Chapter

Epigenetic Landscape in Leukemia and Its Impact on Antileukemia Therapeutics

Bingzhi He, Julia Cathryn Hlavka-Zhang, Richard B. Lock and Duohui Jing

Abstract

Epigenomic landscape mapping in leukemia cells supports germ line mutation studies to understand pathogenicity and treatment plans. The differential regulation of gene expression and heterogeneity between cell types during hematopoiesis and leukemia development is important in understanding oncogenesis. Oncogenesis in leukemia occurs at both genomic and epigenomic levels in order for hematological cells to evade lineage commitment. To ensure that therapies target the entire malignancy, it is important to consider the regulatory network that drives malignancy caused by mutations. Therapies tailored to respond to a patient-specific epigenetic landscape have the potential to minimize risk in administering chemotherapies that may not work. In this chapter, a focused study on childhood acute lymphoblastic leukemia (ALL) will be used as an example of the current research in the field of epigenetics in leukemia and the impact it carries on our understanding of the disease and treatment plans.

Keywords: epigenome, childhood acute lymphoblastic leukemia, chemotherapy, glucocorticoid, epigenetic drugs

1. Epigenetics overview

Epigenetics is a biological system that describes phenotypes and occurs due to differential regulation of genes (as opposed to genetic mutations). Current epigenetic studies include the control of basic biological functions [1], developmental biology [2, 3], the origin of disease [4], and cancer therapeutics [5]. Investigating the regulatory mechanisms driving these results involves genome-wide mapping of chromatin accessibility and conformation, transcription factor (TF) binding, DNA methylation, and histone modifications. Leukemia oncogenesis is a result of both genetic and epigenetic factors, whereby hematopoietic pathways are disrupted and leukemia cells are able to evade lineage commitment. This interdependence between altered genes and gene regulation is critical in cancer development. Consequently, a broader understanding inclusive of genomics and epigenomics will allow for development of drug targets which may limit cancer progression, improve therapeutic response, and find novel targeted therapies.

1.1 Regulation of gene expression

Multicellular eukaryotic organisms consist of a complex variety of different cells, all containing identical genomic DNA. The ability to create such diversity from identical duplicates of DNA is attributed to differential gene expression regulation. Gene expression is as product of epigenetic regulatory systems. Differences within these regulations result in differential expressions and subsequently, cell types that differ in structure and function [6].

The three-dimensional organization of DNA is central to gene expression, as it depends on physical access to the gene to be expressed. Genomic DNA is condensed and wrapped around histone proteins, forming chromatin [7]. Condensed chromatin structures inhibit gene transcription by making the gene physically inaccessible for transcriptional machinery to access (**Figure 1**). To unfold chromatin structure and expose the gene for transcription, endogenous mechanisms and drugs modify the histone proteins around which the DNA is bound [8, 9]. Modifications which result in gene silencing include histone deacetylation and DNA methylation. To achieve differential expression profiles between cell types, cells have different gene access profiles controlled by protein mediation, external stimuli, and development of cells [10].

1.1.1 Histone deacetylation

Histone deacetylation involves the switching of histone proteins from a positive to neutral charge via the addition of an acetyl to a lysine residue within the histone tail. This change in charge will result in the DNA (negatively charged) separation from the histone due to repulsion forces of like charges. Separated DNA regions become accessible to transcriptional machinery as a result. When this mechanism is reversed and lysines are deacetylated, DNA is attracted back toward the protein, resulting in tight DNA wrapping, thus inaccessible for transcriptional machinery (**Figure 2**) [11]. The enzyme responsible for acetylation of histone proteins is called histone acetyltransferase (HAT), which opens the chromatin structure and allows transcription. Deacetylating enzymes which cause compacting of chromatin are called histone deacetylases (HDAC).

1.1.2 DNA methylation

DNA methylation is another mechanism of chromatin remodeling. CpG islands are target sites in the genome for methylation by the DNA methyltransferase

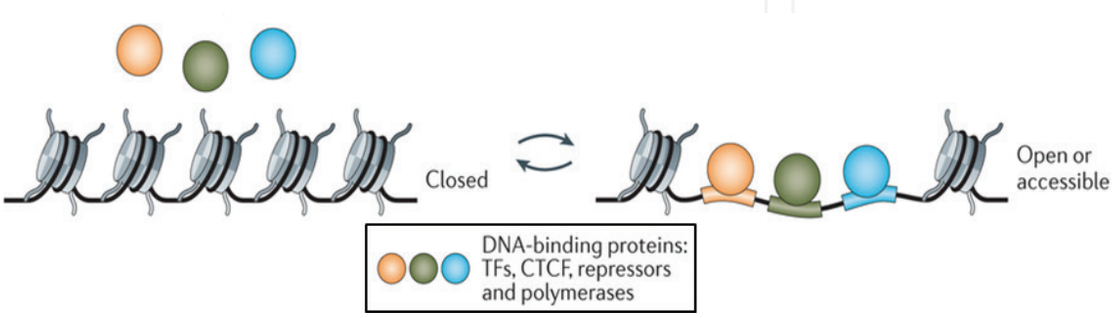

Figure 1.
Chromatin accessibility. Chromatin accessibility modulates gene expression. Densely packed chromatin (left) is inaccessible, preventing transcription factors (TFs), CCCTC-binding factor (CTCF), and polymerases from binding and subsequent gene expression. Opened chromatin structure (right) can be accessed by TFs, CTCF, and polymerases bindings, resulting in ability for gene transcription. Opened and closed chromatin structures are regulated by the acetylation status of histone proteins. Acetylated histones provide an open chromatin structure, while deacetylated histones form a closed chromatin structure. Adapted from Shlyueva et al., 2014 [10].

