Seminars in Cancer Biology 51 (2018) 129–138

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

Review

Epigenetics in pediatric acute lymphoblastic leukemia

Jessica Nordlund*, Ann-Christine Syvänen

Department of Medical Sciences and Science for Life Laboratory, Uppsala University, Sweden

ARTICLE INFO

Keywords:
Acute lymphoblastic leukemia (ALL)
DNA methylation
Epigenetics
Epigenomics
Subtyping

ABSTRACT

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. ALL arises from the malignant transformation of progenitor B- and T-cells in the bone marrow into leukemic cells, but the mechanisms underlying this transformation are not well understood. Recent technical advances and decreasing costs of methods for high-throughput DNA sequencing and SNP genotyping have stimulated systematic studies of the epigenetic changes in leukemic cells from pediatric ALL patients. The results emerging from these studies are increasing our understanding of the epigenetic component of leukemogenesis and have demonstrated the potential of DNA methylation as a biomarker for lineage and subtype classification, prognostication, and disease progression in ALL. In this review, we provide a concise examination of the epigenetic studies in ALL, with a focus on DNA methylation and mutations perturbing genes involved in chromatin modification, and discuss the future role of epigenetic analyses in research and clinical management of ALL.

1. Acute lymphoblastic leukemia (ALL)

Acute lymphoblastic leukemia (ALL) is the most common form of pediatric cancer. Structural chromosomal rearrangements, which can lead to expressed fusion genes, are together with clinical features such as white blood cell count at diagnosis and minimal residual disease status, the main basis for diagnosis, risk stratification, and prognosis of pediatric ALL [1]. Patients with ALL are classified into genetic subtypes based on the occurrence of recurrent chromosomal abnormalities detected by karyotyping (G-banding), fluorescent in situ hybridization (FISH), and/or polymerase chain reaction (PCR) amplification. ALL arises from hematopoietic cells in either the B-cell precursor (BCP-ALL) or T-cell lineages (T-ALL). Both the BCP-ALL and T-ALL immunophenotype groups comprise multiple subtypes defined by chromosomal alterations that are believed to be the leukemia-initiating lesions [1]. In most protocols, the important subtypes for prognosis of ALL are T-ALL and the BCP-ALL subtypes high hyperdiploidy (HeH), t(12;21)*ETV6-RUNX1*, t(1;19)*E2A-PBX1*, t(9;22)*BCR-ABL1*, dic(9;20), iAMP21, hypodiploidy (< 45 chr), and *KMT2A* (also known as *MLL1*) rearrangements.

However, 20% of newly diagnosed BCP-ALL cases do not belong to any of the known genetic subtypes. These patients, whose subtype is referred to as B-other, are limited to clinical and minimal residual disease data for informing treatment decisions and also typically lack leukemia-specific genetic changes that can be used for disease monitoring during treatment [2]. During 2016–2017, several previously unknown recurrent genomic rearrangements involving the *DUX4*, *ZNF384*, or *MEF2D* genes were discovered in the B-other subgroup [3,4,5,6]. These and other emerging subtypes of ALL have been reviewed in detail elsewhere [1].

Normal hematopoietic cell development requires tightly controlled regulation of DNA methylation, chemical modification of histones, and expression of non-coding RNAs, all of which may be deregulated during leukemic transformation. DNA methylation is by far the most well characterized epigenetic modification, and is involved in the regulation of gene expression, maintenance of genome stability, and cellular differentiation. Many studies have implicated aberrant epigenetic regulation in the pathogenesis, treatment outcome and recurrence of ALL. This review will summarize and discuss the functions and consequences of epigenetic alterations with a focus on DNA methylation and somatic mutational signatures in epigenetic regulating genes in pediatric ALL as elucidated by recent studies.

Abbreviations: 5mc, 5-methyl cytosine; 5hmc, 5-hydroxymethylcytosine; ALL, Acute Lymphoblastic Leukemia; ASE, Allele-specific gene expression; B-All, B-cell precursor lineage ALL; CpG, CG dinucleotide; CGI, CpG island; CIMP, CpG island methylator phenotype; DMCs, Differentially methylated CpG sites; DMRs, Differentially methylated regions; DNAm, DNA methylation; EFS, Event free survival; ETP-ALL, Early T-cell precursor ALL; FISH, Fluorescent in situ hybridization; HELP, HpaII tiny fragment Enrichment by Ligation-mediated PCR; NGS, Next generation sequencing; OS, Overall survival; PCR, Polymerase chain reaction; RRBS, Reduced representation bisulfite sequencing; T-ALL, T-cell lineage ALL; WGBS, Whole genome bisulfite sequencing

* Corresponding author at: Molecular Medicine, Box 1432, BMC, 75144 Uppsala, Sweden.
E-mail address: Jessica.Nordlund@medsci.uu.se (J. Nordlund).

http://dx.doi.org/10.1016/j.semcancer.2017.09.001
Received 12 June 2017; Received in revised form 21 August 2017; Accepted 2 September 2017
Available online 05 September 2017

2. Technology for DNA methylation analysis

The human genome contains 28 million CpG sites that are targets for DNA methylation. The discovery of epigenetic biomarkers has significantly expanded owing to the development of methods for interrogating DNA methylation on a genome-wide scale using hybridization microarrays and next generation sequencing (NGS). Methods for detecting DNA methylation can be divided into three broad classes; those involving (I) enrichment of methylated genomic positions, (II) digestion with methylation- sensitive restriction enzymes, and (III) utilization of sodium bisulfite treatment, which converts unmethylated cytosine residues into thymine, while methylated cytosines are protected against conversion. These approaches vary in many aspects, such as required DNA input, degree of genomic resolution and coverage, and ability of quantification. Thus, when comparing results from different DNA methylation studies the method(s) used for determination of DNA methylation levels should be considered. Here, we will briefly summarize methodologies applied for genome-wide DNA methylation analysis in studies of ALL patient cohorts.

The earliest genome-wide methods implemented in ALL involved enrichment of methylated DNA fragments, either by a combination of methylation specific restriction enzyme digestion and PCR [7,8] or immunoprecipitation of methylated DNA fragments [9], followed by reading out the results using hybridization microarrays. The relative abundance of the enriched DNA regions is an estimate of the amount of cytosine methylation at any given region of the genome that is represented by probes on the array. The strength of these methods is that they cover relatively large genomic regions, but a limitation is that enrichment methods do not provide single-base resolution of DNA methylation at individual CpG sites.

Sodium bisulfite treatment of DNA enables measurement of the methylation status of individual cytosine residues at a single-base resolution. Targeted analysis of DNA methylation in bisulfite-treated DNA can in principle be performed by any SNP genotyping method. The MassArray system (Agena, formerly Sequenom) has been used during more than two decades because it allows robust quantification of the DNA methylation levels at targeted CpG sites [10]. A custom designed Golden Gate assay (Illumina) for 1536 CpG sites in candidate gene promoter regions was used in an early study of allele-specific regulation of gene expression by DNA methylation in primary ALL cells [11]. Today the most frequently used methods with capacity of single-base resolution of bisulfite-converted DNA with high-throughput analysis of many CpG sites and samples in parallel are the Infinium BeadChip assays (Illumina). The BeadChip assays interrogate the methylation status of cytosine residues by genotyping cytosine or thymine (methylated vs unmethylated cytosine residues) using a predetermined set of probes in a microarray format. The Infinium assays offer quantitative measurement of DNA methylation and have been launched with increasing numbers of target CpG sites over the last decade, starting with the HumanMethylation 27 K BeadChip (27k array) that mostly targeted CpG islands [12], followed by the HumanMethylation 450 K (450k array) [13] and Infinium MethylationEPIC (850k array) BeadChips [14], which in addition to CpG islands and genes, also assay CpG island shores, gene bodies, enhancers, and other non-coding genomic regions. The BeadChips provide a user friendly and straightforward approach for analyzing hundreds of thousands of CpG sites in many samples at a relatively low cost.

Although the targeted approaches using BeadChip assays for DNA methylation analysis offer advantages for analysis of large patient cohorts, they target only up to 3% of the 28 million CpG sites in the genome, while complete genome-wide DNA methylome maps can only be achieved by whole-genome sequencing of bisulfite–treated DNA (WGBS) [15]. Several approaches for creating WGBS libraries from genomic DNA in combination with bisulfite conversion followed by sequencing by next generation sequencing (NGS) have been described. The different WGBS library construction approaches may affect the genomic coverage and the accuracy of the methylation calling, as shown in a recent bench-marking study [16]. Although the reagent costs for whole-genome sequencing (WGS) are decreasing, the large amount of DNA required in the first generation WGBS methods together with the high cost and limited availability of user-friendly methods for analysis of the WGBS data have so far limited the size of the patients cohorts subjected to WGBS in ALL [17,18].

A potential source of error in interpretation of DNA methylation levels using bisulfite conversion is that both 5-methyl cytosine (5mC) and 5-hyroxylmethyl cytosine (5hmC) are read as cytosine, and thus cannot be discriminated. This drawback of bisulfite treatment can be circumvented by introduction of an oxidation step in the bisulfite treatment procedure [19]. From a biological point of view discrimination between 5hmC and 5mC at single-base resolution in DNA is desirable because 5hmC may have opposite functions to 5mC, for example by how it affects gene expression. Demethylation of 5hmC occurs as a result of oxidation of 5mC by enzymes of the TET (ten-eleven translocation) family [20]. TET mutations are rare, but have been detected in about 1% of ALL cases [21,22]. Mutations in the *TET2* enzyme are associated with reduced 5hmC levels in acute myeloid leukemia [23], however it is unclear whether 5hmC occurs in ALL cells and hence it is not known to what extent 5hmC may influence the results of the bisulfite conversion-based assays commonly used to study DNA methylation in ALL.

There are several factors that can affect the interpretation of quantitative DNA methylation in the analyses of ALL cells, such as the sample number in the studies, tumor heterogeneity due to presence of DNA from subclonal populations of leukemic blasts, and presence of DNA from normal hematopoietic or blood cells within the leukemic DNA samples. It is difficult to experimentally identify and define epigenetic states within a population of bulk cells because of the heterogeneity within the leukemic cell population. Methods are needed to ascertain the uniqueness of the ALL methylome in the presence of normal cells when bone marrow or peripheral blood samples from ALL patients are analyzed in bulk [24,25]. In combination with recent reductions in DNA sequencing costs, single-cell sequencing offers a breakthrough for future analysis of DNA methylation in single cells from heterogeneous tumor populations [26,27,28].

