Metallothionein III (MT3) is a tumor inhibitor. MTs have been proposed to play significant roles in protecting against DNA damage, apoptosis and oxidative stress [29].

Overexpression of MT3 may inhibit proliferation and stimulate apoptosis in AML cells. Epigenetic inactivation of MT3 via promoter hypermethylation has been detected in both AML cell lines and pediatric AML samples. Patients with methylated MT3 have displayed lower levels of MT3 expression compared to those with unmethylated MT3 [30].

In AML cells, the EphB1 transcript was reversely correlated with EphB1 promoter methylation [31]. The presence of EphB1 allowed EfnB1 ligand-mediated p53 DNA binding, leading to the recovery of DNA damage response cascade by the activation of ATR, Chk1, p53, p21, p38, CDK1^{tyr15}, and Bax, and down-regulation of heat shock protein 27 and Bcl2. Comparatively, the reintroduction of EphB1 expression in EphB1-methylated AML cells increased the same cascade of ATR, Chk1, p21, and CDK1^{tyr15}, which consequently induced programmed cell death. Interestingly, in pediatric AML, EphB1 peptide phosphorylation and mRNA expression are actively suppressed, and a considerable percentage of the primary AML has EphB1 promoter hypermethylation [32].

GATA-1 and PU.1 are two significant hematopoietic transcription factors that mutually inhibit each other in progenitor cells to direct entrance into the erythroid or myeloid lineage, respectively. PU.1 is controlled during myelopoiesis by binding to the distal URE enhancer whose deletion leads to AML. Moreover, GATA-1 together with DNMT1 mediates the suppression of the *PU.1* gene through the URE. Suppression of the *PU.1* gene

includes both DNA methylation at the URE and its histone H3 lysine-K9 methylation and deacetylation as well as the H3K27 methylation at extra DNA elements and the promoter [33].

Chronic myeloid leukemia

The *SHP-1* gene is situated on human chromosome 12p13 and is a non-receptor type protein-tyrosine phosphatase negatively adjusting growth-promoting signaling molecules [34]. Up-regulated DNMT1 may contribute to the disease development in CML by inducing improper hypermethylation of *SHP-1* promoter. Decreased expression of *SHP-1* may play an essential role in the progression of CML to blast crisis [35].

The *human Homeobox (HOX)* gene regulates the progression process, hematopoietic differentiation, and leukemogenesis. Silencing of *HOX* genes by DNA methylation is thought to disrupt the normal progression of blood cells and therefore be involved in leukemic transformation [36]. Increased epigenetic silencing of potential tumor inhibitor genes correlates with disease development in some CML patients treated with Imatinib and this suggests relevance between epigenetic silencing and resistance progression. *HOXA4* hypermethylation is related to a higher risk for Imatinib resistance [37]. Another study indicated *HOXA4* promoter hypermethylation in CLL and AML [38]. The repression of *HOXA4* expression by hypermethylation induced gene silencing can be one of the potential mechanisms in BCR-ABL independent pathway inducing Imatinib resistance in CML patients [39].

PU.1 is a member of Ets family transcription factor which plays a principal role in the progression of lymphoid and myeloid cells and regulation of expression of lineage-specific genes [40]. Down-regulation of PU.1 expression at the mRNA and protein levels has shown a relation with aberrant methylation. Aberrant methylation has shown the promoter region of transcription factor PU.1 in CML patients both in chronic phase and blast crisis phase. Methylation of the proximal promoter of the *ABL1* oncogene is a prevalent epigenetic alteration associated with the clinical development of CML. *ABL1* methylation has showed a majority of colonies from blast crisis, but not chronic-phase CML. Specific methylation of the Ph-associated *ABL1* allele accompanies clonal progression in CML [41].

Myelodysplastic syndrome

Glutathione peroxidase 3 (GPX3) located on the 5q23, plays an important role in preventing oxidative damages by reducing extra reactive oxygen species [42]. *GPX3* methylation has shown 15% of MDS patients which is lower than AML patients. *GPX3* methylated patients had a higher frequency of DNMT3A mutation and have shown remarkably shorter overall survival. *GPX3* methylation is associated with incompatible prognosis and leukemia transformation in MDS [43].

Suppressor of cytokine signaling-1 (SOCS-1) is a significant factor in the transition of extracellular cytokine signals to the nucleus and adjust cellular processes involved in cell growth, differentiation and transformation [44]. Aberrant methylation of SOCS-1 induces transcriptional silencing in myeloid cells and

the activity of the Janus kinase/STAT pathway and expression *BCL2L1* increases [45].