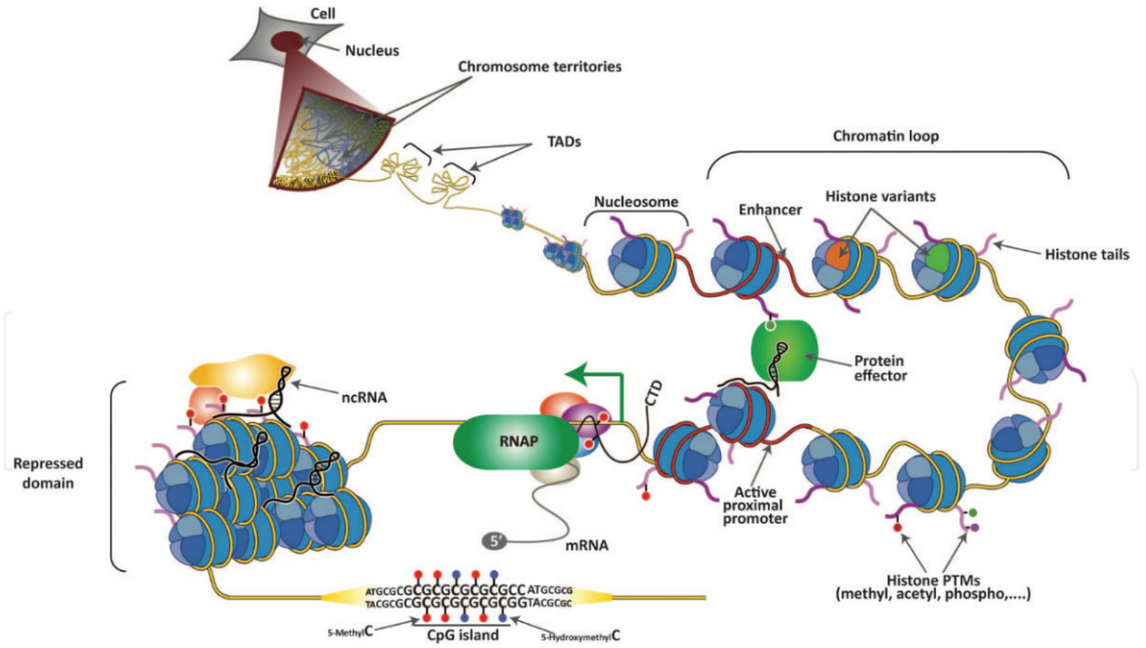

Figure 2.
Hierarchical layers of chromatin organization in mammalian cells. Adapted from Aranda et al., 2015 [11].

(DNMT) enzyme. Of the CpG sites in gene promoter regions, 70% are primary targets for methylation. DNMT prevents gene transcription via physically preventing transcription factor binding and by methylating DNA. Methylated DNA binds to methyl-CpG-binding domain (MBD) proteins that in turn recruit proteins such as histone deacetylase and other chromatin-remodeling proteins. In this new environment, chromatin becomes compact and inactive, termed heterochromatin (repressed domain in **Figure 2**).

1.2 Epigenetics in leukemia

ALL population studies indicated a trend in disease peak around the age of 5, after which there is no increase in prevalence (**Figure 3**). Evidences suggest hematopoietic regulatory network probably most highly involved in leukemia development at their highest in children <5 years old. Case-control studies have shown that the occurrence of childhood ALL is inversely linked to the degree of exposure to infections in the first few months of life [12, 13]. This suggests that there may be certain oncogenic factors present in the early days of a child's life that lead to the development of ALL, rather than being present later on or in adulthood. Addressing these up- and downregulations of oncogenic factors in this critical stage of hematopoiesis will provide insight into pathogenesis and progression of ALL beyond the genetic level.

1.2.1 Oncogenesis driven by epigenetics (in ALL)

Epigenetics is still in the early stages of investigation and translational clinical use. Primary testing in clinics focuses on cytogenic studies, categorizing disease based on genetic abnormalities and cell markers, and then treating the patient accordingly; screening gene expression to map out regulatory profiles of a tumor are less established. In acute lymphoblastic leukemia (ALL), subtypes are diagnosed based on cytogenic testing and testing for markers [14]. In vivo mouse studies, however, have indicated that in almost 75% of diagnosed cases, chromosomal changes alone are insufficient to induce ALL [15]. Investigation of

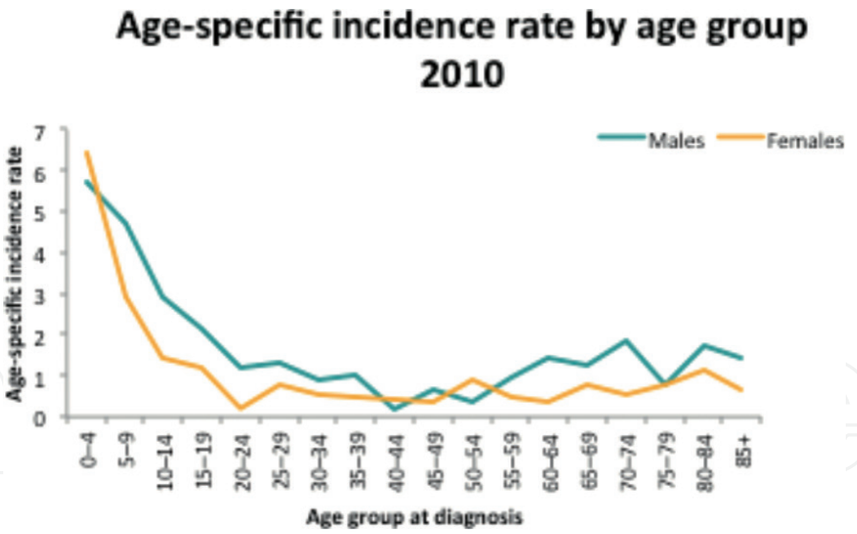

Figure 3.
Incidence of ALL per 100,000 populations in 2010. Source: Australian Institute of Health and Welfare (AIHW), 2014 [97].

