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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 causeed by adult T-cell leukemia/lymphoma have been fully discussed.

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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 (*P14ARF*, *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

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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].

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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, CDK1tyr15, 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 CDK1tyr15, 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].

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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 myelopro-liferative 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

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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 leukemi

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

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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-κB shows high expression in ATL that results from a HTLV-I infection [66]. It has been revealed that NF-κB plays several roles in proliferation, inflammation, and especially anti-apoptotic mechanism [67] all of which are important in oncogenesis [68]. NF-κB signaling can be activated by NF-κB 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-κB 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

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activating NF-κB signaling. NF-κB 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 el 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-depended NF-κB 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

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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 methyl-ation 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 concernung these drugs.

Conflict of Interest

The authors declare no conflict of interest.

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References

[1]. Fazzari MJ, Greally JM. Introduction to epigenomics and epigenome-wide analysis. In Statistical Methods in Molecular Biology 2010; Humana Press, Totowa, NJ, pp. 243-265.

[2]. Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. Nat Rev Genetics 2016; 17(8): 487.

[3]. Williams K, Christensen J, Helin K. DNA methylation: TET proteins-guardians of CpG islands? EMBO Reports. 2012; 13(1): 28-35.

[4]. Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. Trends biochem sci. 2006; 31(2): 89-97.

[5]. Bradner JE, Hnisz D, Young RA. Transcriptional addiction in cancer. Cell 2017; 168(4): 629-43.

[6]. Shahrabi S, Khosravi A, Shahjahani M, Rahim F, Saki N. Genetics and epigenetics of myelodysplastic syndromes and response to drug therapy: new insights. Oncol Rev. 2016; 10(2): 311.

[7]. Jones PA, Issa JPJ, Baylin S. Targeting the cancer epigenome for therapy. Nat Rev Genet. 2016; 17(10): 630.

[8]. Law JA, Jacobsen SE. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nat Rev Genet 2010; 11(3): 204.

[9]. Tulisiak CT, Harris RA, Ponomarev I. DNA modifications in models of alcohol use disorders. Alcohol 2017; 60: 19-30.

[10]. Anastasiadi D, Esteve-Codina A, Piferrer F. Consistent inverse correlation between DNA methylation of the first intron and gene expression across tissues and species. Epigene Chromat. 2018; 11(1): 37.

[11]. Picard CL, Gehring M. Proximal methylation features associated with nonrandom changes in gene body methylation. Genome Biol. 2017; 18(1): 73.

[12]. Wu H, Zhang Y. Reversing DNA methylation: mechanisms, genomics, and biological functions. Cell 2014; 156(1): 45-68.

[13]. Tate PH, Bird AP. Effects of DNA methylation on DNA-binding proteins and gene expression. Curr Opinion Genetics Dev. 1993; 3(2): 226-31.

[14]. Moarefi AH, Chédin F. ICF syndrome mutations cause a broad spectrum of biochemical defects in DNMT3B-mediated de novo DNA methylation. J Mol Biol. 2011; 409(5): 758-72.

[15]. Zhang J, Liu Y, Xia EH, Yao QY, Liu XD, Gao LZ. Autotetraploid rice methylome analysis reveals methylation variation of transposable elements and their effects on gene expression. Proceed Nation Acad Sci. 2015; 112(50): 7022-7029.

[16]. Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 1999; 99(3): 247-57.

[17]. Ketabchi N, Paridar M, Mohammadi Asl J, Abooali A, Kavianpour M, Saki N. Analysis of DNA methyltransferase 3A gene mutations in patients with Philadelphia-negative myeloproliferative neoplasms. Clinic Cancer Investig J. 2017; 6(1): 81.

[18]. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid leukemia. New Eng J Med. 2016; 374(23): 2209-221.

[19]. Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. New Eng J Med. 2015; 373(12): 1136-152.

[20]. Daver N, Boddu P, Garcia-Manero G, Yadav SS, Sharma P, Allison J, et al. Hypomethylating agents in combination with immune checkpoint inhibitors in acute myeloid leukemia and myelodysplastic syndromes. Leukemia 2018; 32(5): 1094.