Myelodysplastic/Myeloproliferative neoplasm

Characteristics of both groups of myeloproliferative diseases and myelo-dysplastic syndromes are shown with the increasing variability in cell count, cytopenia, and morphology of dysplasia. These disorders also involve epigenetic changes, including DNA methylation listed below.

Chronic myelomonocytic leukemia (CMML)

p15INK4b is a regulator of cell-cycle ceased in the G1 phase of the cell cycle through the inhibition of cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 6 (CDK6) [46]. Novel small RNAs, including microRNA-29b [47] and *p15-AS* [48], are as regulators of *p15INK4b* expression and *p15INK4b* DNA methylation simultaneous with repressive histone modifications. Hypermethylation of *p15INK4b* occurs in more than 75% of the case of AML [49]. *p15INK4b* gene methylation occurs mostly in high-risk MDS with an increased tendency to advance to blast transformation [50]. Aberrant *p15INK4b* gene methylation occurs in up to 58% of the cases of CMML and a high degree of methylation has demonstrated a great decrease or nearly a complete lack of *p15INK4b* expression. Upregulation of all three DNA methyltransferases has been detected in CMML with a high degree of *p15INK4b* gene methylation [51].

Juvenile myelomonocytic leukemia

Six genes including *BMP4*, *CALCA*, *CDKN2A*, *CDKN2B*, *H19*, and *RARB* in JMML undergo methylation [52] and four genes *BMP4*, *CALCA*, *CDKN2A*, and *RARB* are significantly associated

with poor prognosis [52]. Studies have shown that DNA hypermethylation is related to poor overall survival and a high risk of treatment failure [53].

Lymphoid leukemia

Lymphoid leukemia has been divided into three categories including B-cell leukemia, T-cell leukemia, and NK-cell leukemia [54]. The acute form of both B and T lineage can be seen in both adolescence and childhood. The common chromosomal anomalies in pediatric ALL include t(12;21)(p12;q22)/ETV6-RUNX1, t(1;19)(q23;p13)/TCF3-PBX1, t(9;22)(q34;q11)/BCR-ABL and t(4;11)(q21;q23)/MLL-AF4 [55]. In general, prognosis in children is better than adults, and the rate of relapse in adults is higher than that of children [56]. Moreover, DNA methylation occurs in these disorders; and we explain some of these below.

CLL is a chronic clonal disorder which is characterized by progressive accumulation of lymphocytes and clonal B cells arrest differential in the naive B cell stage [57]. Common cytogenetic abnormalities included del(13)(q12.3), del(17)(p13) and trisomy 12 [58]. Also, DNA methylation occurs in these disorders some of which have been explained below.

Adult T-cell leukemia (ATL) is one of the important types of lymphoid leukemia which is caused by human T-cell leukemia virus type I (HTLV-I) [59]. ATL has attracted increasing attention because of the new findings in the signaling pathways and HTLV-1 caused epigenetics alterations [60]. In this subsection, epigenetic alterations, chromatin remodeling, transcriptomic alterations, and genomic

alterations which are caused by HTLV-1 are completely covered.

Acute lymphoblastic leukemia

P57KIP2 encodes a cyclin-dependent kinase inhibitor (CDKI) that belongs to the CIP/KIP family and is considered a putative tumor suppressor gene [61]. Methylation of a region in close proximity to the transcription start position of *p57KIP2* is related to gene silencing. Aberrant methylation of *p57KIP2* has been observed at initial presentation and at relapse in adult ALL and methylation of a cell-cycle regulatory pathway involving *p73*, *p15*, and *p57KIP2* has been detected as a subgroup of patients with Philadelphia chromosome (Ph)– a negative disease with poor prognosis [62].

Ras-association domain family 1 isoform A (RASSF1A) regulates several essential biological processes including cell-cycle development and apoptosis [63]. P53 connects the RASSF1A promoter, recruiting DAXX as well as DNA methyltransferase 1 (DNMT1) for DNA methylation, which eventually results in inactivation of RASSF1A in wild-type p53 ALL cell and induces overexpression of DAXX leading to enhanced *RASSF1A* promoter methylation. p53/DAXX-mediated RASSF1A methylation regulates murine double minute 2 (MDM2) protein constancy in ALL [64].