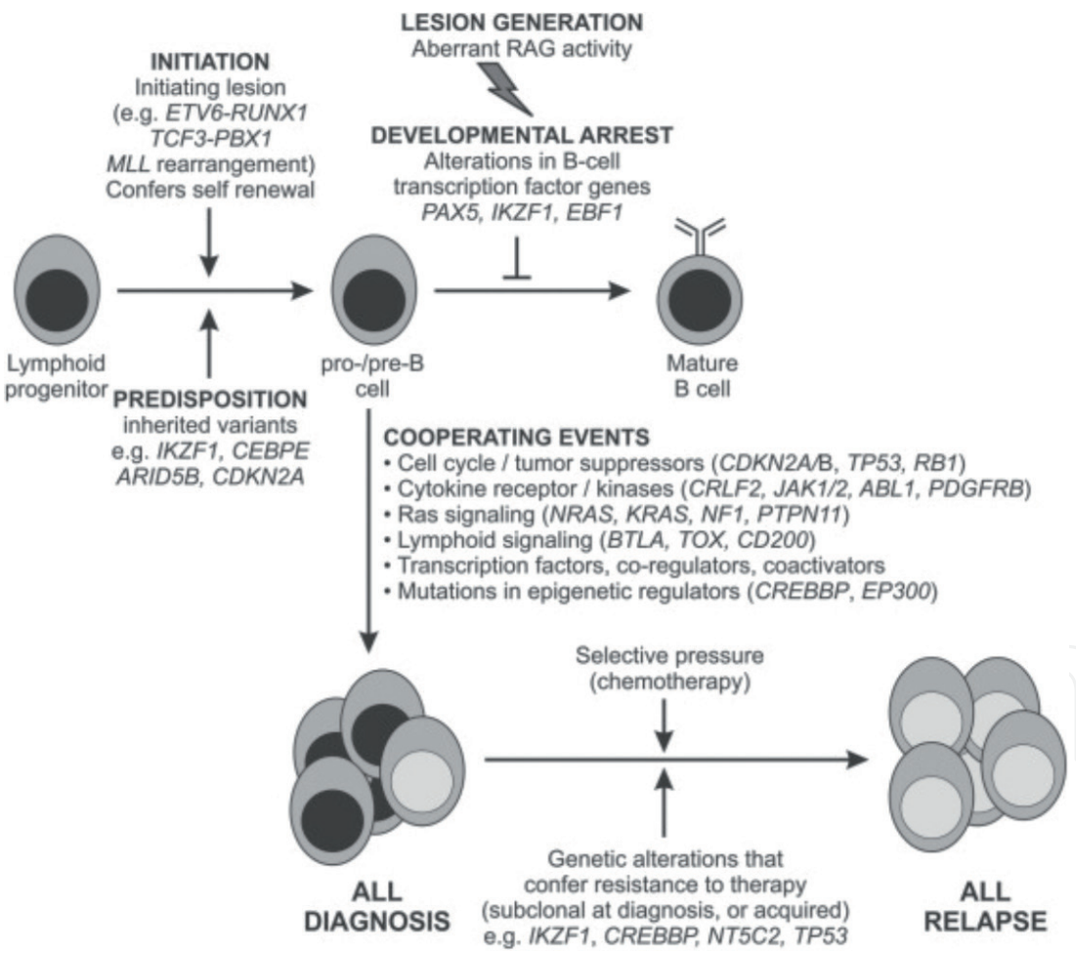

Figure 4.
B-ALL development. Multiple mutations contribute to the development of ALL. Mutations in ALL predisposing genes, e.g., IKZF1, and initiating genes, e.G., ETV6-RUNX1, and MLL rearrangement will promote ALL development. Alterations in B-cell development genes, e.G., PAX5 and IKZF1, inhibit cell maturation, resulting in the accumulation of immature cells. This alone however is not enough to cause ALL pathogenesis. Cell cycle and lymphoid lineage regulatory gene expression must also be altered to promote its survival. Further alterations may induce chemoresistance. Adapted from Mullighan, 2013 [15].

genetic alterations alongside epigenetics microarrays of gene expression suggested an association between mutations and altered regulation in gene expressions during hematopoiesis in both B-ALL and T-ALL [14, 15]. Prenatal lesions and

postnatal-acquired mutations have also shown impaired regulation and development of progenitor B cells or T cells [16–18]. Together, these studies suggest that it is not mutations alone that act as oncogenic drivers but also an altered gene regulatory network.

Thus far, ALL oncogenic studies have reported prenatal genetic lesions and inherited genetic predisposition which neither can stand alone to account for the disease. Prenatal genetic lesions have been reported with an unknown pathogenesis [18], and only 5% of ALL patients reported with an inherited genetic predisposition such as Down's syndrome and Bloom's syndrome [17, 19]. **Figure 4** indicates multiple genetic lesions contributing to an altered regulatory network in healthy lymphoid development toward a pathogenic ALL pathway [20–23]. Incomplete evidence regarding prenatal genetic lesions in ALL supports research into the epigenetic regulatory system in the development of ALL. Prenatal genetic lesions suggest the preleukemic states. Despite studies suggesting preleukemic state in utero, other studies show that the development of ALL in monozygotic twins follows a different time course. The difference in postnatal disease progression despite an identical prenatal state suggests the role of epigenetics in ALL manifestation [24–27].

2. Targeting epigenome of ALL in chemotherapy

Since drugs target distinct cell pathways relying on gene expression, access to target genes is crucial for the treatment to work. A common ALL chemotherapy regimen of glucocorticoids relies on activation of the glucocorticoid receptor (GR), which binds to glucocorticoid-response elements (GREs) in gene promoters, to induce expression of pro-apoptotic pathways [28]. Using a DNase hypersensitivity assay (DHA) to determine chromatin accessibility [29], the majority of GR binding to GREs were identified in open chromatin [8, 30]. Thus, glucocorticoid therapy is dependent on GRE accessibility which is defined by chromatin accessibility. Resistance and patient response to such drugs is thought to be dependent on their gene accessibility profiles.