[21]. Steensma DP. Myelodysplastic syndromes: diagnosis and treatment. Mayo Clinic Proceed. 2015; 90(7): 969-83.

[22]. Heidari N, Abroun S, Bertacchini J, Vosoughi T, Rahim F, Saki N. Significance of inactivated genes in leukemia: pathogenesis and prognosis. Cell J (Yakhteh). 2017; 19(S 1): 9.

[23]. Paul R, Ewing C, Jarrard D, Isaacs W. The cadherin cell-cell adhesion pathway in prostate cancer progression. British J Urolo. 1997; 79(S1): 37-43.

[24]. Melki JR, Vincent PC, Brown RD, Clark SJ. Hypermethylation of E-cadherin in leukemia. Blood 2000; 95(10): 3208-213.

[25]. Treppendahl MB, Möllgård L, Hellström-Lindberg E, Cloos P, Grønbaek K. Downregulation but lack of promoter hyperme-thylation or somatic mutations of the potential tumor suppressor CXXC5 in MDS and AML with deletion 5q. Euro J Heematol. 2013; 90(3): 259-60.

[26]. Kühnl A, Valk PJ, Sanders MA, Ivey A, Hills RK, Mills KI, et al. Down-regulation of the Wnt inhibitor CXXC5 predicts a better prognosis in acute myeloid leukemia. Blood 2015: 613703.

[27]. Grimwade D. Down-regulation of the Wnt inhibitor CXXC5 predicts a better prognosis in acute myeloid leukemia. 2015.

[28]. Shi Jl, Fu L, Ang Q, Wang Gj, Zhu J, Wang Wd. Overexpression of ATP1B1 predicts an adverse prognosis in cytogenetically normal acute myeloid leukemia. Oncotarget 2016; 7(3): 2585.

[DOI: 10.18502/ijml.v6i3.1396]

