Chapter

Epigenetic Landscape in Leukemia and Its Impact on Antileukemia Therapeutics

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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
(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
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 to 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 transcriptional 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.
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 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].

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 binding 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...
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 group 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 acetylated 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 TGFβ/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′-deoxyctydine-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/m² 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 $\textit{BIM}$ 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 $\textit{BIM}$ 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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References

[1] Amin V et al. Epigenomic footprints across 111 reference epigenomes reveal tissue-specific epigenetic regulation of lincRNAs. Nature Communications. 2015;6:6370

[2] Ziller MJ et al. Dissecting neural differentiation regulatory networks through epigenetic footprinting. Nature. 2015;518(7539):355-359

[3] Dixon JR et al. Chromatin architecture reorganization during stem cell differentiation. Nature. 2015;518(7539):331-336

[4] De Jager PL et al. Alzheimer's disease: Early alterations in brain DNA methylation at ANK1, BIN1, RHBDL2 and other loci. Nature Neuroscience. 2014;17(9):1156-1163

[5] Jing D et al. Lymphocyte-specific chromatin accessibility pre-determines glucocorticoid resistance in acute lymphoblastic leukemia. Cancer Cell. 2018;34(6):906-921 e8

[6] Alvarez-Errico D et al. Epigenetic control of myeloid cell differentiation, identity and function. Nature Reviews. Immunology. 2015;15(1):7-17

[7] Luger K, Dechassa ML, Tremethick DJ. New insights into nucleosome and chromatin structure: An ordered state or a disordered affair? Nature Reviews. Molecular Cell Biology. 2012;13(7):436-447

[8] John S et al. Chromatin accessibility pre-determines glucocorticoid receptor binding patterns. Nature Genetics. 2011;43(3):264-268

[9] Margueron R, Reinberg D. Chromatin structure and the inheritance of epigenetic information. Nature Reviews. Genetics. 2010;11(4):285-296

[10] Shlyueva D, Stampfel G, Stark A. Transcriptional enhancers: From properties to genome-wide predictions. Nature Reviews. Genetics. 2014;15(4):272-286

[11] Aranda S, Mas G, Di Croce L. Regulation of gene transcription by polycomb proteins. Science Advances. 2015;1(11):e1500737

[12] Wiemels J. Perspectives on the causes of childhood leukemia. Chemico-Biological Interactions. 2012;196(3):59-67

[13] Gilham C et al. Day care in infancy and risk of childhood acute lymphoblastic leukaemia: Findings from UK case-control study. BMJ. 2005;330(7503):1294

[14] Mullighan CG. The genomic landscape of acute lymphoblastic leukemia in children and young adults. Hematology. American Society of Hematology. Education Program. 2014;2014(1):174-180

[15] Mullighan CG. Genomic characterization of childhood acute lymphoblastic leukemia. Seminars in Hematology. 2013;50(4):314-324

[16] Dang J et al. PAX5 is a tumor suppressor in mouse mutagenesis models of acute lymphoblastic leukemia. Blood. 2015;125(23):3609-3617

[17] Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. Lancet. 2008;371(9617):1030-1043

[18] Roberts KG, Mullighan CG. Genomics in acute lymphoblastic leukaemia: Insights and treatment implications. Nature Reviews. Clinical Oncology. 2015;12(6):344-357

[19] Bercovich D et al. Mutations of JAK2 in acute lymphoblastic leukaemias
associated with down's syndrome. Lancet. 2008;372(9648):1484-1492

[20] Levine RL. Inherited susceptibility to pediatric acute lymphoblastic leukemia. Nature Genetics. 2009;41(9):957-958

[21] Zuckerman T, Rowe JM. Pathogenesis and prognostication in acute lymphoblastic leukemia. F1000Prime Reports. 2014;6:59

[22] Hunger SP, Mullighan CG. Acute lymphoblastic leukemia in children. The New England Journal of Medicine. 2015;373(16):1541-1552

[23] Swaminathan S et al. Mechanisms of clonal evolution in childhood acute lymphoblastic leukemia. Nature Immunology. 2015;16(7):766-774

[24] Ford AM et al. Fetal origins of the TEL-AML1 fusion gene in identical twins with leukemia. Proceedings of the National Academy of Sciences of the United States of America. 1998;95(8):4584-4588

