Clinical Epigenetics

REVIEW

Open Access

DNA methylation in monozygotic twins discordant for acute lymphoblastic leukemia: a case report and systematic review

Mao-ling Sun[1], Yang Li[2], Rong-xi Man[1], Si-wen Wang[1], Rong Guo[1], Ying Yang[3*] and Yu-qing Pan[1*]

Abstract

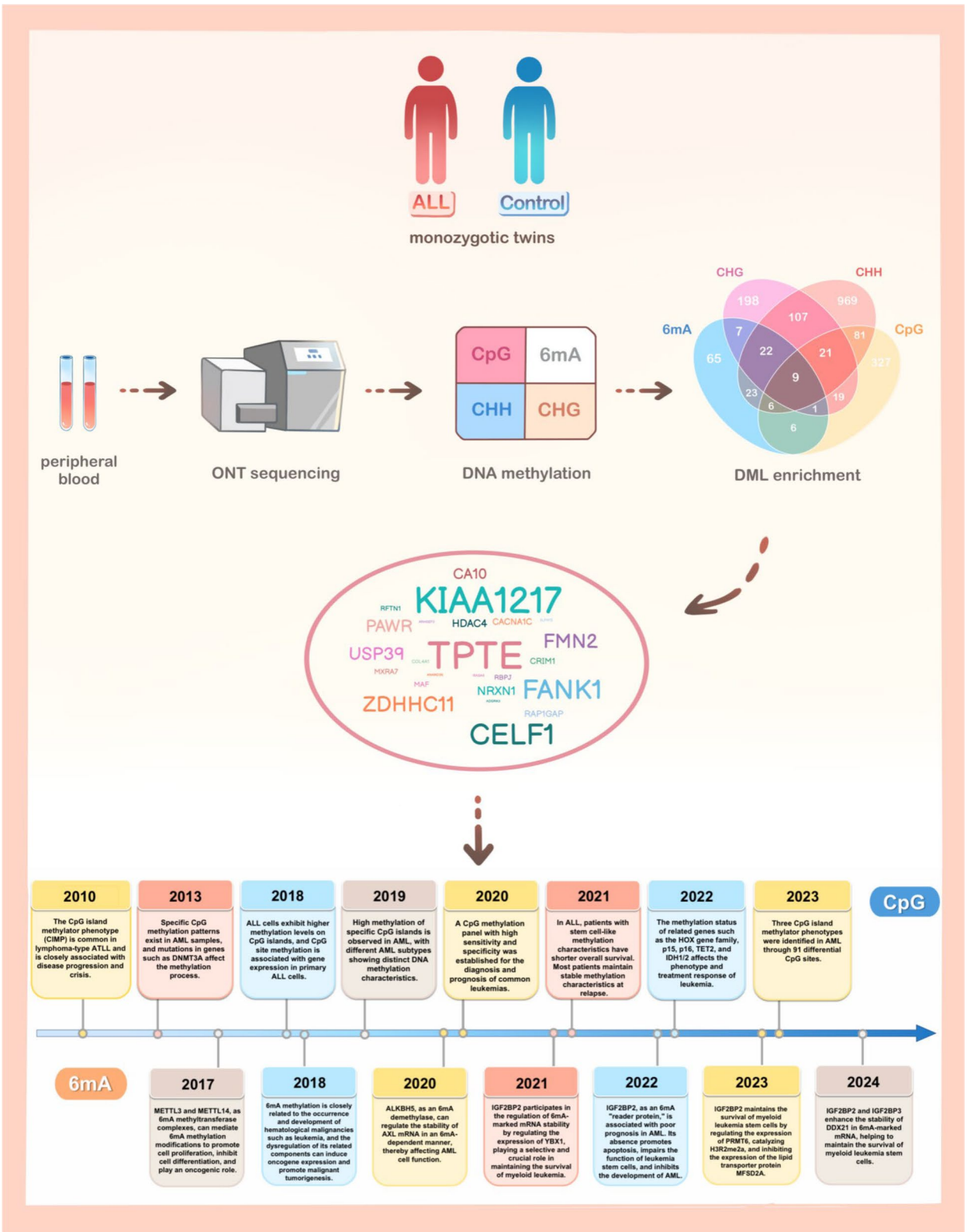
Acute lymphoblastic leukemia (ALL) is a prevalent malignant hematologic disease characterized by the abnormal proliferation and accumulation of immature lymphocytes in bone marrow and lymphoid tissues. In our study, Oxford Nanopore Technologies (ONT) sequencing was performed to investigate four types of methylation modifications—6 mA, CHG, CHH, and CpG—in a pair of monozygotic twins, where one twin has ALL and the other is healthy. The results showed the significant global hypomethylation of CpG sites and an increase in 6 mA, CHG, and CHH methylation in the twin diagnosed with ALL. Notably, the hypomethylation of CpG was particularly increased in the open sea, gene body, and 3'UTR regions, while 6 mA and CHG modifications exhibited high methylation levels in the gene body, TSS1500, TSS200, and 3'UTR regions. Additionally, CHH modifications showed high methylation across all genomic regions. Within the differential methylation loci (DML), we identified several genes related to tumorigenesis and progression (such as ZDHHC11, NBPF1, and TPTE). Furthermore, we systemically reviewed the literatures on leukemia and DNA methylation modifications, providing a comprehensive description of their correlation. In summary, these findings indicate that DNA methylation plays a crucial role in the onset and progression of ALL, offering valuable insights for future research into its impact on leukemia development.