[29]. Howells C, West AK, Chung RS. Neuronal growth-inhibitory factor (metallothionein-3): evaluation of the biological function of growth-inhibitory factor in the injured and neurodegenerative brain. FEBS J . 2010; 277(14): 2931-939.
[30]. Tao YF, Xu LX, Lu J, Cao L, Li ZH, Hu SY, et al. Metallothionein III (MT3) is a putative tumor suppressor gene that is frequently inactivated in pediatric acute myeloid leukemia by promoter hypermethylation. J of translational medicine. 2014; 12(1): 182.
[31]. Kampen K, Scherpen F, Garcia-Manero G, Yang H, Kaspers G, Cloos J, et al. EphB1 Suppression in Acute Myeloid Leukemia: Regulating the DNA Damage Control System. Mol Cancer Res. 2015; 13(6): 982-92.
[32]. Kampen K, Scherpen F, Garcia-Manero G, Yang H, Kaspers G, Cloos J, et al. EphB1 suppression in acute myelogenous leukemia: regulating the dna damage control system. Mol Cancer Res. 2015; 13(6): 982-92.
[33]. Burda P, Vargova J, Curik N, Salek C, Papadopoulos GL, Strouboulis J, et al. GATA-1 inhibits PU. 1 Gene via DNA and histone H3K9 methylation of its distal enhancer in erythroleukemia. PloS one 2016; 11(3): 152234.
[34]. Zhang J, Somani AK, Siminovitch KA. Roles of the SHP-1 tyrosine phosphatase in the negative regulation of cell signalling. Seminars in immunology; 2000: Elsevier.
[35]. Li Y, Yang L, Pan Y, Yang J, Shang Y, Luo J. Methylation and decreased expression of SHP-1 are related to disease progression in chronic myelogenous leukemia. Oncol Reports 2014; 31(5): 2438-446.
[36]. Issa JPJ. DNA methylation as a therapeutic target in cancer. Clinic Cancer Res. 2007; 13(6): 1634-637.
[37]. Elias MH, Baba AA, Husin A, Sulong S, Hassan R, Sim GA, et al. HOXA4 gene promoter hypermethylation as an epigenetic mechanism mediating resistance to imatinib mesylate in chronic myeloid leukemia patients. BioMed Res Int. 2012; 2013.
[38]. Strathdee G, Holyoake TL, Sim A, Parker A, Oscier DG, Melo JV, et al. Inactivation of HOXA genes by hypermethylation in myeloid and lymphoid malignancy is frequent and associated with poor prognosis. Clinic Cancer Res. 2007; 13(17): 5048-5055.
[39]. Fournier M, Lebert-Ghali CÉ, Krosl G, Bijl JJ. HOXA4 induces expansion of hematopoietic stem cells in vitro and confers enhancement of pro-B-cells in vivo. Stem cells Dev. 2011; 21(1): 133-42.
[40]. Ito T, Nishiyama C, Nakano N, Nishiyama M, Usui Y, Takeda K, Kanada S, et al. Roles of PU. 1 in monocyte-and mast cell-specific gene regulation: PU. 1 transactivates CIITA pIV in cooperation with IFN-γ. Int Immunol. 2009; 21(7): 803-16.