[25] Wasserman R et al. Predominance of fetal type DJH joining in young children with B precursor lymphoblastic leukemia as evidence for an in utero transforming event. The Journal of Experimental Medicine. 1992;176(6):1577-1581

[26] Francis SS et al. Mode of delivery and risk of childhood leukemia. Cancer Epidemiology, Biomarkers & Prevention. 2014;23(5):876-881

[27] Copley MR, Eaves CJ. Developmental changes in hematopoietic stem cell properties. Experimental and Molecular Medicine. 2013;45:e55

[28] Jing D et al. Opposing regulation of BIM and BCL2 controls glucocorticoid-induced apoptosis of pediatric acute lymphoblastic leukemia cells. Blood. 2015;125(2):273-283

[29] Crawford GE et al. Genome-wide mapping of DNase hypersensitive sites using massively parallel signature sequencing (MPSS). Genome Research. 2006;16(1):123-131

[30] Wiench M et al. DNA methylation status predicts cell type-specific enhancer activity. The EMBO Journal. 2011;30(15):3028-3039

[31] Schindler JW et al. TEL-AML1 corrupts hematopoietic stem cells to persist in the bone marrow and initiate leukemia. Cell Stem Cell. 2009;5(1):43-53

[32] Zelent A, Greaves M, Enver T. Role of the TEL-AML1 fusion gene in the molecular pathogenesis of childhood acute lymphoblastic leukaemia. Oncogene. 2004;23(24):4275-4283

[33] Armstrong SA, Look AT. Molecular genetics of acute lymphoblastic leukemia. Journal of Clinical Oncology. 2005;23(26):6306-6315

[34] Di Cello F et al. Inactivation of the Cdkn2a locus cooperates with HMGA1 to drive T-cell leukemogenesis. Leukemia & Lymphoma. 2013;54(8):1762-1768

[35] Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. The New England Journal of Medicine. 2004;350(15):1535-1548

[36] Mullighan CG et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature. 2007;446(7137):758-764

[37] Speck NA, Gilliland DG. Core-binding factors in haematopoiesis and leukaemia. Nature Reviews Cancer. 2002;2(7):502-513

[38] Torpy JM, Lynam C, Glass RM. JAMA patient page. Acute lymphoblastic leukemia. JAMA. 2009;301(4):452
[39] Goulding NJ, Flower RJ. Glucocorticoids, Milestones in drug therapy. Basel, Switzerland: Boston: Birkhäuser Verlag; 2001. p. 205

[40] Dalakas MC. Inflammatory muscle diseases. The New England Journal of Medicine. 2015;372(18):1734-1747

[41] Nair P et al. Oral glucocorticoid-sparing effect of benralizumab in severe asthma. The New England Journal of Medicine. 2017;376(25):2448-2458

[42] Inaba H, Pui CH. Glucocorticoid use in acute lymphoblastic leukaemia. The Lancet Oncology. 2010;11(11):1096-1106

[43] Kim IK et al. Glucocorticoid-induced tumor necrosis factor receptor-related protein co-stimulation facilitates tumor regression by inducing IL-9-producing helper T cells. Nature Medicine. 2015;21(9):1010-1017

[44] Palumbo A et al. Daratumumab, bortezomib, and dexamethasone for multiple myeloma. The New England Journal of Medicine. 2016;375(8):754-766

[45] Pui CH, Evans WE. A 50-year journey to cure childhood acute lymphoblastic leukemia. Seminars in Hematology. 2013;50(3):185-196

[46] Klumper E et al. In vitro cellular drug resistance in children with relapsed/refractory acute lymphoblastic leukemia. Blood. 1995;86(10):3861-3868

[47] Howard SC et al. Urolithiasis in pediatric patients with acute lymphoblastic leukemia. Leukemia. 2003;17(3):541-546

[48] Australian Institute of Health and Welfare. A Picture of Australia’s Children 2012. Canberra: Australian Institute of Health and Welfare; 2012

[49] Guenther MG et al. A chromatin landmark and transcription initiation at most promoters in human cells. Cell. 2007;130(1):77-88

[50] Li G et al. Extensive promoter-centered chromatin interactions provide a topological basis for transcription regulation. Cell. 2012;148(1-2):84-98