3. DNA methylation in ALL cells

Methylation of cytosine residues in CpG dinucleotides plays a pivotal role in the establishment of cellular identity by influencing gene expression [29] and is a widespread and common feature of all human cancers, including leukemias [30,31]. The DNA methylomes of cancer cells have been found to contain large hypomethylated blocks and to display deregulation of the tightly controlled boundaries between methylated and unmethylated genomic regions [32]. Hypermethylation of CpG dense regions also known as CpG islands (CGIs) is the most systematically studied type of aberrant methylation across human cancers [31]. Accumulating evidence suggests that the pathogenesis and phenotypic characteristics of leukemic cells are the results of a combination of specific targeted and genome-wide alterations of DNA methylation [33,34].

The earliest studies to investigate aberrant DNA methylation in primary ALL cells analyzed CGIs nearby or in individual candidate genes. Although the small number of CGIs and patients included in these initial studies limits the interpretation of their results, they suggest that aberrant promoter methylation is associated with prognosis [35], cytogenetic alterations [36], cytogenetic subtype [37], and relapse [38]. These early findings combined with technological advances outlined in Section 2 above, have spurred several groups to study aberrant changes in DNA methylation of ALL cells on a genome-wide scale. The design of these studies in terms of methods for DNA methylation analysis, number of ALL samples, type of control cells used, and their main results are summarized in Table 1.

The consensus of the studies listed in Table 1 is that ALL cells generally have higher methylation levels in CpG sites that are located within CGIs than matched remission bone marrow and normal B/T-cells or hematopoietic precursor cells. Several independent studies discovered recurrent DNA methylation alterations, predominantly hypermethyated CGIs, which are observed across all genetic or immunophenotypic subtypes [39,44,8,47,49]. This finding suggests that some specific epigenetic changes are essential for leukemic transformation across all subtypes of ALL. Many of these changes occur *de novo* in regions that are co-occupied by the histone modifications H3K4me3 and H3K27me3 in embryonic stem cells, which indicates that leukemic transformation in ALL is assisted epigenetically via loss of plasticity and retention of an unlimited capacity of cellular self-renewal [47,17], as has been observed also in other hematological malignancies and solid tumors [31].

Most DNA methylation studies published to date rely on targeted approaches with a focus on CGIs and thus do not present an unbiased view of the DNA methylome in ALL cells. Approaches that use NGS can in principle circumvent the bias of targeted analyses, but yet the few NGS studies from primary ALL cells offer somewhat conflicting results. Almamum et al. used an NGS approach where DNA fragments spanning methylated DNA regions are enriched and sequenced [50]. Their study comprising 20 ALL patients of various subtypes and pre-B cell controls found that CGIs in ALL cells had a larger number of methylated CpG sites than CGIs in control cells, but they also noted significant genome-wide hypomethylation. Interrogation of two BCP-ALL genomes by WGBS revealed that one patient with the t(12;21)*ETV6-RUNX1* subtype had similar global methylation levels as pre-B cells, while the second patient with a HeH subtype showed 4% lower total global methylation than pre-B cells [17]. WGBS of seven primary ALL patients of four different subtypes (T-ALL, t(12;21)*ETV6-RUNX1*, B-other, and normal karyotype) showed that each of the genomes were approximately 5% more abundantly methylated than control blood cells [18]. Based on discordant DNA methylation patterns in the WGBS reads, this study also concluded that the increase and large-variability of DNA methylation in CGIs is a result of stochastically occurring aberrant DNA methylation on individual chromosomes, as opposed to coordinated clonal methylation of the ALL cells. Notably this study did not find evidence for mega-base long segments of continuous hypomethylation, like those that have been frequently observed in other cancer types [60,32].

4. DNA methylation in ALL subtypes

DNA methylation studies of the recurrent ALL subtypes have identified subtype-specific signatures [41,8,47,53,17,18]. Thus, it is well-established that each genetic subtype of ALL has a set of specific differentially methylated CpG sites that are useful for distinguishing between subtypes, similarly subtype-specific gene expression signatures measured by microarray- or NGS-based methods [61,62]. Fig. 1 shows an example of subtype-specific DNA methylation patterning in established and emerging ALL subtypes. Subtype-specific DNA methylation signatures have been consistently reported across the ALL cohorts examined independently of the methods used to interrogate DNA methylation. These observations are perhaps not surprising since the subtype-defining primary genetic abnormalities define the key features of the cell and are ubiquitous across all clones. Such signatures have been used to design DNA methylation classifiers [48] and have potential use as future biomarkers. DNA methylation patterns in the BCP-ALL subtypes have been characterized in greater detail than in T-ALL, which often is viewed as a single subtype, although there are known subtypes of T-ALL [63].

4.1. High hyperdiploidy (HeH)

HeH is a common subtype of pediatric ALL accounting for approximately 25% of diagnoses [64]. HeH is characterized by the non-random gain of chromosomes X, 4, 6, 8, 10, 14, 17, 18 and 21 for a total modal chromosome number greater than 50 [65]. The most striking epigenetic feature of HeH is a strong hypomethylation signature compared to the other ALL subtypes. One of the first DNA methylation studies in ALL described DNA hypomethylation hotspots and lower DNA methylation levels of aneuploid chromosomes in HeH patients [9]. Studies in larger cohorts reproduced the findings of hypomethylation, but did not observe preferential hypomethylation of trisomic chromosomes [8,47,53]. Furthermore, WGBS revealed a 4.4% lower genome-wide CpG methylation level in one HeH patient than in normal pre-B cells [17]. Whole genome sequencing suggests that chromosomal gains are early driving events in HeH pathogenesis [66]. Because the DNA methylation profiles are so similar between patients harboring various combinations of chromosomal aneuploidies, it is plausible that the subtype-specific methylation signatures may predate or have occurred simultaneously as the chromosomal gains [48].

4.2. t(12;21)/ETV6-RUNX1

The *ETV6-RUNX1* fusion gene is detected in approximately 20% of ALL diagnoses and is a favorable prognostic marker [64]. CGI-centric experimental designs have shown a bias towards hypermethylation of this subtype [43,8], whereas studies using 450k BeadChips and WGBS revealed relatively equal numbers of differentially methylated CpG sites with higher and lower DNA methylation levels in patients with *ETV6-RUNX1* when compared to normal blood cells [45,47,53,18]. There is a generally good overlap between the differentially methylated genes in this subgroup. Of note, *IGF2BP1*, *EPOR*, *FUCA1*, and *HLA-DPB1* were found to be hypometylated and have increased mRNA expression in *ETV6-RUNX1* in four independent studies [45,8,47,49].

4.3. KMT2A-r

Lysine-specific methyl transferase 2A (*KMT2A*), also known as *MLL1*, is a promiscuous gene with over 80 different gene-fusion partners [67]. *KMT2A* rearrangements (*KMT2A*-r) are detected in 5–6% of ALL cases, most frequently in infants (1 < year), which comprise a distinctive disease entity with an aggressive disease with poor prognosis [68]. Infants with *KMT2A*-r exhibit few additional genetic abnormalities [69], but display typical DNA methylation profiles, both as a subgroup distinct from other BCP-ALL subgroups, and within the *KMT2A*-r subgroup depending on the fusion partner [40,58]. Two independent studies have shown that infants with t(11;19) and t(4;11) translocations differ from each other based on their DNA methylation pattern, yet both have increased DNA methylation in relation to normal bone marrow. It was also observed that samples from patients with t(9;11) resembled normal bone marrow samples [40]. These findings suggest that the DNA methylation pattern might underlie functionally relevant changes depending on the translocation partner of *KMT2A*.

4.4. t(1;19)/TCF3-PBX1

The translocation t(1;19)(q23;p13.3) resulting in the *TCF3-PBX1* fusion gene is observed in approximately 4% of ALL cases and is associated with an intermediate risk [64]. According to DNA methylation analysis using 450k BeadChips the t(1;19)-specific DNA methylation signature is mostly hypomethylated [47,53]. However, as determined by the HELP assays based on restriction site cleavage and ligation-assisted PCR, a subtype-specific signature in t(1;19) patients displayed nearly equal amounts of hyper- and hypomethylation [8].

4.5. dic(9;20)

The chromosomal aberration dic(9;20)(p13.2;q11.2) occurs in up to 5% of BCP-ALL cases [70]. The translocation results in the loss of chromosome arms 9p and 20q with heterogeneity of the breakpoint,

Table 1
Large-scale DNA methylation studies in pediatric ALL.