2.1 Glucocorticoid-based chemotherapy: Focused study on ALL

2.1.1 Acute lymphoblastic leukemia (ALL)

ALL is a malignant disease in both adults and children, with mutations developing along the lymphoid lineage starting at the lymphoid progenitor cells. Normally, lymphoid cells have the potential to differentiate into B or T cells, which under oncogenic conditions give rise to either B-ALL or T-ALL [17, 21]. Hematopoietic stem cells (HSCs), in the bone marrow, are the origin of both lymphoid and myeloid lineages. The tight regulation of gene expression in HSCs determines the lineage pathway and development. During oncogenesis, molecular defects and abnormal genes regulation may alter the differentiation of HSC; these factors may also contribute to further alterations downstream in hematopoiesis [17, 31–36]. Alterations along the lymphoid lineage result in abnormal pre-lymphoid cells called lymphoblasts; these aggressively proliferate and gradually replace the normal hematopoietic cells in the bone marrow and blood. Accumulation of lymphoblasts results in immunity retardation due to the insufficient amounts of mature lymphoid cells. Patients thus become immunocompromised and prone to various infectious diseases normally fought off by the immune system's lymphocytes [21, 37, 38].

2.1.2 Glucocorticoids in the clinic

Glucocorticoids are naturally occurring steroid hormones that are widely recognized for their anti-inflammatory and immunosuppressing activities [39–41]. In leukemia, glucocorticoids are able to induce apoptosis in lymphoid cells. As such, glucocorticoid drugs such as dexamethasone and prednisolone are used as part of multi-agent chemotherapy regimens treating hematological malignancies [42–44], including ALL, chronic lymphocytic leukemia, multiple myeloma, and lymphoma. Due to pro-apoptotic pathway activation, glucocorticoids have remained the pivot point in chemotherapy treatment to combat ALL for 50 years [38, 45].

Glucocorticoids play a role in all three phases of treatment phases. During the remission-induction phase, glucocorticoids make up a significant portion of drug when administered in combination with vincristine and asparaginase and/or anthracycline. This initial high glucocorticoid portion aims to relieve at least 99% of the leukemic burden; the patient's response is critical in determining the future course of treatment and determining chance of relapse and prognosis [46]. The following two chemotherapy phases are less intensive, involving re-administration of remission-induction drugs in addition to methotrexate and mercaptopurine [17]. To note, some patients do not need to be administered pulses of glucocorticoids in phases two and three of the therapy, due to patients' contraindications [47]. This three-phase glucocorticoid regimen has seen an increase in ALL 5-year survival rate from 73–90% in the past 20 years [48], yet there still exits a subset of ALL patients who are resistant to glucocorticoids, resulting in poor prognosis.

2.1.3 Glucocorticoid mechanism of action

Glucocorticoid mode of action involves the activation of specific cellular pathways specific in lymphoid cells to induce cell death. Depending on cell type, cell-specific chromatin conformation provides the structural framework for transcription factor (TF) binding to regulate gene transcription that determines the ability of a cell to activate a pathway [49–51]. A cell-type-specific conformation is generated [11, 52] with each type having approximately 70,000–100,000 accessible chromatin domains and a network of cell-type-specific binding of transcriptional regulators [10, 53, 54]. Glucocorticoids are able to target intracellular pathways by interacting with the glucocorticoid receptor (GR) in the cytoplasm [55, 56]. The complex then translocates into the nucleus and binds at accessible chromatin domains containing glucocorticoid-response elements (GREs) at proximal promoter regions and/or distal sites of a gene [57–59]. GR binding to GREs induces chromatin remodeling and activates gene transcription via recruitment of other transcription proteins [60, 61]. To keep gene transcription tightly regulated, GR binding is highly selective and predetermined by chromatin accessibility in different cell types [8, 62]. Currently, the GR-binding landscape in different cell subsets, as well as between glucocorticoid-sensitive and resistant leukemia subsets, is yet to be established. Understanding this epigenetic landscape is crucial in understanding patient relapse or chemoresistance. Preliminary studies have started this investigation in pediatric ALL, to understand the mechanism of drug resistance in B-ALL.

2.1.4 Limitations to glucocorticoid treatment

While the glucocorticoid induces apoptosis pathway is still unclear, it is a pro-apoptotic pathway exclusive to lymphoid cells despite widespread expressions of GR in most human tissues [63, 64]. Glucocorticoids are rarely efficacious in treating myeloid leukemia [65]. Due to this lymphoid-specific apoptosis pathway,

it is hypothesized that glucocorticoid-sensitive cells have a distinguished chromatin structure allowing for specific GR binding at GREs that glucocorticoid-resistant lymphoids, myeloid cells, and other tissue cells do not have [8, 66-69]. Therefore, understanding the lymphocyte-specific mechanisms of glucocorticoid-induced apoptosis, as well as the development of resistance to this class of steroid hormones, is critical in optimizing glucocorticoid-based therapies in the clinic.

The actions of glucocorticoids are cell type specific [65–67], although the exact molecular basis for this differential function remained elusive. While certain signaling pathways resulting from ALL oncogenes appear to interfere with glucocorticoid actions resulting in resistance, epigenetic evidence suggests that in addition to genetic alterations, epigenetic factors contribute to resistance. For instance, inhibition of GR expression or its translocation to the nucleus in vitro and in vivo models via *BTG1* or *PTEN* loss can cause glucocorticoid resistance by [70, 71]; however, GR function is rarely blocked in resistant ALL PDXs [72]. Mutations in epigenetic regulators such as *KMT2D*, *CREBBP*, and *HDAC7*, in two of five resistant PDX models, could not account for abnormal epigenetic changes. Mutations in various signaling pathways [73–76] have been reported to impair glucocorticoid-induced apoptosis in ALL by downregulating the GR-activated pro-apoptotic gene, *BIM* expression. The importance to study beyond gene mutations, toward epigenetic mapping of GR binding, will provide a deeper understanding into individual drug response.