[41]. Asimakopoulos FA, Shteper PJ, Krichevsky S, Fibach E, Polliack A, Rachmilewitz E, et al. ABL1 methylation is a distinct molecular event associated with clonal evolution of chronic myeloid leukemia. Blood 1999; 94(7): 2452-460.
[42]. Brigelius-Flohé R, Maiorino M. Glutathione peroxidases. Biochimica et Biophysica Acta 2013; 1830(5): 3289-303.
[43]. Zhou JD, Lin J, Zhang TJ, Ma JC, Yang L, Wen XM, et al. GPX3 methylation in bone marrow predicts adverse prognosis and leukemia transformation in myelodysplastic syndrome. Cancer Med. 2017; 6(1): 267-74.
[44]. Fujimoto M, Naka T. Regulation of cytokine signaling by SOCS family molecules. Trends Immunol. 2003; 24(12): 659-66.
[45]. Neumann M, Greif PA, Baldus CD. Mutational landscape of adult ETP-ALL. Oncotarget 2013; 4(7): 954.
[46]. Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. Gene Dev. 1999; 13(12): 1501-512.
[47]. Garzon R, Liu S, Fabbri M, Liu Z, Heaphy CE, Callegari E, et al. MicroRNA-29b induces global DNA hypomethylation and tumor suppressor gene reexpression in acute myeloid leukemia by targeting directly DNMT3A and 3B and indirectly DNMT1. Blood 2009; 113(25): 6411-418.
[48]. Yu W, Gius D, Onyango P, Muldoon-Jacobs K, Karp J, Feinberg AP, et al. Epigenetic silencing of tumour suppressor gene p15 by its antisense RNA. Nature 2008; 451(7175): 202-206.
[49]. Aggerholm A, Guldberg P, Hokland M, Hokland P. Extensive intra-and interindividual heterogeneity of p15INK4B methylation in acute myeloid leukemia. Cancer Res. 1999; 59(2): 436-41.
[50]. Tien HF, Tang JL, Tsay W, Liu MC, Lee FY, Wang CH, et al. Methylation of the p15INK4B gene in myelodysplastic syndrome: it can be detected early at diagnosis or during disease progression and is highly associated with leukaemic transformation. British J Haematol. 2001; 112(1): 148-54.
[51]. Tessema M, Länger F, Dingemann J, Ganser A, Kreipe H, Lehmann U. Aberrant methylation and impaired expression of the p15INK4b cell cycle regulatory gene in chronic myelomonocytic leukemia (CMML). Leukemia 2003; 17(5): 910-18.
[52]. Sakaguchi H, Muramatsu H, Okuno Y, Makishima H, Xu Y, Furukawa-Hibi Y, et al. Aberrant DNA methylation is associated with a poor outcome in juvenile myelomonocytic leukemia. PloS one 2015; 10(12): 145394.
[53]. Olk-Batz C, Poetsch AR, Nöllke P, Claus R, Zucknick M, Sandrock I, et al. Aberrant DNA