Study	Method	ALL subtype (n patients)	Non-leukemia controls (n)	Main result	Data availability
Taylor et al. [7]	Restriction-PCR-Chip	NA (16)	BM (4)	Hypermethylation in ALL and differences in BCP-ALL and T-ALL	NA
Milani et al. [11]	Custom Array (1536 CpG sites)	NA (197)	–	DNAm is associated with ASE	NA
Dunwell et al. [39]	Chr 3 NotI array	T-ALL and BCP-ALL (88)	Peripheral blood, BM (12)	Identification of DNAm in ALL	NA
Davidsson et al. [9]	MeDIP-Chip	HeH and t(12;21) (20)	–	Differences between *ETV6-RUNX1* and HeH	NA
Stumpel et al. [40]	Differential DNAm hybridization	KMT2A-r (57)	Pediatric BM (8)	DNAm signatures in *KMT2A-r* subtypes, correlation with outcome	GSE18400
Milani et al. [41]	Custom Array (1536 CpG sites)	T-ALL, HeH, t(12;21), normal karyotype, B-other, t (1;19), t(9;22), dic(9;20), KMT2A-r, iAMP21, hypodiploid (401)	–	Subtype-specific DNAm signature, correlation with relapse and OS	NA
Hogan et al. [42]	Infinium 27k	HeH, t(12;21), normal karyotype. Diagnosis-relapse pairs (33)	–	Increased promoter DNAm at relapse	GSE28461
Wong et al. [43]	Infinium 27k	t(12;21) (19)	Matched remission BM, fractionated cells (30)	*ETV6-RUNX1* DNAm signature, DNAm differences between diagnosis and remission	GSE29189
Nordlund et al. [44]	Custom Array (1536 CpG sites)	T-ALL, HeH, t(12;21), t(1;19), t(9;22), KMT2A-r, normal karyotype, B-other, iAMP21 (20)	Matched remission BM, pediatric BM (30)	Promoters are hypermethylated in ALL compared to controls	Table S1
Sandoval et al. [86]	Infinium 450k	Non-high risk BCP-ALL (29)	B-cells (4)	DNAm is associated with relapse	GSE39141
Busche et al. [45]	Infinium 450k	HeH, t(12;21), t(9;22), t(1;19), KMT2A-r, normal karyotype, hypodiploid (46)	Matched remission BM (46)	Subtype-specific DNAm, *ETV6-RUNX1* signature	GSE38235
Borssen et al. [46]	Infinium 27k	T-ALL (43)	–	CIMP status associated with relapse and OS	GSE42079
Figueroa et al. [8]	HELP	T-ALL, HeH, t(12;21), t(1;19), t(9;22), KMT2A-r, CRLF2-r, ERG deleted (167)	B-cells, T-cells (19)	Common DNAm signature in ALL, subtype-specific DNAm, correlation with gene expression	GSE44862
Nordlund et al. [47]; Nordlund et al. [48]	Infinium 450k	T-ALL, HeH, t(12;21), t(1;19), t(9;22), KMT2A-r, dic (9;20), iAMP21 (764, 27 diagnosis-relapse pairs)	Matched remission BM, fractionated cells (137)	Common DNAm signature in ALL, subtype-specific DNAm, correlation with gene expression and outcome, increased DNAm at relapse	GSE49031
Chatterton et al. [49]	Infinium 27k and 450k	HeH, t(12;21), B-other (69)	Matched remission BM, fractionated cells (48)	Common DNAm signature in ALL, subtype-specific DNAm correlated with gene expression	NA
Almamun et al. [50]; Almamun et al. [51]; Arthur et al. [52]	MIRA-seq	HeH, B-other, normal karyotype (20)	Umbilical cord blood (10)	Promoter DNAm correlated with gene expression, intronic and intergenic DMRs in ALL, hypermethylation of ncRNAs	SRP058314
Gabriel et al. [53]	Infinium 450k	HeH, t(12;21), t(1;19), dic(9;20) (52)	–	Confirms subtype-specific DNAm, but not associations with outcome	GSE69229
Lee et al. [17]	WGBS, Infinium 450k	HeH, t(12;21), t(1;19), KMT2A-r, B-other (227)	Fractionated cells (NA)	Subtype-specific DNAm, role of polycomb in de novo DNAm	GSE56602
Safavi et al. [54]	Infinium 450k	Hypodiploid (9)	–	DNAm dependent on modal chromosome number	NA
Kunz et al. [55]	Infinium 450k	T-ALL, diagnosis-relapse pairs (13)	Matched remission BM (13)	Hypomethylation at relapse	NA
Borssen et al. [56]	Infinium 450k	T-ALL (65)	–	CIMP status associated with EFS	GSE69954
Marincevic-Zuniga et al. [57]	Infinium 450k	t(9;14)*PAX5-ESRRB* (4) and BCP-ALL subtypes (28)	–	DNAm signature in *PAX5-ESRRB*	GSE49031
Wahlberg et al. [18]	WGBS	T-ALL, B-ALL: t(12;21), normal karyotype, B-other (7)	Fractionated cells (25)	Stochastic de novo DNAm in CpG islands	GSE76270
Bergmann et al. [58]	Infinium 450k	KMT2A-r (26)	Fractionated cells (22)	DNAm signatures in *KMT2A-r* subtypes	GSE76585
Abdullah et al. [59]	Infinium 27k	NA (10)	Peripheral blood (4)	Hypermethylation in resistant (relapsed) patients	NA
Marincevic-Zuniga et al. [77]	Infinium 450k	*DUX4-IGH* (8) and *ZNF384* rearrangements (6)	–	Subtype-specific DNAm signatures in DUX4-IGH and ZNF384 rearranged subtypes	GSE49031

Abbreviations: Not available (NA); bone marrow (BM), DNA methylation (DNAm), Differentially methylated region (DMR), Whole genome bisulfite sequencing (WGBS), HpaII tiny fragment Enrichment by Ligation-mediated PCR Assay (HELP), methylated CpG island recovery assay followed by NGS (MIRA-seq), non-coding RNA (ncRNA), B-lineage precursor ALL (BCP-ALL), minimal residual disease (MRD), event-free survival (EFS), overall survival (OS).

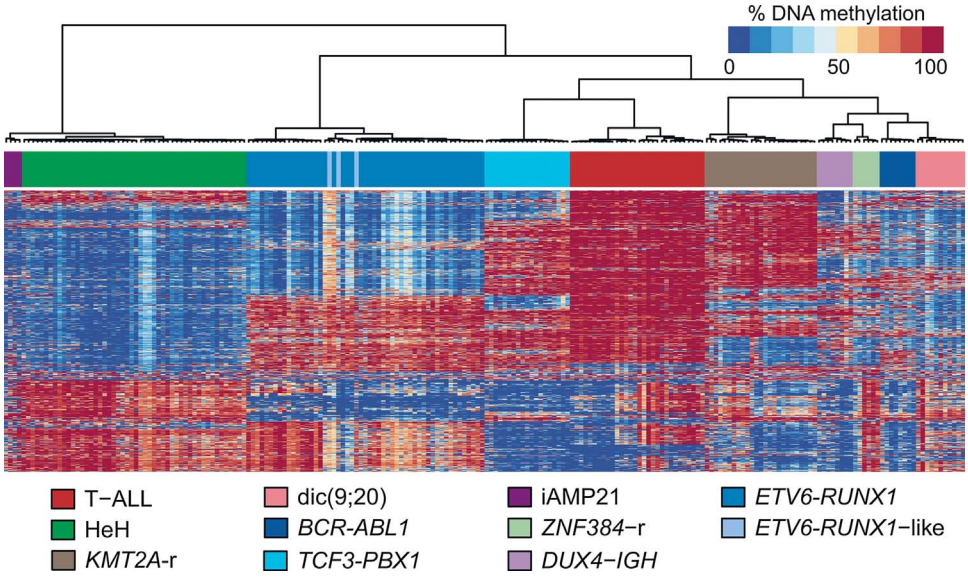

Fig. 1. Hierarchical clustering of primary ALL samples (GSE49031) with known subtype based on the methylation levels of the 1000 most variable CpG sites in the dataset [47]. The patients are color-coded by cytogenetic subtype and are clustered along the horizontal axis and the CpG sites are clustered along the vertical axis. In the heatmap, blue indicates low, yellow indicates intermediate, and red indicates high DNA methylation levels.

which in some cases produces a fusion gene involving *PAX5* [71]. Besides studies on fusion genes, dic(9;20) has been a less frequently studied molecular subtype of BCP-ALL and it is uncertain whether the oncogenic mechanism underlying the dic(9;20) subtype is a gene fusion, loss of DNA from 9p and 20q, or a combination of both [72]. Only two independent studies have analyzed DNA methylation in dic(9;20) cases as a unique molecular entity and these studies agree that dic(9;20) displays a preferentially hypermethylated pattern [47,53].

4.6. t(9;22)/BCR-ABL1

Philadelphia chromosome (Ph+) or t(9;22)*BCR-ABL1* is detected in 3–5% of pediatric ALL cases [64]. *BCR-ABL1* is a prognostic indicator of an adverse disease and a biomarker for targeted therapy with imatinib or dasatinib [73]. Like other subtypes of ALL, patients with *BCR-ABL1* can be distinguished by subtype-specific DNA methylation profiles. However, only 271 subtype-specific differentially methylated CpG sites in 36 genes were detected in *BCR-ABL1*, whilst other ALL subtypes typically displayed ~2000 differentially methylated CpG sites [47] and in a follow-up study of the same patients, *BCR-ABL1* was the most difficult subtype of ALL to classify based on DNA methylation [48]. In another study, 365 differentially methylated regions were described in *BCR-ABL1*, which is a similar number as in other subtypes [8]. Unlike most of the other BCP-ALL subtypes, the effect on DNA methylation by the BCR-ABL1 fusion protein is most likely indirect, as it does not involve direct aberration of transcription factors or histone modifications. Both of the studies mentioned above included 19 patients, but the method used to determine the DNA methylation levels differed (HELP and 450k, respectively). Thus the DNA methylation signatures for *BCR-ABL1* need to be further clarified.

4.7. iAMP21

Intra chromosomal amplification of chromosome 21 (iAMP21) occurs in approximately 2% of ALL diagnoses [1]. The unifying feature of all iAMP21 cases is the amplification of the *RUNX1* locus on chromosome 21, which is readily identifiable by FISH. This subtype was initially associated with high risk of relapse, but with intensified treatment protocols the prognosis has improved [73]. Given the low incidence of this subtype, our group has been the only one to report DNA methylation patterns specifically for this group [47,48]. Interestingly, in our DNA methylation classifiers, we found that all iAMP21 cases classified both as HeH and iAMP21, and thus there is an overlapping signature between the iAMP21 and HeH cases, a pattern that is also seen based on unsupervised hierarchical clustering (Fig. 1). Given that most HeH cases have extra copies of chromosome 21, it is likely the source of the methylation signature, but given the low number of patients (n = 8) analyzed, additional studies are needed.

4.8. Hypodiploidy

Hypodiploidy is defined based on a reduced number (< 45) of chromosomes and occurs at a frequency of 1–2% of ALL cases [64]. The modal number of chromosomes allows further classification of hypodiploidy into high hypodiploidy (40–44 chromosomes), low hypodiploidy (31–39 chromosomes), and near-haploidy (25–30 chromosomes). Only a single DNA methylation study has specifically addressed hypodiploid ALL and shown that the patients cluster together depending on the modal chromosome number per cell. In this study, like in HeH, the differences in methylation levels did not appear to arise from the chromosomal copy number [54].

4.9. T-ALL

Compared to BCP-ALL, T-ALL has a worse outcome. Despite increasing knowledge of genetic aberrations in ALL, there are currently no other known reliable molecular genetic markers than minimal residual disease (MRD) for identifying patients with higher risk of relapse specifically in T-ALL [74]. The CpG island methylator phenotype (CIMP), defined by extensive DNA hypermethylation of cytosines within CGIs, has been documented in many cancers, including T-ALL [75,76]. CIMP status can be denoted as CIMP+ or CIMP-, for high or low DNA methylation levels, respectively. T-ALL patients with CIMP- had significantly worse outcome compared to CIMP+ cases [46] and CIMP classification appears to predict relapse independently of MRD [56]. This pattern has been observed in relatively small T-ALL sample sets and needs to be replicated in larger, prospective cohorts before it can be implemented in clinical routine for treatment decisions. The question also remains if CIMP contributes to leukemogenesis or if is it merely a surrogate for epigenetic deregulation or cell proliferation in this subtype [76].

4.10. DNA methylation signatures in emerging BCP-ALL subtypes

The group of patients without an established subtype-defining abnormality is collectively referred to as B-other. There remains a great

deal of heterogeneity in this group, but recent transcriptome sequencing studies discovered a network of recurrent fusion genes that in some cases identify the pathogenetic change and define new, prognostically relevant subtypes.