[51] Corces MR et al. Lineage-specific and single-cell chromatin accessibility charts human hematopoiesis and leukemia evolution. Nature Genetics. 2016;48(10):1193-1203

[52] Hnisz D, Day DS, Young RA. Insulated neighborhoods: Structural and functional units of mammalian gene control. Cell. 2016;167(5):1188-1200

[53] Thurman RE et al. The accessible chromatin landscape of the human genome. Nature. 2012;489(7414):75-82

[54] Perera D et al. Differential DNA repair underlies mutation hotspots at active promoters in cancer genomes. Nature. 2016;532(7598):259-263

[55] Greenstein S et al. Mechanisms of glucocorticoid-mediated apoptosis in hematological malignancies. Clinical Cancer Research. 2002;8(6):1681-1694

[56] Watson LC et al. The glucocorticoid receptor dimer interface allosterically transmits sequence-specific DNA signals. Nature Structural & Molecular Biology. 2013;20(7):876-883

[57] John S et al. Interaction of the glucocorticoid receptor with the chromatin landscape. Molecular Cell. 2008;29(5):611-624

[58] Paakinaho V et al. Glucocorticoid receptor activates poised FKBP51 locus through long-distance interactions. Molecular Endocrinology. 2010;24(3):511-525

[59] Vockley CM et al. Direct GR binding sites potentiate clusters of TF
binding across the human genome. Cell. 2016;166(5):1269-1281 e19

[60] Guo B et al. Glucocorticoid hormone-induced chromatin remodeling enhances human hematopoietic stem cell homing and engraftment. Nature Medicine. 2017;23(4):424-428

[61] Swinstead EE et al. Steroid receptors reprogram FoxA1 occupancy through dynamic chromatin transitions. Cell. 2016;165(3):593-605

[62] Love MI et al. Role of the chromatin landscape and sequence in determining cell type-specific genomic glucocorticoid receptor binding and gene regulation. Nucleic Acids Research. 2017;45(4):1805-1819

[63] Gross KL, Lu NZ, Cidlowski JA. Molecular mechanisms regulating glucocorticoid sensitivity and resistance. Molecular and Cellular Endocrinology. 2009;300(1-2):7-16

[64] Wasim M et al. PLZF/ZBTB16, a glucocorticoid response gene in acute lymphoblastic leukemia, interferes with glucocorticoid-induced apoptosis. The Journal of Steroid Biochemistry and Molecular Biology. 2010;120(4-5):218-227

[65] Klein K et al. Glucocorticoid-induced proliferation in untreated pediatric acute myeloid leukemic blasts. Pediatric Blood & Cancer. 2016;63(8):1457-1460

[66] Gruver-Yates AL, Cidlowski JA. Tissue-specific actions of glucocorticoids on apoptosis: A double-edged sword. Cell. 2013;2(2):202-223

[67] Cain DW, Cidlowski JA. Immune regulation by glucocorticoids. Nature Reviews. Immunology. 2017;17(4):233-247

[68] Ploner C et al. Glucocorticoid-induced apoptosis and glucocorticoid resistance in acute lymphoblastic leukemia. The Journal of Steroid Biochemistry and Molecular Biology. 2005;93(2-5):153-160

[69] Schmidt S et al. Glucocorticoid resistance in two key models of acute lymphoblastic leukemia occurs at the level of the glucocorticoid receptor. The FASEB Journal. 2006;20(14):2600-2602

[70] Piovan E et al. Direct reversal of glucocorticoid resistance by AKT inhibition in acute lymphoblastic leukemia. Cancer Cell. 2013;24(6):766-776

[71] van Galen JC et al. BTG1 regulates glucocorticoid receptor autoinduction in acute lymphoblastic leukemia. Blood. 2010;115(23):4810-4819

[72] Bachmann PS et al. Divergent mechanisms of glucocorticoid resistance in experimental models of pediatric acute lymphoblastic leukemia. Cancer Research. 2007;67(9):4482-4490

[73] Jones CL et al. MAPK signaling cascades mediate distinct glucocorticoid resistance mechanisms in pediatric leukemia. Blood. 2015;126(19):2202-2212

[74] Serafin V et al. Glucocorticoid resistance is reverted by LCK inhibition in pediatric T-cell acute lymphoblastic leukemia. Blood. 2017;130(25):2750-2761