[DOI: 10.18502/ijml.v6i3.1396]

methylation characterizes juvenile myelomonocytic leukemia (JMML) with poor outcome. Blood 2011; 117(18): 4871-880.
[54]. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia. Blood 2016: 643544.
[55]. Lundin C, Heldrup J, Ahlgren T, Olofsson T, Johansson B. B-cell precursor t(8; 14) (q11; q32)-positive acute lymphoblastic leukemia in children is strongly associated with Down syndrome or with a concomitant Philadelphia chromosome. Euro J Haematol. 2009; 82(1): 46-53.
[56]. Kahn JM, Keegan TH, Tao L, Abrahão R, Bleyer A, Viny AD. Racial disparities in the survival of American children, adolescents, and young adults with acute lymphoblastic leukemia, acute myelogenous leukemia, and Hodgkin lymphoma. Cancer 2016; 122(17): 2723-730.
[57]. Fabbri G, Dalla-Favera R. The molecular pathogenesis of chronic lymphocytic leukaemia. Nat Rev Cancer 2016; 16(3): 145.
[58]. Laurie CC, Laurie CA, Smoley SA, Carlson EE, Flinn I, Fridley BL, et al. Acquired chromosomal anomalies in chronic lymphocytic leukemia patients compared with more than 50,000 quasi-normal participants. Cancer Genet. 2014; 207(1): 19-30.
[59]. Kataoka K, Nagata Y, Kitanaka A, Shiraishi Y, Shimamura T, Yasunaga JI, et al. Integrated molecular analysis of adult T cell leukemia/ lymphoma. Nature genet. 2015; 47(11): 1304.
[60]. Bangham CR. Human T cell leukemia virus type 1: persistence and pathogenesis. Ann Rev Immunol. 2018; 36: 43-71.
[61]. Watanabe H, Pan ZQ, Schreiber-Agus N, DePinho RA, Hurwitz J, Xiong Y. Suppression of cell transformation by the cyclin-dependent kinase inhibitor p57KIP2 requires binding to proliferating cell nuclear antigen. Proceed Nation Acad Sci. 1998; 95(4): 1392-397.
[62]. Shen L, Toyota M, Kondo Y, Obata T, Daniel S, Pierce S, et al. Aberrant DNA methylation of p57KIP2 identifies a cell-cycle regulatory pathway with prognostic impact in adult acute lymphocytic leukemia. Blood 2003; 101(10): 4131-136.
[63]. Donninger H, Vos MD, Clark GJ. The RASSF1A tumor suppressor. J Cell Sci. 2007; 120(18): 3163-172.
[64]. Zhang H, He J, Li J, Tian D, Gu L, Zhou M. Methylation of RASSF1A gene promoter is regulated by p53 and DAXX. The FASEB J. 2013; 27(1): 232-42.
[65]. Morales-Martinez M, Franco-Cea LA, Vargas LM, Martinez-Maza O, Huerta-Yepez S, Vega MI. Bifunctional role of kruppel-like factor 4 in hematological malignancies. Forum on immunopathological diseases and therapeutics; 2016: Begel House Inc.
[66]. Bangham CR. Human T cell leukemia virus type 1: persistence and pathogenesis. Annual review of immunology. 2018; 36: 43-71.
[67]. Taniguchi K, Karin M. NF-κB, inflammation, immunity and cancer: coming of age. Nat Rev Immunol. 2018; 18(5): 309-21.
[68]. Zou K, Li Z, Zhang Y, Zhang Hy, Li B, Zhu W-l, et al. Advances in the study of berberine and its derivatives: a focus on anti-inflammatory and anti-tumor effects in the digestive system. Acta Pharmacol Sinica. 2017; 38(2): 157.
[69]. Harhaj EW, Giam CZ. NF-κB signaling mechanisms in HTLV-1-induced adult T-cell leukemia/lymphoma. The FEBS J. 2018.
[70]. Yamagishi M, Nakano K, Miyake A, Yamochi T, Kagami Y, Tsutsumi A, et al. Polycomb-mediated loss of miR-31 activates NIK-dependent NF-κB pathway in adult T cell leukemia and other cancers. Cancer Cell 2012; 21(1): 121-35.
[71]. Nicot C. Tumor suppressor inactivation in the pathogenesis of adult T-cell leukemia. J Oncol. 2015; 2015.
[72]. Nakashima M, Kobayashi S, Tanaka Y, Iwanaga M, Utsunomiya A, Uchimaru K, et al. Polycomb-dependent epigenetic landscape in adult T-cell leukemia. Blood 2016; 127(14): 1790-802.
[73]. Pichler K, Schneider G, Grassmann R. MicroRNA miR-146a and further oncogenesis-related cellular microRNAs are dysregulated in HTLV-1-transformed T lymphocytes. Retrovirol. 2008; 5(1): 100.
[74]. Rahman S, Quann K, Pandya D, Singh S, Khan ZK, Jain P. HTLV-1 Tax mediated downregulation of miRNAs associated with chromatin remodeling factors in T cells with stably integrated viral promoter. PLoS One 2012; 7(4): 34490.
[75]. Tanaka-Nakanishi A, Yasunaga JI, Takai K, Matsuoka M. HTLV-1 bZIP factor suppresses apoptosis by attenuating the function of FoxO3a and altering its localization. Cancer Res. 2014; 74(1): 188-200.
[76]. Ma G, Yasunaga JI, Matsuoka M. Multifaceted functions and roles of HBZ in HTLV-1 pathogenesis. Retrovirol. 2016; 13(1): 16.
[77]. Clerc I, Polakowski N, André-Arpin C, Cook P, Barbeau B, Mesnard JM, et al. An interaction between the human T cell leukemia virus type 1 basic leucine zipper factor (HBZ) and the KIX domain of p300/CBP contributes to the down-regulation of tax-dependent viral transcription by HBZ. J Biologic Chem. 2008; 283(35): 23903-3913.
[78]. Murphy J, Hall WW, Ratner L, Sheehy N. Novel interactions between the HTLV antisense proteins HBZ and APH-2 and the NFAR protein