2.2 Epigenetic landscape shapes the response to glucocorticoids in leukemia

Lymphocyte-specific enhancers associated with glucocorticoid-induced apoptosis were identified in cell-wide studies [5, 28]. Moreover, aberrations at these enhancers were observed in glucocorticoid-resistant ALL cells. Similarly, nonlymphoid cells also exhibited inaccessible chromatin at these enhancers, providing insights into the cell-type-specific actions of glucocorticoids. A link between epigenetic differences and cell-type-specific actions of glucocorticoids are essential in the treatment determining treatment approaches for lymphoid malignancies. Lymphocyte-specific epigenetic modifications pre-determine glucocorticoid resistance in ALL and may account for the lack of glucocorticoid sensitivity in other cell types. Recent findings suggest that in glucocorticoid-sensitive cells, GR cooperates with the structural protein, CTCF, at lymphocyte-specific regulatory domains to mediate the formation of a transcriptionally active DNA loop to trigger gene transcription, which can be inhibited by increased DNA methylation in glucocorticoid-resistant ALL. By using a comprehensive map of chromatin accessibility, CTCF binding, histone modifications, and DNA methylation in normal and malignant cell types, there is evidence of regulatory heterogeneity in the epigenome of different cell types. Azacytidine, a DNA demethylating drug that is routinely used in the clinic, could partially reverse these changes and restore glucocorticoid-induced gene expression and glucocorticoid sensitivity. This indicates that reversal of epigenetic changes may lead to improvements in the use of glucocorticoids for the management of lymphoid malignancies.

3. Epigenetic drugs

3.1 What are epigenetic drugs?

Epigenetic drugs inhibit and manipulate different epigenetic regulators involved in histone remodeling. Drugs which target epigenetic regulators can open closed chromatin structures commonly found in chemotherapy-resistant patients.

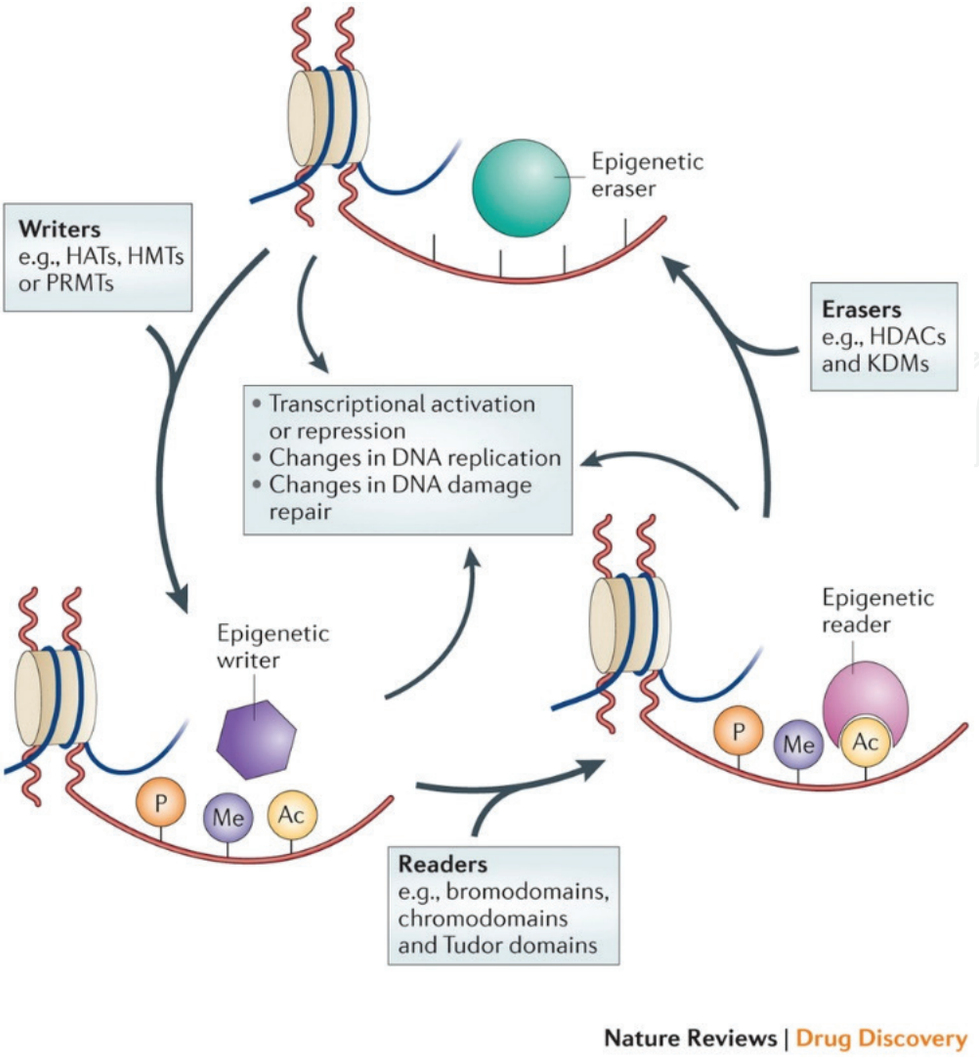

Figure 5.
Epigenetic reader, writer, and eraser. Epigenetic writers such as histone acetyltransferases (HATs), histone methyltransferases (HMTs), protein arginine methyltransferases (PRMTs), and kinases lay down epigenetic marks on amino acid residues on histone tails. Epigenetic readers are proteins that contain bromodomains, chromodomains, and Tudor domains, allowing them to bind to these epigenetic marks. Epigenetic erasers such as histone deacetylases, lysine demethylases, and phosphatases catalyze the removal of epigenetic marks. Together they modulate chromatin structures and regulate various DNA-dependent biological processes such as DNA synthesis and replication. Adapted from Falkenberg and Johnstone, 2014 [77].