Our group described a subset of B-other patients with DNA methylation signatures akin to t(12;21)*ETV6-RUNX1*, but that were negative for the *ETV6-RUNX1* fusion gene by FISH or PCR [48]. Such *ETV6-RUNX1*-like cases have also been described based on gene expression data [3]. Transcriptome sequencing studies of *ETV6-RUNX1*-like cases has since identified several, previously unknown in-frame fusion genes frequently involving *ETV6* and another gene, including *CBX3*, *AK125726*, *BORCS5*, *NID1*, or *PMEL* [3,77]. Similarly, *ERG*-altered "B-other" ALL cases have a DNA methylation signature distinct from other ALL subtypes [8]. Recently, rearrangements with the *DUX4* gene (*IGH-DUX4* or *DUX4-ERG*) were recognized as the initiating event in leukemic transformation in this emerging subgroup previously defined by *ERG*-alterations [3,4]. Patients with *DUX4* rearrangements along with another newly described group characterized by *ZNF384* rearrangements also harbor distinct subtype-specific DNA methylation patterns [77].

PAX5 on chromosome 9p is one of the most frequently altered genes in ALL. A rare subgroup of B-other patients harboring der(9)t(9;14) *PAX5-ESRRB* also displays distinct DNA hypomethylation in comparison to known BCP-ALL subtypes, including patients with other types of *PAX5* gene fusions [57]. It is likely that most groups of cases within the B-other group with recurrent, leukemia-initiating genetic aberrations will have distinctive DNA methylation patterns.

Several independent studies have identified a distinct subgroup among the patients with BCP-other that lack the *BCR-ABL1* translocation, but yet have a similar expression signature as *BCR-ABL1* positive cases [78,79,80]. This group, referred to as *BCR-ABL1*-like, is now recognized as a new leukemia entity by WHO classification and may comprise as many as 8% of ALL patients, as has been reviewed in detail elsewhere [73,1,64]. These patients have several genomic features in common, like rearrangement or mutation of *CRLF2*, *ABL1, ABL2, PDGFRB*, *JAK2*, *EPOR*, etc. To our knowledge, no study has yet reported a *BCR-ABL1*-like DNA methylation signature, but this would be an important avenue for future research.

5. Relationship between DNA methylation and gene expression

Methylation of CpG sites in gene promoter regions has been found to correlate with gene expression in primary ALL cells. This is convincingly illustrated by a quantitative correlation between methylation levels of CpG sites in promoter regions and bidirectional allele-specific gene expression (ASE) observed for a subset of genes with large variability in DNA methylation levels [11]. This finding suggests that bidirectional ASE occurs as a consequence of CpG site methylation that is randomly distributed between the two chromosomes and causes allele-specific silencing or reduction of the expression of one of the alleles and is in agreement with observations from WGBS data from ALL cells [18]. The relationship between DNA methylation and gene expression is however complex, with multiple other epigenetic factors than DNA methylation that affect gene expression, like non-coding RNAs and chemical modification of histone proteins in regulatory (enhancer) regions of the genome. The subtype-specific DNA methylation changes in ALL are more frequently localized to enhancer regions and are associated with changes in gene expression, in contrast to the core set of differentially methylated CpG sites that are shared across ALL subtypes [8,47]. Aberrant *de novo* DNA methylation in enhancers is associated with down- regulation of enhancer-derived RNA (eRNA) in BCP-ALL [51] and furthermore the ETV6-RUNX1 fusion protein alters the expression of eRNAs in enhancers containing RUNX1 binding motifs [81].

Similarly to protein coding RNA expression, long-non-coding (lnc) or antisense RNAs are also differentially expressed in subtype-specific patterns [62,82,83,84]. Moreover, aberrant expression of lnc antisense RNAs in BCP-ALL have been found to be associated with DNA hypermethylation [52]. Similarly as for lncRNAS, miRNA expression is common in ALL with subtype-specific signatures in most subtypes (reviewed in [33], which is exemplified by a study demonstrating a specific miRNA expression signature in patients harboring *DUX4*-rearrangements (ERG deleted) [85].

6. DNA methylation and relapse of ALL

Although the survival rate for patients with pediatric ALL treated on modern protocols is high (> 90%), the outcome of ALL patients that relapse is poor [1]. Thus it would be highly beneficial if new biomarkers that predict relapse of ALL at diagnosis could be identified. As DNA methylation marks cellular states and is reversible, DNA methylation has good potential both as biomarker and as a potential treatment target.

6.1. DNA methylation as biomarker for prediction of relapse in ALL

Several studies have attempted to use DNA methylations signatures that are detectable at diagnosis for predicting relapse of ALL [40,41,46,47,86,56,59]. However, interpretation of the results from these studies is hampered by the minimal overlap of the DNA methylation signatures detected or CpG sites analyzed and by the low number of ALL patients in each study, subtype, or treatment group. Few studies have attempted replication of previous finings, or replication has failed [53]. An exception is a study on T-ALL that replicated a CpG island methylator phenotype (CIMP) signature in two independent, although relatively small sets of Nordic T-ALL patients that were treated on different protocols [46,56]. One common finding in most of these studies is that the patients with lower methylation levels at diagnosis were more likely to relapse, compared to patients that escaped relapse. Examples of such findings are the T-ALL CIMP-negative signature of 1347 CpG sites [46,56] and hypomethylation signatures based on small sets of genes (< 30) in HeH, *ETV6-RUNX1*, *KMT2A*-r, and *BCR-ABL1* subgroups [41,47]. One reason for the lack of reproducible prediction of relapse based on DNA methylation signatures at diagnosis could be that the clones which evolve to relapse are present at diagnosis only as minor clones at varying amounts in different patients and therefore do not expose a strong DNA methylation signature. Other reasons could be additional cellular heterogeneity of normal cells present in the sample that dampen the signatures, the different treatment protocols used across studies, or the relatively small cohorts examined.

6.2. Differential DNA methylation at diagnosis and at relapse of ALL

Matched cell samples collected at diagnosis and at relapse from ALL patients have been analyzed in three ALL cohorts using Infinium BeadArrays to evaluate differences in the methylome of ALL cells prior to and after therapeutic intervention [42,47,55]. The first study measured DNA methylation differences at diagnosis and relapse in 33 BCP-ALL cases and found higher DNA methylation levels in CGIs at relapse [42]. Genes of interest for relapsed ALL that emerged from this study included *CDKN2A*, *COL6A2*, *PTPRO*, and *CSMD1*. The second study involving 27 paired samples from diagnosis-relapse ALL patients of multiple subtypes replicated these findings [47]. An additional finding in the second study was that similarly to a common DNA methylation signature observed in ALL cells independently of cytogenetic subtype, the relapse-associated CpG sites overlapped with bivalent epigenetic marks of chromatin states and with genes regulated by REST, SOX2, NANOG, and OCT4 [47]. Conversely, in the third study where paired diagnosis-relapse samples from 13 T-ALL patients were analyzed, hypomethylation of promoter regions was more frequent than hypermethylation in relapsed samples [55]. Additional studies specifically focusing on T-ALL are needed to confirm this finding.

7. Chromatin modifications as epigenetic marks in ALL

Posttranslational modifications of histone proteins that together with DNA constitute the chromatin of each cell play an important role for temporal and cell-specific regulation of gene expression. Dynamic modification of chromatin as a consequence of interaction between histone marks and DNA methylation is likely to play a role for the malignant transformation of normal hematopoietic precursor cells into ALL cells. Chemical modifications of histone proteins as epigenetic marks have been less studied in ALL than DNA methylation (reviewed in [87]), probably due to the technically more demanding methods for cell sampling and detection of histone marks. On the other hand the recent rapid development of NGS technologies have enabled identification of somatic mutations in ALL by comparison of matched tumor and normal DNA samples from ALL patients, either by analyzing panels of cancer genes, the entire coding region (exome) or whole genomes of the patients [88,89,90,91,92,69,55,93,54,94,21,95,22,87]. Compared to other cancers, ALL genomes typically harbor few somatic mutations, but somatic alteration by mutation or translocation of genes encoding chromatin modifier genes that introduce or erase epigenetic marks from chromatin are enriched in ALL [96].

The most frequently altered genes in ALL involved in epigenetic regulation are *KMT2A*, *CREBBP*, *EP300*, *ASXL1*, *NSD2, SETD2*, *KMT2D*, *ATRX*, and members of the Polycomb repressive complex 2 (PRC2) [88,89,91,92,94,21,95,22,87]. Somatic point mutations or small insertion-deletions (indels) occur across most subtypes of ALL, and do not appear to be mutually exclusive or enriched to any specific subtype, with the exception of the higher incidence of PRC2 mutations in T-ALL [96]. On the contrary, in-frame fusion genes involving *KMT2A*, *CREBBP*, and *EP300* delineate specific subgroups of BCP-ALL with specific molecular and clinical phenotypes [1]. Mutations in DNA methyltransferases, on the other hand, appear to be rare in ALL [97,21].

KMT2A-r (MLL rearrangements) are a prototypic example of leukemia driven by deregulation of epigenetic processes, in this case by a fusion protein that disrupts the normal function of KMT2A, a H3K4-specific methyltransferase. In infants with the *KMT2A*-r subtype, few or perhaps no additional somatic coding mutations or copy number aberrations are required to cause overt leukemia, and thus the KMT2A fusion protein is thought to be a powerful cancer driver gene [69]. The *KMT2A-AF4* fusion gene resulting from the translocation t(4;11)(q21,q23) is the most common *KMT2A* rearrangement in infant-ALL [1]. The downstream targets of KMT2A–AF4 have been characterized genome-wide in ALL cell lines by chromatin immunoprecipitation coupled with NGS [98]. The fusion protein binds to fewer genomic regions than wild-type KMT2A and extensive chromatin remodeling occurs in the KMT2A-AF4-bound regions, which results in large domains of aberrant H3K79 methylation However, KMT2A fusion proteins retain the ability to bind HOX genes and other promoter regions and are associated with enhanced gene expression [99]. Recurrent mutations in *KMT2A* along with *KMT2D* (*MLL2*), and *KMT2C* (*MLL3*) have been identified in ALL patients of various subtypes [21,22].