[DOI: 10.18502/ijml.v6i3.1396]

family: Implications for the HTLV lifecycles. Virol. 2016; 494: 129-42.
[79]. Kato M, Slack FJ. microRNAs: small molecules with big roles. C. elegans to human cancer. Biol Cell 2008; 100(2): 71-81.
[80]. Yeung ML, Yasunaga JI, Bennasser Y, Dusetti N, Harris D, Ahmad N, et al. Roles for microRNAs, miR-93 and miR-130b, and tumor protein 53–induced nuclear protein 1 tumor suppressor in cell growth dysregulation by human T-cell lymphotrophic virus 1. Cancer Res. 2008; 68(21): 8976-985.
[81]. Kress AK, Kalmer M, Rowan AG, Grassmann R, Fleckenstein B. The tumor marker Fascin is strongly induced by the Tax oncoprotein of HTLV-1 through NF-kappaB signals. Blood 2011; 117(13): 3609-612.
[82]. Zheng Y, Sethi R, Mangala LS, Taylor C, Goldsmith J, Wang M, et al. Tuning microtubule dynamics to enhance cancer therapy by modulating FER-mediated CRMP2 phosphorylation. Nat Communic. 2018; 9(1): 476.
[83]. Varrin-Doyer M, Nicolle A, Marignier R, Cavagna S, Benetollo C, Wattel E, et al. Human T lymphotropic virus type 1 increases T lymphocyte migration by recruiting the cytoskeleton organizer CRMP2. J Imunol. (Baltimore, Md: 1950). 2012; 188(3): 1222-233.
[84]. Twizere JC, Springael JY, Boxus M, Burny A, Dequiedt F, Dewulf JF, et al. Human T-cell leukemia virus type-1 Tax oncoprotein regulates G-protein signaling. Blood 2007; 109(3): 1051-60.
[85]. Kawaguchi A, Orba Y, Kimura T, Iha H, Ogata M, Tsuji T, et al. Inhibition of the SDF-1alpha-CXCR4 axis by the CXCR4 antagonist AMD3100 suppresses the migration of cultured cells from ATL patients and murine lymphoblastoid cells from HTLV-I Tax transgenic mice. Blood 2009; 114(14): 2961-968.
[86]. Suzuki S, Zhou Y, Refaat A, Takasaki I, Koizumi K, Yamaoka S, et al. Human T cell lymphotropic virus 1 manipulates interferon regulatory signals by controlling the TAK1-IRF3 and IRF4 pathways. J biolchem. 2010; 285(7): 4441-446.
[87]. Chen J, Petrus M, Bryant BR, Phuc Nguyen V, Stamer M, Goldman CK, et al. Induction of the IL-9 gene by HTLV-I Tax stimulates the spontaneous proliferation of primary adult T-cell leukemia cells by a paracrine mechanism. Blood 2008; 111(10): 5163-172.
[88]. Tattermusch S, Skinner JA, Chaussabel D, Banchereau J, Berry MP, McNab FW, et al. Systems biology approaches reveal a specific interferon-inducible signature in HTLV-1 associated myelopathy. PLoS Pathogens 2012; 8(1): 1002480.
[89]. Taylor JM, Ghorbel S, Nicot C. Genome wide analysis of human genes transcriptionally and post-transcriptionally regulated by the HTLV-I protein p30. BMC Genomics 2009; 10: 311.
[90]. Currer R, Van Duyne R, Jaworski E, Guendel I, Sampey G, Das R, et al. HTLV tax: a fascinating multifunctional co-regulator of viral and cellular pathways. Frontiers Microbiol. 2012; 3(1): 406.
[91]. Vernin C, Thenoz M, Pinatel C, Gessain A, Gout O, Delfau-Larue MH, et al. HTLV-1 bZIP factor HBZ promotes cell proliferation and genetic instability by activating OncomiRs. Cancer Res. 2014.
[92]. Tong WG, Wierda WG, Lin E, Kuang SQ, Bekele BN, Estrov Z, et al. Genome-wide DNA methylation profiling of chronic lymphocytic leukemia allows identification of epigenetically repressed molecular pathways with clinical impact. Epigenetics 2010; 5(6): 499-508.
[93]. Herman JG, Graff JR, Myöhänen S, Nelkin BD, Baylin SB. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proceedings Nation Acad Sci. 1996; 93(18): 9821-826.
[94]. Xiong Z, Laird PW. COBRA: a sensitive and quantitative DNA methylation assay. Nucleic Acid Res. 1997; 25(12): 2532-534.
[95]. Galm O, Rountree MR, Bachman KE, Jair KW, Baylin SB, Herman JG. Enzymatic regional methylation assay: a novel method to quantify regional CpG methylation density. Genome Res. 2002; 12(1): 153-57.
[96]. Ramsahoye B. Measurement of genome wide DNA methylation by reversed-phase high-performance liquid chromatography. Methods 2002; 27(2): 156-61.
[97]. Oakes CC, Martin-Subero JI. Insight into origins, mechanisms, and utility of DNA methylation in B-cell malignancies. Blood 2018; 132(10): 999-1006.
[98]. Li S, Garrett-Bakelman FE, Chung SS, Sanders MA, Hricik T, Rapaport F, et al. Distinct evolution and dynamics of epigenetic and genetic heterogeneity in acute myeloid leukemia. Nat Med. 2016; 22(7): 792-99.
[99]. Hájková H, Fritz MHY, Haškovec C, Schwarz J, Šálek C, Marková J, et al. CBFBMYH11 hypomethylation signature and PBX3 differential methylation revealed by targeted bisulfite sequencing in patients with acute myeloid leukemia. J Hematol Oncol. 2014; 7(1): 66.

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