Adult T-cell leukemia

Kruppel-like factor 4 (KLF4) gene is a cell cycle regulator and *early growth response 3 (EGR3)* gene is an essential transcriptional factor for the excitation of Fas ligand (FasL) expression. DNA methylation of *KLF4* gene is related to its silencing in ATL and *EGR3* gene is silenced by histone deacetylation rather than

by DNA methylation showing a commensurate increase in the methylation density of these regions with disease development [65].

Polycomb-dependent epigenetic alteration in ATL

NF- κ B shows high expression in ATL that results from a HTLV-I infection [66]. It has been revealed that NF- κ B plays several roles in proliferation, inflammation, and especially anti-apoptotic mechanism [67] all of which are important in oncogenesis [68]. NF- κ B signaling can be activated by NF- κ B inducing kinase (NIK) [69]. NIK can be targeted and consequently regulated by miR-31. Interestingly, the YY1 binding motif is located in the miR-31 gene and causes polycomb repressive complex 2 (PRC2) recruiting and then suppression of *miR-31* expression through histone H3Lys27 (H3K27me3) trimethylation. PRC2 consists of three core subunits: Eed, Suz12, and Ezh2. Hence, by silencing miR-31, Ezh2 can indirectly activate NIK and NF- κ B signaling and lead to apoptosis resistance [70]. HTLV-1 oncoprotein Tax is an influential activator of Ezh2 [71]. As a result, it can suppress many genes including miR-31 and KDM family, thus encoding the H3K27me3 demethylase, by affecting Ezh2 [72].

The effect of HTLV-1 proteins on chromatin remodeling

It has been shown that HTLV-1 Tax can cause chromatin remodeling by interfering with the miRNA machinery [73]. miRNA microarray analysis has revealed suppression of three miRNAs (has-miRs-135b, 149, and 872) from nine identified miRNAs for P/CAF, and also shown down-regulation in specific miRNAs for

p300 including hsa-miRs-149, 872, and 873 after introducing Tax protein [74].

HTLV-1 can inhibit apoptosis by HTLV-1 bZIP factor (HBZ) [75]. HBZ targets FoxO3a and so leads to down-regulation of Bim and FasL [76]. For inhibiting FoxO3a, two mechanism have been shown by Clerc et.al: HBZ interplay with FoxO3a and inhibition of phosphorylated FoxO3a nuclear export [77]. The first one is a more important mechanism associated with chromatin remodeling. Apoptosis can be suppressed by the LXXLL-like motif of HBZ, while interaction with FoxO3a can occur by the central domain [78]. Moreover, the interaction between LXXLL-like motif and the KIX domain of histone acetyltransferase p300/CBP has been reported, which results in a decrease in the level of histone acetylation. Furthermore, HBZ very likely plays an important role in CpGs hypermethylation in *Bim* promoter and causes long-term suppression of *Bim* gene [75].

HTLV1 caused transcriptomic alteration

miRNAs regulate the expression of a variety of genes; that's why they can modulate apoptosis, cell proliferation, cell-cycle timeline, and signaling [79]. To evaluate the effect of HTLV-1 on aforementioned biological activities, miRNA expression in determined ATL cell lines was profiled by Yeung et al. They indicated up-regulation of six miRNAs and the targeting of tumor protein 53-induced nuclear protein 1 (TP53INP1) by two of them including miR-93 and miR-130b. They concluded that, miR-93 and miR-130b can increase cell survival and proliferation by TP53INP1 suppression [80]. It has been established that HTLV-1 Tax is associated with metastasis by

activating NF- κ B signaling. NF- κ B can also induce Fascin (FSCN-1). FSCN-1 is a 54-58 kilodalton actin-bundling protein and, on the other hand, play an important role in migration and metastasis [81]. Collapsin response mediator protein 2 (CRMP2) can organize the cytoskeleton and has a key role in migratory of lymphocyte to the central nervous system [82]. The effect of HTLV-1 Tax on a greater phosphorylation level and, as a result, higher activation of CRMP2 has been revealed by Varrin-Doyer et al. Furthermore, they showed that the axis of CRMP2/PI3K/Akt is the key pathway in increasing lymphocyte migration and cytoskeleton organization [83]. In the end, all of these results elucidate the aforementioned axis having a major role in metastasis. The effect of Tax protein on the SDF-1/CXCR4 axis activation has also been observed [84]. Moreover, the SDF-1/CXCR4 axis was shown as a central pathway in the migration of the leukemic cells. Therefore, it could be as another Tax-based metastasis mechanism [85]. Two studies played crucial roles in broadening our insight into the effect of the HTLV-1 on the interferon and interleukin signaling. In the first one, it has been revealed that interferon regulation factor 3 (IRF3) can be regulated both positively and negatively by two different pathways. In a positive pathway, Tax activates transforming growth factor- β -activated kinase 1 (TAK1) and then this kinase induces the activation of the TBK1-IRF3 axis and surely some IFN-stimulated genes such as *CCL5* and *CXCL10*. On the other hand, in the negative pathway, the up-regulation of *IRF4* can suppress *TAK1* [86]. In another study, it was

revealed that Tax-dependent NF- κ B activation can increase the expression of Interleukin-9 and, as a consequence, the cell-proliferation in the primary ATL cells [87].