The *BRD9-NUTM1* fusion gene resulting from t(5;15)(p15;q14) has been described in two infant-ALL cases negative for *KMT2A*-r to date [69,48]. BRD9 is one of the less studied bromodomain (BRD)-containing proteins, but it likely functions in chromatin remodeling and regulation of gene expression and has recently been implicated in acute myeloid leukemia [100]. NUTM1 enhances the histone acetyltransferase activity of EP300 and is well known for its fusion with BRD4 in an aggressive malignancy called NUT midline carcinoma [101]. Interestingly, sporadic ALL cases with other *NUTM1* fusion genes have been described in recent transcriptome sequencing studies (*IKZF1-NUTM1, AFF1-NUTM1*, *ZNF618-NUTM1)* [3,6]. These recent findings identify a rare subset of ALL patients who may benefit from targeted small molecule inhibitors [102].

Loss of function mutations in the *CREBBP* (CBP) gene have been observed in 18% of relapsed ALLs, particularly in the HeH subgroup [88]. EP300 (p300) is a CREBBP homolog with loss of function mutations in relapsed BCP-ALL [21] and in an aggressive subtype referred to as early T-cell precursor ALL (ETP-ALL) [90]. *ZNF384* rearrangements have recently been described in a new subtype of BCP-ALL in which up to 80% of the patients have a mutation or translocation involving an epigenetic regulating gene [5,6]. The fusions between *ZNF384* and *CREBBP* or *EP300* in this subtype result in dominant-negative loss of histone lysine acetyltransferase activity, which results in a global reduction of histone acetylation, and increased sensitivity to histone deacetylase inhibitors in *in vitro* assays [6]. Hense, a pattern is emerging in ALL where the genes with epigenetic functions that harbor recurrent single nucleotide variants (point mutations) or small indels and are also involved in gene fusions. Examples in addition to those described above include *CBX3* mutations at relapse [103] and the *ETV6-CBX3* fusion gene [48] and *ASXL1* mutations and the *RUNX1-ASXL1* [48] and *NOL4L-ASXL1* [6] fusion genes.

Mutations in the genes encoding the three core subunits of the polycomb repressive complex 2 (PRC2), which methylates H3K27, have also been described in ETP-ALL [104,90]. These genes include embryonic ectoderm development (*EED*), enhancer of zeste 2 (*EZH2*), and suppressor of zeste 12 (*SUZ12*). The *EZH2* promoter is hypermethylated in T-ALL patients, however no promoter methylation changes were identified for the *SUZ12* or *EED* genes [95]. Moreover, disruption of *EZH2* is sufficient to induce T-ALL in mice [105]. Contrary to the function of H3K27 methylation, loss of function mutations in lysine demethylase 6A (*KMT6A*, also known as *UTX*), which specifically demethylates lysine 27 of histone H3, have also been observed in T-ALL [96,106]. Interestingly, *KMT6A* mutated leukemias appear to be sensitive to treatment with an H3K27me3 inhibitor in an in vitro assay [107].

Given that somatic mutations in ALL occur frequently in genes encoding epigenetic regulators, it is surprising that chemical modifications of histone proteins have not been mapped systematically on a genome-wide scale in primary ALL cells nor have the consequences of somatic mutations in epigenetic regulator genes on posttranslational modification of histone proteins been thoroughly examined. However, deregulated H3K9ac [108], global acetylation [109], and increased histone deacetylase activity [110] have been observed in ALL cells. Recent mechanistic studies in cell lines or mouse models have also linked perturbed patters of histone modifications to leukemogenesis. For example, an association between somatic alteration of the histone deacetylase gene *KDM5C* and increased global levels of H3K4me3 has been observed in a murine model of *ETV6-RUNX1* driven ALL [111]. In another study, the chromatin mark H3K36me2 that is methylated by the ASH1L histone methyltransferase was linked to MLL-associated leukemogenesis in a human leukemia cell line, while KDM2A, which is a H3K36me2 demethylase and Polycomb group silencing protein antagonizes MLL-associated leukemogenesis [112]. Importantly, the Blueprint Consortium (www.blueprint-epigenome.eu) for mapping human blood cell epigenomes [113] has recently made available genome-wide data on histone modifications from primary ALL cells from 15 B-cell precursor ALL patients via the European Genome-Phenome Archive (accession #EGAD00001002421). Using this data, a regulatory mechanism for hereditary risk of HeH ALL has been proposed. A risk allele located in an enhancer element on chromosome 10p21.2 marked by H3K4me1, H3K4me3, and H3K27ac disrupts RUNX3 binding and reduces *ARID5B* expression, which in turn could arrest normal lymphocyte development leading to leukemia [114]. The data from Blueprint made available to the research community will be a valuable data source for future epigenetic studies in ALL.

8. Future perspectives and conclusions

The results from the studies discussed herein provide evidence for the importance of epigenetic alterations in pediatric ALL and their possible implications for the development of ALL, disease progression,

and relapse. Combined with the heterogeneity of the cytogenetic subtypes of ALL, the characteristic patterns of DNA methylation and other epigenetic features render almost every patient unique. This heterogeneity poses challenges for diagnosis and treatment stratification. On the other, hand DNA methylation is highly informative regarding the cellular origin of the leukemia in each patient and the cytogenetic features underlying leukemogenesis. Moreover, DNA methylation is stable and easily detectable by relatively simple assays even in degraded DNA and historical biobanked samples of variable quality. Taken together these characteristics render epigenetic alterations, and especially DNA methylation excellent biomarkers for ALL.

The studies on epigenetics in primary ALL cells have so far been dominated by analysis of DNA methylation and more recently by the overrepresentation of mutations in epigenetic regulator genes. Collectively, the studies described above emphasize the importance of epigenetic control in leukemogenesis, but the phenotypic consequences of these mutations remain largely uncharacterized in primary ALL patients. A challenge for the future will be to translate our understanding of the relationship between mutations or gene fusions involving chromatin modifier genes, specific DNA methylation changes, and patterning of histone modifications in primary ALL cells into new therapeutic approaches.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

We thank our colleagues within the Nordic Society of Pediatric Hematology and Oncology (NOPHO), especially Yanara Marincevic-Zuniga for assistance with the figure and Vasilios Zachariadis for helpful discussions. The Swedish Cancer Society and the Swedish Childhood Cancer Foundation supported this work.