Epigenetic regulators can be divided into different categories based on their method of modification: epigenetic writers, readers, and erasers (**Figure 5**).

Epigenetic regulators determine gene expression, and an understanding of them allows for the development of drugs to regulate gene expression over epigenetic marks. Epigenetic writers lay down epigenetic marks on DNA or amino acid residues on histones tails [77]. Examples include histone acetyltransferases (HATs), histone methyltransferases (HMTs), and protein arginine methyltransferases (PRMTs). Drugs which target these are DNA hypomethylating agents, bromodomains, and HDAC inhibitors. Epigenetic readers are proteins that contain bromodomains, chromodomains, and Tudor domains allowing them to bind to specific epigenetic marks on chromatin. Epigenetic erasers such as histone deacetylases, lysine demethylases, and phosphatases catalyze the removal of epigenetic marks.

3.2 Categories of epigenetic modifying drugs

The epigenetic drugs to be discussed are designed to target different epigenetic regulators responsible for gene silencing.

3.2.1 DNMT inhibitors

During preparation of genetic information in the S phase of the cell cycle, replication machinery is responsible for DNA replication, and DNMT functions duplicate methylation status by adding methyl groups to the DNA accordingly (**Figure 6**). DNMT inhibitors, azacytidine and decitabine, prevent DNMT methylation. Azacytidine and decitabine are metabolized inside cells into 5-aza-2′-deoxycytidine-triphosphate. The difference between DNMT bounding to 5-aza-2′-deoxycytidine-triphosphate and DNMT bound to cytosine is used to inhibit DNMT. As illustrated in **Figure 7**, DNMT reversibly binds with cytosine, which allows DNMT to be released from DNA through beta-elimination once methylation is completed. DNMT binding to 5-aza-2′-deoxycytidine-triphosphate establishes a covalent bond preventing beta-elimination; therefore, DNMT remains bond to the DNA. Subsequently, the error triggers DNA damage signaling and the trapped DNMT is degraded. As a result, methylation markers get lost during DNA replication [78]. Demethylated DNA allows for an open chromatin structure to be accessed by transcription factors induce by chemotherapeutic drugs such as glucocorticoids.

Single-agent study conducted by Khaldoun Al-Romaih's group showed that decitabine therapy had a cytotoxic effect mediated by the removal of hypomethylation of the CpG position both in vivo and in vitro. The cells treated with decitabine showed significant cell death in vitro, and six pro-apoptotic genes (GADD45A, HSPA9B, PAWR, PDCD5, NFKBIA, and TNFAIP3) were induced to ≥ twofold in vivo [79]. Combination therapy trials revealed the value of DNMT inhibition in addition to current therapeutic regimens. In one clinical trial in refractory ALL patients, decitabine combined with hyper-CVAD (fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with high-dose methotrexate and cytarabine) was able to achieve complete remission in patients that did

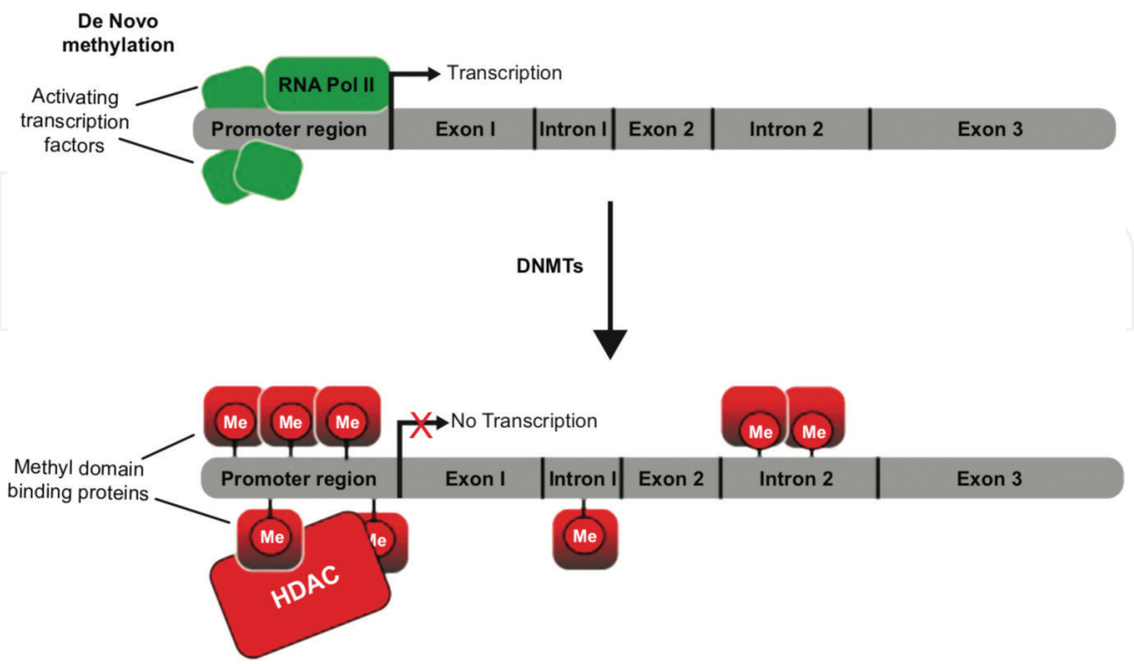

Figure 6.
Effect of epigenetic modification on gene expression. Activation of gene transcription needs transcription factor binding to the promoter region of the gene. Without DNA methylation, transcription factors and RNA polymerase II (RNA pol II) can bind to DNA segments; however, when methyl group is added to the DNA by DNA methyltransferase (DNMT), the methylation not only impedes the binding of transcription factors to DNA but also recruits histone deacetylase (HDAC) causing chromatin structure to become compacted, which places spatial limitation for transcription factor binding [83].