Furthermore, it has been reported that more expression of IFN-inducible genes in chronic HTLV-1 infection not only fails to eliminate the infection but interestingly can cause HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) because they cannot down-regulate the Tax protein as a viral transcriptional transactivator [88]. HTLV-1 P30 protein can change the expression of many genes. Tylor et al. used microarray analysis and showed a 2.5-fold enhancement in the expression of 15 genes and a reduction in the expression of 65 genes [89].

HTLV1 caused genomic alteration

It has been revealed that HTLV1 can cause genetic instability in established ATL cell lines [90]. In one of the most important studies which supports this idea, it has indicated that miR17 and miR21 targets the DNA-damage effector OBFC2A-hSSB2 and these miRNAs can themselves be downregulated by HBZ [91].

Chronic lymphoid leukemia

A study has shown that 22 genes undergo methylation in CLL patients. These genes include *SOX11*, *DLX1*, *FAM62C*, *SOX14*, *RSPO1*, *ADCY5*, *HAND2*, *SPOCK*, *MLL*, *ING1*, *PRIMA1*, *BCL11B*, *LTBP2*, *BNC1*, *NR2F2*, *SALL1*, *GALGT2*, *LHX1*, *DLX4*, *KLK10*, *TFAP2* and *APP* and has shown that IgVH mutational status or zeta-chain associated protein-70 expression is not related to particular methylation profiles, methylation of LINE and APP is associated with a shorter overall

survival, and methylation of *LINE* and *SALL1* is accompanied by a poor prognosis [92].

Diagnosis and prognosis

There are various methods to detect DNA methylation containing methylation-specific polymerase chain reaction which can monitor the state of methylation of CpG on an island [93]. Methylation-sensitive single nucleotide primer extension evaluates the types of methylation at specific CpG location. combined bisulfite restriction analysis determines methylation levels in the locus-specific gene with a small amount of DNA [94]. Methylight is a high-sensitivity method that detects methylated alleles in the presence of more than 10,000 nonmethyl alleles, quantitative analysis of methylated alleles, and enzymatic regional methylation assay which determines the precise size of the methylation concentration of the region under study [95]. MethylQuant is a method which can determine the exact amount of specific cytosine methylation in the genome complex and reverse-phase high-performance liquid chromatography determines 5-methyl cytosine levels at low DNA levels [96].

Many studies have shown that DNA methylation can predict clinical outcomes and serve as a marker for risk classification. In CLL, DNA aberrant methylation is valuable for prognosis and treatment. For example, methylation of *LINE* and *APP* is associated with shorter overall survival and methylation of *LINE* and *SALL1* is accompanied by a poor prognosis [97], or in AML, patients with a high degree of CpG methylation pattern have shown a shorter time to relapse than low CpG methylation pattern

[98]. Furthermore, hypomethylation of the regulatory region of *PBX3* is associated with the higher rates of relapse and shorter relapse-free survival in AML patients while not associated with overall survival [99].

Conclusion

Epigenome and genome are changed by several cancers especially lymphoid leukemia and lead to numerous drastic phenotypic alterations like drug resistance and immune system escape. The use of new technologies including next-generation sequencing for analyses of global genomics increases our knowledge about lymphoid leukemia and mechanisms involved in epigenetic alterations. Understanding the epigenetic pathways and DNA methylation mechanisms can help us find the Achilles heel of the many cancer types. Therefore, a new insight was established into the development of the drugs that target molecules involved in epigenetic alterations. Recent clinical trials show that these drugs have great efficacy in lymphoid leukemia treatment when used with other therapeutic approaches such as chemotherapy or especially immunotherapy. In spite of the mentioned improvements, the available epigenetic drugs have some potential risks and develop new innovative epigenetic drugs thus requiring more research. Moreover, there is a critical need for more clinical trials concerning these drugs.

Conflict of Interest

The authors declare no conflict of interest.

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