References

[1] I. Iacobucci, C.G. Mullighan, Genetic basis of acute lymphoblastic leukemia, J. Clin. Oncol. 35 (2017) 975–983.
[2] C.H. Pui, J.J. Yang, S.P. Hunger, R. Pieters, M. Schrappe, A. Biondi, A. Vora, A. Baruchel, L.B. Silverman, K. Schmiegelow, et al., Childhood acute lymphoblastic leukemia: progress through collaboration, J. Clin. Oncol. 33 (2015) 2938-U2924.
[3] H. Lilljebjorn, R. Henningsson, A. Hyrenius-Wittsten, L. Olsson, C. Orsmark-Pietras, S. von Palffy, M. Askmyr, M. Rissler, M. Schrappe, G. Cario, et al., Identification of ETV6-RUNX1-like and DUX4-rearranged subtypes in paediatric B-cell precursor acute lymphoblastic leukaemia, Nat. Commun. 7 (2016) 11790.
[4] J. Zhang, K. McCastlain, H. Yoshihara, B. Xu, Y. Chang, M.L. Churchman, G. Wu, Y. Li, L. Wei, I. Iacobucci, et al., Deregulation of DUX4 and ERG in acute lymphoblastic leukemia, Nat. Genet. 48 (2016) 1481–1489.
[5] S. Hirabayashi, K. Ohki, K. Nakabayashi, H. Ichikawa, Y. Momozawa, K. Okamura, A. Yaguchi, K. Terada, Y. Saito, A. Yoshimi, et al., ZNF384-related fusion genes define a subgroup of childhood B-cell precursor acute lymphoblastic leukemia with a characteristic immunotype, Haematologica 102 (2017) 118–129.
[6] M. Qian, H. Zhang, S.K. Kham, S. Liu, C. Jiang, X. Zhao, Y. Lu, C. Goodings, T.N. Lin, R. Zhang, et al., Whole-transcriptome sequencing identifies a distinct subtype of acute lymphoblastic leukemia with predominant genomic abnormalities of EP300 and CREBBP, Genome Res. 27 (2017) 185–195.
[7] K.H. Taylor, K.E. Pena-Hernandez, J.W. Davis, G.L. Arthur, D.J. Duff, H. Shi, F.B. Rahmatpanah, O. Sjahputera, C.W. Caldwell, Large-scale CpG methylation analysis identifies novel candidate genes and reveals methylation hotspots in acute lymphoblastic leukemia, Cancer Res. 67 (2007) 2617–2625.
[8] M.E. Figueroa, S.C. Chen, A.K. Andersson, L.A. Phillips, Y. Li, J. Sotzen, M. Kundu, J.R. Downing, A. Melnick, C.G. Mullighan, Integrated genetic and epigenetic analysis of childhood acute lymphoblastic leukemia, J. Clin. Invest. 123 (2013) 3099–3111.
[9] J. Davidsson, H. Lilljebjorn, A. Andersson, S. Veerla, J. Heldrup, M. Behrendtz, T. Fioretos, B. Johansson, The DNA methylome of pediatric acute lymphoblastic leukemia, Hum. Mol. Genet. 18 (2009) 4054–4065.
[10] M. Ehrich, S. Zoll, S. Sur, D. van den Boom, A new method for accurate assessment of DNA quality after bisulfite treatment, Nucleic Acids Res. 35 (2007) e29.
[11] L. Milani, A. Lundmark, J. Nordlund, A. Kiialainen, T. Flaegstad, G. Jonmundsson, J. Kanerva, K. Schmiegelow, K.L. Gunderson, G. Lonnerholm, et al., Allele-specific gene expression patterns in primary leukemic cells reveal regulation of gene expression by CpG site methylation, Genome Res. 19 (2009) 1–11.
[12] M. Bibikova, J. Le, B. Barnes, S. Saedinia-Melnyk, L.X. Zhou, R. Shen, K.L. Gunderson, Genome-wide DNA methylation profiling using infinium (R) assay, Epigenomics 1 (2009) 177–200.
[13] J. Sandoval, H. Heyn, S. Moran, J. Serra-Musach, M.A. Pujana, M. Bibikova, M. Esteller, Validation of a DNA methylation microarray for 450,000 CpG sites in the human genome, Epigenetics-Us 6 (2011) 692–702.
[14] R. Pidsley, E. Zotenko, T.J. Peters, M.G. Lawrence, G.P. Risbridger, P. Molloy, S. Van Djik, B. Muhlhausler, C. Stirzaker, S.J. Clark, Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling, Genome Biol. 17 (2016) 208.
[15] J. Soto, C. Rodriguez-Antolin, E. Vallespin, J. de Castro Carpeno, I. Ibanez de Caceres, The impact of next-generation sequencing on the DNA methylation-based translational cancer research, Transl. Res. 169 (2016) 1–18 e11.
[16] A. Raine, E. Manlig, P. Wahlberg, A.C. Syvanen, J. Nordlund, SPlinted Ligation Adapter Tagging (SPLAT), a novel library preparation method for whole genome bisulphite sequencing, Nucleic Acids Res. 45 (2017) e36.
[17] S.T. Lee, M.O. Muench, M.E. Fomin, J. Xiao, M. Zhou, A. de Smith, J.I. Martin-Subero, S. Heath, E.A. Houseman, R. Roy, et al., Epigenetic remodeling in B-cell acute lymphoblastic leukemia occurs in two tracks and employs embryonic stem cell-like signatures, Nucleic Acids Res. 43 (2015) 2590–2602.
[18] P. Wahlberg, A. Lundmark, J. Nordlund, S. Busche, A. Raine, K. Tandre, L. Ronnblom, D. Sinnett, E. Forestier, T. Pastinen, et al., DNA methylome analysis of acute lymphoblastic leukemia cells reveals stochastic de novo DNA methylation in CpG islands, Epigenomics 8 (2016) 1367–1387.
[19] S.K. Stewart, T.J. Morris, P. Guilhamon, H. Bulstrode, M. Bachman, S. Balasubramanian, S. Beck, oxBS-450K: a method for analysing hydroxymethylation using 450K BeadChips, Methods 72 (2015) 9–15.
[20] R.M. Kohli, Y. Zhang, TET enzymes, TDG and the dynamics of DNA demethylation, Nature 502 (2013) 472–479.
[21] C.M. Lindqvist, A. Lundmark, J. Nordlund, E. Freyhult, D. Ekman, J. Carlsson Almlof, A. Raine, E. Overnas, J. Abrahamsson, B.M. Frost, et al., Deep targeted sequencing in pediatric acute lymphoblastic leukemia unveils distinct mutational patterns between genetic subtypes and novel relapse-associated genes, Oncotarget 7 (2016) 64071–64088.
[22] L.W. Ding, Q.Y. Sun, K.T. Tan, W. Chien, A.M. Thippeswamy, A. Eng Juh Yeoh, N. Kawamata, Y. Nagata, J.F. Xiao, X.Y. Loh, et al., Mutational landscape of pediatric acute lymphoblastic leukemia, Cancer Res. 77 (2017) 390–400.
[23] M. Ko, Y. Huang, A.M. Jankowska, U.J. Pape, M. Tahiliani, H.S. Bandukwala, J. An, E.D. Lamperti, K.P. Koh, R. Ganetzky, et al., Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2, Nature 468 (2010) 839–843.
[24] M. Duran-Ferrer, R. Beekman, J.I. Martin-Subero, In silico deconvolution and purification of cancer epigenomes, Oncoscience 4 (2017) 25–26.
[25] S.C. Zheng, S. Beck, A.E. Jaffe, D.C. Koestler, K.D. Hansen, A.E. Houseman, R.A. Irizarry, A.E. Teschendorff, Correcting for cell-type heterogeneity in epigenome-wide association studies: revisiting previous analyses, Nat. Methods 14 (2017) 216–217.
[26] M. Farlik, N.C. Sheffield, A. Nuzzo, P. Datlinger, A. Schonegger, J. Klughammer, C. Bock, Single-cell DNA methylome sequencing and bioinformatic inference of epigenomic cell-state dynamics, Cell Rep. 10 (2015) 1386–1397.
[27] K. Wang, X. Li, S. Dong, J. Liang, F. Mao, C. Zeng, H. Wu, J. Wu, W. Cai, Z.S. Sun, Q-RRBS: a quantitative reduced representation bisulfite sequencing method for single-cell methylome analyses, Epigenetics-US 10 (2015) 775–783.
[28] G.X. Zheng, J.M. Terry, P. Belgrader, P. Ryvkin, Z.W. Bent, R. Wilson, S.B. Ziraldo, T.D. Wheeler, G.P. McDermott, J. Zhu, et al., Massively parallel digital transcriptional profiling of single cells, Nat. Commun. 8 (2017) 14049.
[29] A.M. Deaton, A. Bird, CpG islands and the regulation of transcription, Genes Dev. 25 (2011) 1010–1022.
[30] J. Sandoval, M. Esteller, Cancer epigenomics: beyond genomics, Curr. Opin. Genet. Dev. 22 (2012) 50–55.
[31] H. Easwaran, H.C. Tsai, S.B. Baylin, Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance, Mol. Cell 54 (2014) 716–727.
[32] K.D. Hansen, W. Timp, H.C. Bravo, S. Sabunciyan, B. Langmead, O.G. McDonald, B. Wen, H. Wu, Y. Liu, D. Diep, et al., Increased methylation variation in epigenetic domains across cancer types, Nat. Genet. 43 (2011) 768–775.
[33] M.J. Burke, T. Bhatla, Epigenetic modifications in pediatric acute lymphoblastic leukemia, Front. Pediatr. 2 (2014) 42.
[34] V. Hale, G.A. Hale, P.A. Brown, E.K. Amankwah, A review of DNA methylation and microRNA expression in recurrent pediatric acute leukemia, Oncology 92 (2017) 61–67.
[35] I.H.N. Wong, M.H.L. Ng, D.P. Huang, J.C.K. Lee, Aberrant p15 promoter methylation in adult and childhood acute leukemias of nearly all morphologic subtypes: potential prognostic implications, Blood 95 (2000) 1942–1949.
[36] P.J. Shteper, Z. Siegfried, F.A. Asimakopoulos, G.A. Palumbo, E.A. Rachmilewitz, Y. Ben-Neriah, D. Ben-Yehuda, ABL1 methylation in Ph-positive ALL is exclusively associated with the P210 form of BCR-ABL, Leukemia 15 (2001) 575–582.
[37] S.C. Zheng, X.M. Ma, L.P. Zhang, L. Gunn, M.T. Smith, J.L. Wiemels, K. Leung, P.A. Buffler, J.K. Wiencke, Hypermethylation of the 5 ' CpG island of the FHIT gene is associated with hyperdiploid and translocation-negative subtypes of pediatric leukemia, Cancer Res. 64 (2004) 2000–2006.
[38] C. Matsushita, Y. Yang, S. Takeuchi, M. Matsushita, J.J.M. Van Dongen, T. Szczepanski, C.R. Bartram, H. Seo, H.P. Koeffler, H. Taguchi, Aberrant methylation in promoter-associated CpG islands of multiple genes in relapsed childhood acute lymphoblastic leukemia, Oncol. Rep. 12 (2004) 97–99.
[39] T.L. Dunwell, L.B. Hesson, T. Pavlova, V. Zabarovska, V. Kashuba, D. Catchpoole,