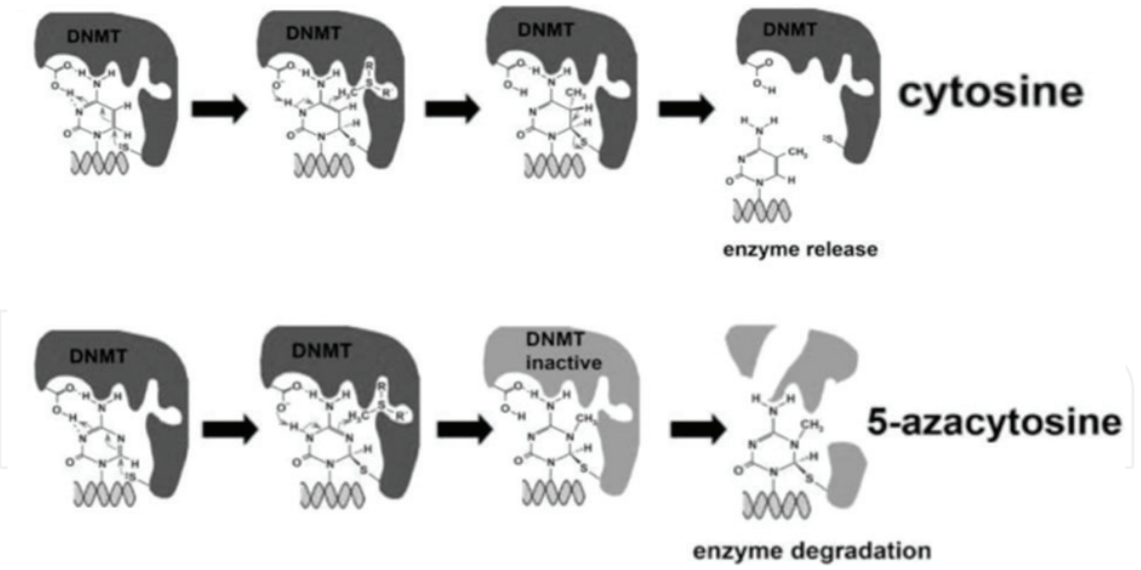

Figure 7.
Reversible binding of cytosine with DNMT in DNA methylation process versus irreversible binding of 5-azacytosine with DNMT when leukemia cells were treated with azacytidine, which leads to degradation of DNMT and subsequent loss of methylation. Adapted from Stresemann and Lyko, 2008 [78].

not respond to hyper-CVAD treatment alone. Patient DNA analysis confirmed that the hypomethylation status in the combination treatment group was the reason for reversal of resistance [80]. A number of other clinical studies provided similar results, showing synergistic effects between hypomethylating agents and several chemotherapy agents. Agents such as prednisolone, etoposides, doxorubicin, and cytarabine were shown to increase chemosensitivity in leukemia cells [81–84]. Although promising not all experimental groups responded, indicating the need for further research into the complex network of interactions.

3.2.2 HDAC inhibitors

An open chromatin structure also relies on the relatively loose interactions between DNA and histone proteins. The addition of an acetyl groups to the lysine amino acids on histone proteins by histone acetyltransferases (HATs) reduces the positive charges on the histone proteins. DNA, negatively charged, is therefore less attracted to these now less positive histones, thus less tightly bound and easier for transcription factors to access [83]. Conversely, the removal of acetylation by histone deacetylase (HDAC) forms condensed heterochromatin and silences critical apoptosis gene transcriptions. HDAC inhibitors prevent this form of gene silencing and are used alongside standard chemotherapy to promote pro-apoptotic pathways.

HDAC enzyme family consists of Class I (HDAC1, HDAC2, HDAC3, HDAC8), Class II (HDAC4, HDAC5, HDAC6, HDAC7, HDAC9, HDAC10), Class III (SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, and SIRT7), and Class IV (HDAC11). HDAC inhibitors are designed as either selective inhibitors or pan-inhibitors (against all types of HDAC). Evidence that some HDACs play a stronger role in cancer development and patient prognosis than others makes specific HDAC inhibitors more appealing for clinical use. A study of 94 pediatric ALL patients showed differential HDAC expressions between the T-ALL and B-ALL subtypes. For T-ALL, HDAC1 and HDAC4 showed a higher expression than in B-ALL. T-ALL patients with HDAC3 expression above the cohort median also displayed a significantly higher 5-year event-free survival (EFS). In B-ALL, HDAC5 had higher expression than

in T-ALL. In both T-cell and B-cell ALL, HDAC7 and HADC9 expression levels higher than the cohort median were associated with a lower 5-year EFS [85]. These trends suggest that Class II HDACs are associated with poorer prognosis; hence, a specific inhibition of this class of HDACs is important.

3.2.3 Bromodomain inhibitors

Bromodomains (BRDs) are epigenetic readers which selectively bind to acetylate lysine of histones tails, regulating gene expression [86]. Bromodomain-containing proteins that target genes are primarily cell cycle M/G1 genes in mitotic chromatin (expressed at the end or immediately after mitosis); late-phase genes were not found to be BRD4 bound. M/G1 gene expression during telophase coincides with histone H3 and H4 acetylation in those genes. BRD binding to M/G1 genes was associated with recruitment of positive transcription elongation factor b (P-TEFb), resulting in translational memory in the daughter cells [87]. BRD binds to MYC and activates enhancer-binding protein 4 (AP4) promoters. AP4 is a key mediator of mitogenicity for proto-oncogene MYC [88]. Upon AP4 activation by MYC, repressing cell cycle arrests gene *P21* [88].