[75] Nagao K, Iwai Y, Miyashita T. RCAN1 is an important mediator of glucocorticoid-induced apoptosis in human leukemic cells. PLoS One. 2012;7(11):e49926

[76] Cialfi S et al. Glucocorticoid sensitivity of T-cell lymphoblastic leukemia/lymphoma is associated with glucocorticoid receptor-mediated inhibition of Notch1 expression. Leukemia. 2013;27(2):485-488
[77] Falkenberg KJ, Johnstone RW. Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. Nature Reviews. Drug Discovery. 2014;13(9):673-691

[78] Stresemann C, Lyko F. Modes of action of the DNA methyltransferase inhibitors azacytidine and decitabine. International Journal of Cancer. 2008;123(1):8-13

[79] Al-Romaih K et al. Modulation by decitabine of gene expression and growth of osteosarcoma U2OS cells in vitro and in xenografts: Identification of apoptotic genes as targets for demethylation. Cancer Cell International. 2007;7:14

[80] Benton CB et al. Safety and clinical activity of 5-aza-2′-deoxycytidine (decitabine) with or without hyper-CVAD in relapsed/refractory acute lymphocytic leukaemia. British Journal of Haematology. 2014;167(3):356-365

[81] Garcia-Manero G et al. DNA methylation of multiple promoter-associated CpG islands in adult acute lymphocytic leukemia. Clinical Cancer Research. 2002;8(7):2217-2224

[82] Garcia-Manero G et al. Epigenetics of acute lymphocytic leukemia. Seminars in Hematology. 2009;46(1):24-32. DOI: 10.1053/j.seminhematol.2008.09.008

[83] Heerboth S et al. Use of epigenetic drugs in disease: An overview. Genetics & Epigenetics. 2014;6:9-19

[84] Lu BY et al. Decitabine enhances chemosensitivity of early T-cell precursor-acute lymphoblastic leukemia cell lines and patient-derived samples. Leukemia & Lymphoma. 2016;57(8):1938-1941

[85] Moreno DA et al. Research paper: Differential expression of HDAC3, HDAC7 and HDAC9 is associated with prognosis and survival in childhood acute lymphoblastic leukaemia. British Journal of Haematology. 2010;150(6):665-673

[86] Fujisawa T, Filippakopoulos P. Functions of bromodomain-containing proteins and their roles in homeostasis and cancer. Nature Reviews Molecular Cell Biology. 2017;18:246

[87] Dey A et al. Brd4 marks select genes on mitotic chromatin and directs postmitotic transcription. Molecular Biology of the Cell. 2009;20(23):4899-4909

[88] Choi SK et al. JQ1, an inhibitor of the epigenetic reader BRD4, suppresses the bidirectional MYC-AP4 axis via multiple mechanisms. Oncology Reports. 2016;35(2):1186

[89] Delmore JE et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell. 2011;146(6):904-917

[90] Jung P, Hermeking H. The c-MYC-AP4-p21 cascade. Cell Cycle. 2009;8(7):982-989

[91] Lock RB et al. Epigenetic silencing of the pro-apoptotic Bim gene in glucocorticoid poor-responsive pediatric acute lymphoblastic leukemia, and its reversal by histone deacetylase inhibition. Blood. 2009;114(22):939

[92] Zhang C et al. Histone acetylation: Novel target for the treatment of acute lymphoblastic leukemia. Clinical Epigenetics. 2015;7:117

[93] Leclerc GJ et al. Histone deacetylase inhibitors induce FPGS mRNA expression and intracellular accumulation of long-chain methotrexate polyglutamates in childhood acute lymphoblastic leukemia: Implications for combination therapy. Leukemia. 2010;24(3):552
[94] Cancer Genome Atlas Research, N et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. The New England Journal of Medicine. 2013;368(22):2059-2074

[95] Hnisz D et al. Activation of proto-oncogenes by disruption of chromosome neighborhoods. Science. 2016;351(6280):1454-1458

[96] Iacobucci I, Mullighan CG. Genetic basis of acute lymphoblastic leukemia. Journal of Clinical Oncology. 2017;35(9):975-983

[97] Australian Institute of Health and Welfare (AIHW). Australian Cancer Incidence and Mortality (ACIM) Books: Acute Lymphoblastic Leukaemia. Canberra: AIHW; 2014