R. Chiaramonte, A.T. Brini, M. Griffiths, E.R. Maher, et al., Epigenetic analysis of childhood acute lymphoblastic leukemia, Epigenetics-US 4 (2009) 185–193.
[40] D.J. Stumpel, P. Schneider, E.H. van Roon, J.M. Boer, P. de Lorenzo, M.G. Valsecchi, R.X. de Menezes, R. Pieters, R.W. Stam, Specific promoter methylation identifies different subgroups of MLL-rearranged infant acute lymphoblastic leukemia, influences clinical outcome, and provides therapeutic options, Blood 114 (2009) 5490–5498.
[41] L. Milani, A. Lundmark, A. Kiialainen, J. Nordlund, T. Flaegstad, E. Forestier, M. Heyman, G. Jonmundsson, J. Kanerva, K. Schmiegelow, et al., DNA methylation for subtype classification and prediction of treatment outcome in patients with childhood acute lymphoblastic leukemia, Blood 115 (2010) 1214–1225.
[42] L.E. Hogan, J.A. Meyer, J. Yang, J.H. Wang, N. Wong, W.J. Yang, G. Condos, S.P. Hunger, E. Raetz, R. Saffery, et al., Integrated genomic analysis of relapsed childhood acute lymphoblastic leukemia reveals therapeutic strategies, Blood 118 (2011) 5218–5226.
[43] N.C. Wong, D. Ashley, Z. Chatterton, M. Parkinson-Bates, H.K. Ng, M.S. Halemba, A. Kowalczyk, J. Bedo, Q. Wang, K. Bell, et al., A distinct DNA methylation signature defines pediatric pre-B cell acute lymphoblastic leukemia, Epigenetics-US 7 (2012) 535–541.
[44] J. Nordlund, L. Milani, A. Lundmark, G. Lonnerholm, A.C. Syvanen, DNA methylation analysis of bone marrow cells at diagnosis of acute lymphoblastic leukemia and at remission, PLoS One 7 (2012) e34513.
[45] S. Busche, B. Ge, R. Vidal, J.F. Spinella, V. Saillour, C. Richer, J. Healy, S.H. Chen, A. Droit, D. Sinnett, et al., Integration of high-resolution methylome and transcriptome analyses to dissect epigenomic changes in childhood acute lymphoblastic leukemia, Cancer Res. 73 (2013) 4323–4336.
[46] M. Borssen, L. Palmqvist, K. Karrman, J. Abrahamsson, M. Behrendtz, J. Heldrup, E. Forestier, G. Roos, S. Degerman, Promoter DNA methylation pattern identifies prognostic subgroups in childhood T-cell acute lymphoblastic leukemia, PLoS One 8 (2013) e65373.
[47] J. Nordlund, C.L. Backlin, P. Wahlberg, S. Busche, E.C. Berglund, M.L. Eloranta, T. Flaegstad, E. Forestier, B.M. Frost, A. Harila-Saari, et al., Genome-wide signatures of differential DNA methylation in pediatric acute lymphoblastic leukemia, Genome Biol. 14 (2013) r105.
[48] J. Nordlund, C.L. Backlin, V. Zachariadis, L. Cavelier, J. Dahlberg, I. Ofverholm, G. Barbany, A. Nordgren, E. Overnas, J. Abrahamsson, et al., DNA methylation-based subtype prediction for pediatric acute lymphoblastic leukemia, Clin. Epigenetics 7 (2015) 11.
[49] Z. Chatterton, L. Morenos, F. Mechinaud, D.M. Ashley, J.M. Craig, A. Sexton-Oates, M.S. Halemba, M. Parkinson-Bates, J. Ng, D. Morrison, et al., Epigenetic deregulation in pediatric acute lymphoblastic leukemia, Epigenetics-US 9 (2014) 459–467.
[50] M. Almamun, B.T. Levinson, A.C. van Swaay, N.T. Johnson, S.D. McKay, G.L. Arthur, J.W. Davis, K.H. Taylor, Integrated methylome and transcriptome analysis reveals novel regulatory elements in pediatric acute lymphoblastic leukemia, Epigenetics-US 10 (2015) 882–890.
[51] M. Almamun, O. Kholod, A.J. Stuckel, B.T. Levinson, N.T. Johnson, G.L. Arthur, J.W. Davis, K.H. Taylor, Inferring a role for methylation of intergenic DNA in the regulation of genes aberrantly expressed in precursor B-cell acute lymphoblastic leukemia, Leuk. Lymphoma (2017) 1–12, http://dx.doi.org/10.1080/10428194.2016.1272683.
[52] G. Arthur, M. Almamun, K. Taylor, Hypermethylation of antisense long noncoding RNAs in acute lymphoblastic leukemia, Epigenomics 9 (2017) 635–645.
[53] A.S. Gabriel, F.M. Lafta, E.C. Schwalbe, S. Nakjang, S.J. Cockell, A. Iliasova, A. Enshaei, C. Schwab, V. Rand, S.C. Clifford, et al., Epigenetic landscape correlates with genetic subtype but does not predict outcome in childhood acute lymphoblastic leukemia, Epigenetics-US 10 (2015) 717–726.
[54] S. Safavi, L. Olsson, A. Biloglav, S. Veerla, M. Blendberg, J. Tayebwa, M. Behrendtz, A. Castor, M. Hansson, B. Johansson, et al., Genetic and epigenetic characterization of hypodiploid acute lymphoblastic leukemia, Oncotarget 6 (2015) 42793–42802.
[55] J.B. Kunz, T. Rausch, O.R. Bandapalli, J. Eilers, P. Pechanska, S. Schuessele, Y. Assenov, A.M. Stutz, R. Kirschner-Schwabe, J. Hof, et al., Pediatric T-cell lymphoblastic leukemia evolves into relapse by clonal selection, acquisition of mutations and promoter hypomethylation, Haematologica 100 (2015) 1442–1450.
[56] M. Borssen, Z. Haider, M. Landfors, U. Noren-Nystrom, K. Schmiegelow, A.E. Asberg, J. Kanerva, H.O. Madsen, H. Marquart, M. Heyman, et al., DNA methylation adds prognostic value to minimal residual disease status in pediatric T-Cell acute lymphoblastic leukemia, Pediatr. Blood Cancer 63 (2016) 1185–1192.
[57] Y. Marincevic-Zuniga, V. Zachariadis, L. Cavelier, A. Castor, G. Barbany, E. Forestier, L. Fogelstrand, M. Heyman, J. Abrahamsson, G. Lonnerholm, et al., PAX5-ESRRB is a recurrent fusion gene in B-cell precursor pediatric acute lymphoblastic leukemia, Haematologica 101 (2016) e20–23.
[58] A.K. Bergmann, G. Castellano, J. Alten, O. Ammerpohl, J. Kolarova, J. Nordlund, J.I. Martin-Subero, M. Schrappe, R. Siebert, DNA methylation profiling of pediatric B-cell lymphoblastic leukemia with KMT2A rearrangement identifies hypomethylation at enhancer sites, PediatR. Blood Cancer 64 (2017).
[59] M. Abdullah, C.W. Choo, H. Alias, E.J. Abdul Rahman, H.M. Ibrahim, R. Jamal, N.H. Hussin, ADAMTSL5 and CDH11: putative epigenetic markers for therapeutic resistance in acute lymphoblastic leukemia, Hematology (2017) 1–6, http://dx.doi.org/10.1080/10245332.2017.1299417.
[60] B.P. Berman, D.J. Weisenberger, J.F. Aman, T. Hinoue, Z. Ramjan, Y. Liu, H. Noushmehr, C.P. Lange, C.M. van Dijk, R.A. Tollenaar, et al., Regions of focal DNA hypermethylation and long-range hypomethylation in colorectal cancer coincide with nuclear lamina-associated domains, Nat. Genet. 44 (2011) 40–46.
[61] E.J. Yeoh, M.E. Ross, S.A. Shurtleff, W.K. Williams, D. Patel, R. Mahfouz, F.G. Behm, S.C. Raimondi, M.V. Relling, A. Patel, et al., Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling, Cancer Cell 1 (2002) 133–143.
[62] J. Nordlund, A. Kiialainen, O. Karlberg, E.C. Berglund, H. Goransson-Kultima, M. Sonderkaer, K.L. Nielsen, M.G. Gustafsson, M. Behrendtz, E. Forestier, et al., Digital gene expression profiling of primary acute lymphoblastic leukemia cells, Leukemia 26 (2012) 1218–1227.
[63] K. Durinck, S. Goossens, S. Peirs, A. Wallaert, W. Van Loocke, F. Matthijssens, T. Pieters, G. Milani, T. Lammens, P. Rondou, et al., Novel biological insights in T-cell acute lymphoblastic leukemia, Exp. Hematol. 43 (2015) 625–639.
[64] S.K. Tasian, S.P. Hunger, Genomic characterization of paediatric acute lymphoblastic leukaemia: an opportunity for precision medicine therapeutics, Br. J. Haematol. 176 (2017) 867–882.
[65] H. Inaba, M. Greaves, C.G. Mullighan, Acute lymphoblastic leukaemia, Lancet 381 (2013) 1943–1955.
[66] K. Paulsson, H. Lilljebjorn, A. Biloglav, L. Olsson, M. Rissler, A. Castor, G. Barbany, L. Fogelstrand, A. Nordgren, H. Sjogren, et al., The genomic landscape of high hyperdiploid childhood acute lymphoblastic leukemia, Nat. Genet. 47 (2015) 672–676.
[67] R.C. Rao, Y. Dou, Hijacked in cancer: the KMT2 (MLL) family of methyltransferases, Nat. Rev. Cancer 15 (2015) 334–346.
[68] M. Stahl, N. Kohrman, S.D. Gore, T.K. Kim, A.M. Zeidan, T. Prebet, Epigenetics in cancer: a hematological perspective, PLoS Genet. 12 (2016) e1006193.
[69] A.K. Andersson, J. Ma, J. Wang, X. Chen, A.L. Gedman, J. Dang, J. Nakitandwe, L. Holmfeldt, M. Parker, J. Easton, et al., The landscape of somatic mutations in infant MLL-rearranged acute lymphoblastic leukemias, Nat. Genet. 47 (2015) 330–337.
[70] V. Zachariadis, F. Gauffin, E. Kuchinskaya, M. Heyman, J. Schoumans, E. Blennow, B. Gustafsson, G. Barbany, I. Golovleva, H. Ehrencrona, et al., The frequency and prognostic impact of dic(9;20)(p13.2;q11.2) in childhood B-cell precursor acute lymphoblastic leukemia: results from the NOPHO ALL-2000 trial, Leukemia 25 (2011) 622–628.
[71] Q. An, S.L. Wright, A.V. Moorman, H. Parker, M. Griffiths, F.M. Ross, T. Davies, C.J. Harrison, J.C. Strefford, Heterogeneous breakpoints in patients with acute lymphoblastic leukemia and the dic(9;20)(p11-13;q11) show recurrent involvement of genes at 20q11. 21, Haematologica 94 (2009) 1164–1169.
[72] M. Shago, Recurrent cytogenetic abnormalities in acute lymphoblastic leukemia, Methods Mol. Biol. 1541 (2017) 257–278.
[73] A.V. Moorman, New and emerging prognostic and predictive genetic biomarkers in B-cell precursor acute lymphoblastic leukemia, Haematologica 101 (2016) 407–416.
[74] K. Karrman, B. Johansson, Pediatric T-cell acute lymphoblastic leukemia, Genes. Chromosomes Cancer 56 (2017) 89–116.
[75] M.D. Kraszewska, M. Dawidowska, N.S.D. Larmonie, M. Kosmalska, L. Sedek, M. Szczepaniak, W. Grzeszczak, A.W. Langerak, T. Szczepanski, M. Witt, et al., DNA methylation pattern is altered in childhood T-cell acute lymphoblastic leukemia patients as compared with normal thymic subsets: insights into CpG island methylator phenotype in T-ALL, Leukemia 26 (2012) 367–371.
[76] B.F. Miller, F. Sanchez-Vega, L. Elnitski, The emergence of pan-cancer CIMP and its elusive interpretation, Biomolecules 6 (2016).
[77] Y. Marincevic-Zuniga, J. Dahlberg, S. Nilsson, A. Raine, S. Nystedt, C.M. Lindqvist, E.C. Berglund, J. Abrahamsson, L. Cavelier, E. Forestier, et al., Transcriptome sequencing in pediatric acute lymphoblastic leukemia identifies fusion genes associated with distinct DNA methylation profiles, J. Hematol. Oncol. 10 (2017) 148.
[78] M.L. Den Boer, M. van Slegtenhorst, R.X. De Menezes, M.H. Cheok, J.G. Buijs-Gladdines, S.T. Peters, L.J. Van Zutven, H.B. Beverloo, P.J. Van der Spek, G. Escherich, et al., A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study, Lancet Oncol. 10 (2009) 125–134.
[79] C.G. Mullighan, X. Su, J. Zhang, I. Radtke, L.A. Phillips, C.B. Miller, J. Ma, W. Liu, C. Cheng, B.A. Schulman, et al., Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia, N. Engl. J. Med. 360 (2009) 470–480.
[80] K.G. Roberts, Y. Li, D. Payne-Turner, R.C. Harvey, Y.L. Yang, D. Pei, K. McCastlain, L. Ding, C. Lu, G. Song, et al., Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia, N. Engl. J. Med. 371 (2014) 1005–1015.
[81] S. Teppo, S. Laukkanen, T. Liuksiala, J. Nordlund, M. Oittinen, K. Teittinen, T. Gronroos, P. St-Onge, D. Sinnett, A.C. Syvanen, et al., Genome-wide repression of eRNA and target gene loci by the ETV6-RUNX1 fusion in acute leukemia, Genome Res. 26 (2016) 1468–1477.
[82] T. Trimarchi, E. Bilal, P. Ntziachristos, G. Fabbri, R. Dalla-Favera, A. Tsirigos, I. Aifantis, Genome-wide mapping and characterization of Notch-regulated long noncoding RNAs in acute leukemia, Cell 158 (2014) 593–606.
[83] F. Ghazavi, B. De Moerloose, W. Van Loocke, A. Wallaert, H.H. Helsmoortel, A. Ferster, M. Bakkus, G. Plat, E. Delabesse, A. Uyttebroeck, et al., Unique long non-coding RNA expression signature in ETV6/RUNX1-driven B-cell precursor acute lymphoblastic leukemia, Oncotarget 7 (2016) 73769–73780.
[84] M. Lajoie, S. Drouin, M. Caron, P. St-Onge, M. Ouimet, R. Gioia, M.H. Lafond, R. Vidal, C. Richer, K. Oualkacha, et al., Specific expression of novel long non-coding RNAs in high-hyperdiploid childhood acute lymphoblastic leukemia, PLoS One 12 (2017) e0174124.
[85] E. Vendramini, M. Giordan, E. Giarin, B. Michielotto, G. Fazio, G. Cazzaniga, A. Biondi, D. Silvestri, M.G. Valsecchi, M.U. Muckenthaler, et al., High expression of miR-and SNORD116 noncoding RNA clusters characterize ERG-related B cell precursor acute lymphoblastic leukemia, Oncotarget (2017) 125b–1252, http://