JQ1 is a BRD inhibitor which acts on the MYC-AP4 axis [89]. Direct inhibition by JQ1 of BRD binding to the MYC and AP4 promoters indirectly results in cell cycle arrest as a *P21*-induced response to DNA damage P53 or TGFb/Smad signaling pathways [90].

3.3 Epigenetic drugs in chemotherapy

Epigenetic drugs are useful in combination with cytotoxic drugs, due to their ability to allow for access to pro-apoptotic pathways, otherwise blocked by epigenetic silencing. In a subset of leukemia patients, resistance to cytotoxic drugs such as glucocorticoids and methotrexate is a result of inaction of pro-apoptotic genes. Theoretically, applying epigenetic drugs such as those mentioned above should remove the epigenetic modification such that pro-apoptotic genes may go from repression to promotion.

3.3.1 Epigenetic drugs to treat glucocorticoid resistance

Chromatin conformation and gene expression studies at the glucocorticoid-induced pro-apoptotic *BIM* gene in drug-sensitive versus resisted lymphoid cells indicated that glucocorticoid resistance in ALL patients may be due to epigenetic modifications.

3.3.2 DNMT inhibitors

Studies showed closed DNMT catalyzed chromatin structure caused by DNA methylation impedes the transcription of *BIM*; lymphocyte-specific open chromatin structure determines *BIM* expression. Therefore, modifying chromatin structure would allow for *BIM* expression. Common cytosine analog hypomethylating drugs, decitabine or azacytidine, used in ALL act by inhibiting DNMT activity thus reactivate silenced genes. Once metabolized inside cells into 5-aza-2′-deoxycytidine-triphosphate, cytosine substrates on DNA replication machinery are replaced by the drug analog. These DNMT inhibitors have been proven to work synergistically with glucocorticoids in glucocorticoid-resistant ALLs, increasing the overall effectiveness of the therapeutic regimens [84].

3.3.3 HDAC inhibitors

Suberoylanilide hydroxamic acid (SAHA; Vorinostat) is an HDAC inhibitor shown to work synergistically with different chemotherapies. Glucocorticoid-resistant ALL cases were associated to a correlation between histone H3K9 deacetylation and pro-apoptotic gene, *BIM*, repression [91, 92]. SAHA acts to increase acetylation at H3K9 for *BIM* expression. Another chemocytotoxic pathway involves FPGS conversion of methotrexate to a cytotoxic product responsible for apoptosis, MTX-PGs. HDAC1 represses *FPGS* via epigenetically silencing. The combination of SAHA with methotrexate was shown to increase *FPGS* expression by two- to fivefolds, thus increasing cytotoxic activity [93].

3.4 Combination therapy

Combination therapy aims to bring synergistic effects by targeting both cell death pathway (chemotherapy, e.g., glucocorticoids) and access to this pathway (epigenetic drug). Of the clinical trials underway, the use of relatively high doses of 5-azacitidine (150 mg/m^2 as a continuous infusion daily × 5 days) is combined with cytarabine in patients relapsed from cytarabine alone. It was hypothesized that treatment with 5-azacitidine could induce expression of deoxycytidine kinase. Two out of the 17 patients achieved complete responses (CR). In another study, decitabine was combined with either amsacrine or idarubicin in patients with acute leukemia. CR was achieved in 36% (23 out 63) of patients, with a median disease-free survival of 8 months [82].

Using a hypomethylating agent such as decitabine, glucocorticoids in resistant ALL patients had the potential to expose the pro-apoptotic gene *BIM*, making it available for GR binding and subsequent transcription; thus reversing patient glucocorticoid resistance [5]. This should especially increase the rate of CR among the patients with glucocorticoid-resistant ALL and prolong event-free survival as suggested in preclinical trial model.

4. Conclusion

Knowledge of gene regulation has deepened the understanding of cellular mechanisms and disease development. In leukemia, genomic and epigenomic landscapes together provide crucial disease mechanism of pathogenicity and drug resistance [94–96]. Epigenetics is the driver of life and diversity of different organisms, and equally able to dysregulate cells and cause diseases such as leukemia. Hematopoiesis is a tightly controlled process essential for life, therefore, unless appropriately regulated, susceptible to regulatory errors as oncogenic drivers alongside mutations. Lineage-specific landscapes have been shown to be involved in hematopoiesis and leukemia evolution [51], providing a backbone for understanding targetable and non-targetable sites within different leukemic subtypes.

Studying a level of cell dysfunction preceding DNA mutations has allowed for understanding into pathogenesis and drug resistance which could not be correlated to DNA sequencing. Understanding resistance to chemotherapies, lowering patient prognosis, has been enlightened in epigenetic studies. For example, actions of glucocorticoids are cell type-specific and can be used in lymphocyte-specific leukemia cells to induce cell death [66, 67]. Analysis between genome-wide lymphocyte-specific open chromatin domains (LSOs) and integrated LSOs with glucocorticoid-induced RNA transcription and chromatin modulation in ALL was performed to causes glucocorticoid resistance beyond gene mutations [5]. LSOs critical for

glucocorticoid-induced apoptosis were identified as well as structural protein CTCF binding in this region. These findings showed that upon GR binding to the LSO and CTCF binding, DNA would loop at the pro-apoptotic *BIM* gene and could be expressed. Crucially, DNA methylation (closed chromatin structure) was present in glucocorticoid-resistant ALL and nonlymphoid cell types, preventing DNA looping and *BIM* expression.

Understanding the importance of chromatin accessibility has allowed for identification of glucocorticoid sensitivity in cells and provides promising drug response predictions. Furthermore, development of epigenetic drugs that may modify chromatin to be accessible is currently being investigated. This would allow for more effective drug treatments to disrupt the oncogenesis driven via dysregulated pathways.

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