dx.doi.org/10.18632/oncotarget.16392.
[86] J. Sandoval, H. Heyn, J. Mendez-Gonzalez, A. Gomez, S. Moran, M. Baiget, M. Melo, I. Badell, J.F. Nomdedeu, M. Esteller, Genome-wide DNA methylation profiling predicts relapse in childhood B-cell acute lymphoblastic leukaemia, Br. J. Haematol. 160 (2013) 406–409.
[87] S. Janczar, K. Janczar, A. Pastorczak, H. Harb, A.J. Paige, B. Zalewska-Szewczyk, M. Danilewicz, W. Mlynarski, The role of histone protein modifications and mutations in histone modifiers in pediatric B-cell progenitor acute lymphoblastic leukemia, Cancers 9 (2017).
[88] C.G. Mullighan, J. Zhang, L.H. Kasper, S. Lerach, D. Payne-Turner, L.A. Phillips, S.L. Heatley, L. Holmfeldt, J.R. Collins-Underwood, J. Ma, et al., CREBBP mutations in relapsed acute lymphoblastic leukaemia, Nature 471 (2011) 235–239.
[89] A. Inthal, P. Zeitlhofer, M. Zeginigg, M. Morak, R. Grausenburger, E. Fronkova, B. Fahrner, G. Mann, O.A. Haas, R. Panzer-Grumayer, CREBBP HAT domain mutations prevail in relapse cases of high hyperdiploid childhood acute lymphoblastic leukemia, Leukemia 26 (2012) 1797–1803.
[90] J. Zhang, L. Ding, L. Holmfeldt, G. Wu, S.L. Heatley, D. Payne-Turner, J. Easton, X. Chen, J. Wang, M. Rusch, et al., The genetic basis of early T-cell precursor acute lymphoblastic leukaemia, Nature 481 (2012) 157–163.
[91] B.G. Mar, L.B. Bullinger, K.M. McLean, P.V. Grauman, M.H. Harris, K. Stevenson, D.S. Neuberg, A.U. Sinha, S.E. Sallan, L.B. Silverman, et al., Mutations in epigenetic regulators including SETD2 are gained during relapse in paediatric acute lymphoblastic leukaemia, Nat. Commun. 5 (2014) 3469.
[92] J.A. Oyer, X. Huang, Y. Zheng, J. Shim, T. Ezponda, Z. Carpenter, M. Allegretta, C.I. Okot-Kotber, J.P. Patel, A. Melnick, et al., Point mutation E1099K in MMSET/NSD2 enhances its methyltranferase activity and leads to altered global chromatin methylation in lymphoid malignancies, Leukemia 28 (2014) 198–201.
[93] C.M. Lindqvist, J. Nordlund, D. Ekman, A. Johansson, B.T. Moghadam, A. Raine, E. Overnas, J. Dahlberg, P. Wahlberg, N. Henriksson, et al., The mutational landscape in pediatric acute lymphoblastic leukemia deciphered by whole genome sequencing, Hum. Mutat. 36 (2015) 118–128.
[94] K.M. Bernt, S.P. Hunger, T. Neff, The functional role of PRC2 in early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) − mechanisms and opportunities, Front. Pediatr. 4 (2016) 49.
[95] V. Schafer, J. Ernst, J. Rinke, N. Winkelmann, J.F. Beck, A. Hochhaus, B. Gruhn, T. Ernst, EZH2 mutations and promoter hypermethylation in childhood acute lymphoblastic leukemia, J. Cancer Res. Clin. Oncol. 142 (2016) 1641–1650.
[96] R. Huether, L. Dong, X. Chen, G. Wu, M. Parker, L. Wei, J. Ma, M.N. Edmonson, E.K. Hedlund, M.C. Rusch, et al., The landscape of somatic mutations in epigenetic regulators across 1, 000 paediatric cancer genomes, Nat. Commun. 5 (2014) 3630.
[97] W. Li, C. Gao, L. Cui, S. Liu, X. Zhao, R. Zhang, M. Wu, H. Zheng, G. Deng, Z. Li, et al., DNMT3A mutations and prognostic significance in childhood acute lymphoblastic leukemia, Leuk. Lymphoma 56 (2015) 1066–1071.
[98] M.G. Guenther, L.N. Lawton, T. Rozovskaia, G.M. Frampton, S.S. Levine, T.L. Volkert, C.M. Croce, T. Nakamura, E. Canaani, R.A. Young, Aberrant chromatin at genes encoding stem cell regulators in human mixed-lineage leukemia, Gene Dev 22 (2008) 3403–3408.
[99] A.V. Krivtsov, Z. Feng, M.E. Lemieux, J. Faber, S. Vempati, A.U. Sinha, X. Xia, J. Jesneck, A.P. Bracken, L.B. Silverman, et al., H3K79 methylation profiles define murine and human MLL-AF4 leukemias, Cancer Cell 14 (2008) 355–368.
[100] A.F. Hohmann, L.J. Martin, J.L. Minder, J.S. Roe, J. Shi, S. Steurer, G. Bader, D. McConnell, M. Pearson, T. Gerstberger, et al., Sensitivity and engineered resistance of myeloid leukemia cells to BRD9 inhibition, Nat. Chem. Biol. 12 (2016) 672–679.
[101] N. Reynoird, B.E. Schwartz, M. Delvecchio, K. Sadoul, D. Meyers, C. Mukherjee, C. Caron, H. Kimura, S. Rousseaux, P.A. Cole, et al., Oncogenesis by sequestration of CBP/p300 in transcriptionally inactive hyperacetylated chromatin domains, EMBO J. 29 (2010) 2943–2952.
[102] S.M. Greenblatt, S.D. Nimer, Chromatin modifiers and the promise of epigenetic therapy in acute leukemia, Leukemia 28 (2014) 1396–1406.
[103] J.A. Meyer, J. Wang, L.E. Hogan, J.J. Yang, S. Dandekar, J.P. Patel, Z. Tang, P. Zumbo, S. Li, J. Zavadil, et al., Relapse-specific mutations in NT5C2 in childhood acute lymphoblastic leukemia, Nat. Genet. 45 (2013) 290–294.
[104] P. Ntziachristos, A. Tsirigos, P. Van Vlierberghe, J. Nedjic, T. Trimarchi, M.S. Flaherty, D. Ferres-Marco, V. da Ros, Z. Tang, J. Siegle, et al., Genetic inactivation of the polycomb repressive complex 2 in T cell acute lymphoblastic leukemia, Nat. Med. 18 (2012) 298–301.
[105] C. Simon, J. Chagraoui, J. Krosl, P. Gendron, B. Wilhelm, S. Lemieux, G. Boucher, P. Chagnon, S. Drouin, R. Lambert, et al., A key role for EZH2 and associated genes in mouse and human adult T-cell acute leukemia, Genes Dev. 26 (2012) 651–656.
[106] P. Ntziachristos, A. Tsirigos, G.G. Welstead, T. Trimarchi, S. Bakogianni, L. Xu, E. Loizou, L. Holmfeldt, A. Strikoudis, B. King, et al., Contrasting roles of histone 3 lysine 27 demethylases in acute lymphoblastic leukaemia, Nature 514 (2014) 513–517.
[107] J. Van der Meulen, V. Sanghvi, K. Mavrakis, K. Durinck, F. Fang, F. Matthijssens, P. Rondou, M. Rosen, T. Pieters, P. Vandenberghe, et al., The H3K27me3 demethylase UTX is a gender-specific tumor suppressor in T-cell acute lymphoblastic leukemia, Blood 125 (2015) 13–21.
[108] M.E. Figueroa, M. Reimers, R.F. Thompson, K. Ye, Y. Li, R.R. Selzer, J. Fridriksson, E. Paietta, P. Wiernik, R.D. Green, et al., An integrative genomic and epigenomic approach for the study of transcriptional regulation, PLoS One 3 (2008) e1882.
[109] K. Janczar, S. Janczar, A. Pastorczak, K. Mycko, A.J. Paige, B. Zalewska-Szewczyk, M. Wagrowska-Danilewicz, M. Danilewicz, W. Mlynarski, Preserved global histone H4 acetylation linked to ETV6-RUNX1 fusion and PAX5 deletions is associated with favorable outcome in pediatric B-cell progenitor acute lymphoblastic leukemia, Leuk. Res. 39 (2015) 1455–1461.
[110] J. Sonnemann, B. Gruhn, S. Wittig, S. Becker, J.F. Beck, Increased activity of histone deacetylases in childhood acute lymphoblastic leukaemia and acute myeloid leukaemia: support for histone deacetylase inhibitors as antileukaemic agents, Br. J. Haematol. 158 (2012) 664–666.
[111] G. Rodriguez-Hernandez, J. Hauer, A. Martin-Lorenzo, D. Schafer, C. Bartenhagen, I. Garcia-Ramirez, F. Auer, I. Gonzalez-Herrero, L. Ruiz-Roca, M. Gombert, et al., Infection exposure promotes ETV6-RUNX1 precursor B-cell leukemia via impaired H3K4 demethylases, Cancer Res. 77 (2017) 4365–4377.
[112] L. Zhu, Q. Li, S.H. Wong, M. Huang, B.J. Klein, J. Shen, L. Ikenouye, M. Onishi, D. Schneidawind, C. Buechele, et al., ASH1L links histone H3 lysine 36 dimethylation to MLL leukemia, Cancer Discov. 6 (2016) 770–783.
[113] J.H. Martens, H.G. Stunnenberg, BLUEPRINT: mapping human blood cell epigenomes, Haematologica 98 (2013) 1487–1489.
[114] J.B. Studd, J. Vijayakrishnan, M. Yang, G. Migliorini, K. Paulsson, R.S. Houlston, Genetic and regulatory mechanism of susceptibility to high-hyperdiploid acute lymphoblastic leukaemia at 10p21.2, Nat. Commun. 8 (2017) 14616.