Keyword Acute lymphoblastic leukemia, DNA methylation, Monozygotic twin, Systematic review

†*Mao-ling Sun and Yang Li are co-first authors.

*Correspondence:
Ying Yang
y18940259125@163.com
Yu-qing Pan
18940256450@163.com
Full list of author information is available at the end of the article

Graphic abstract



Introduction

Acute lymphoblastic leukemia (ALL), a common malignant hematologic disorder with diverse and clinically heterogeneous biology, is characterized by abnormal proliferation and aggregation of immature lymphocytes in the bone marrow and lymphoid tissues [1]. ALL accounts for 15% of all leukemias, and approximately 30%−40% of acute leukemias. The median age of adults with ALL is 30–40 years, and it is usually slightly more common in males than females [2, 3]. Although the etiology of ALL has not been fully elucidated, the presence of disease-defining chromosomal translocations has been demonstrated in the previous study [4]. Monozygotic twin studies have shown concordance rates of approximately 10%, in which twins may have the same starting chromosomal translocation through shared blood chimerism [5, 6]. However, the 90% discordance rate suggests that additional intrauterine or early-life genetic, epigenetic, and environmental factors contribute to a necessary"second hit"in the development of leukemia [7]. Among them, DNA methylation plays a potential role as a predisposing factor [8]. Therefore, it is evident that DNA methylation levels play an indispensable role in the occurrence and development of ALL [9, 10].

DNA methylation is a stable heritable epigenetic imprint that is primarily established during early embryonic development and is subject to genetic, environmental, and stochastic control [11]. Correlated interindividual variant loci (CoRSIV) are sensitive to changes in the perinatal intrauterine environment and may act as metastatic epigenetic alleles, independently of genetic influences, to produce phenotypic variation between individuals. Incongruent monozygotic twins, in which one twin is diseased and the other is not, provide an ideal setting for studying the role of epigenetic influences on disease risk because of their genetic characteristics [12]. In addition, because of the uneven distribution of placental blood flow, monozygotic twins do not necessarily share equally in the nutrients and other factors they receive, thus creating an imbalance in disease risk among genetically identical individuals. The establishment of environmentally sensitive DNA methylation patterns at birth can modulate disease risk, while acquired factors, such as behavior and exposure, can similarly impact DNA methylation level [13].

Compared to normal cells of the same tissue, DNA methylation in tumor cell samples generally consists of two types: demethylation of many regions of the genome versus relative hypermethylation of CpG islands (CGIs) [14, 15]. For example, in the gastric cancer cell line SNU484 and the colon cancer cell lines DKO and SW480, there is a notable occurrence of demethylation in specific gene regions. In contrast, both of the gastric cancer cell line SNU601 and the colon cancer cell line HCT116 exhibit hypermethylation in certain CpG islands of specific genes. On the other hand, there is a hypermethylation of CGIs within specific genes [16]. Similar patterns are also observed in leukemia [17, 18]. Global DNA hypermethylation has been identified in ALL cells compared to healthy bone marrow samples, particularly in CGIs and promoter regions [9].

The previous study shows that the abnormal methylation of CpG islands plays a critical role in the pathogenesis of T-ALL [19]. Normally, CpG islands located in the promoter regions are either unmethylated or only slightly methylated, ensuring normal transcription and expression of the genes. However, CpG islands in promoter regions of key genes, such as PTEN, are often hypermethylated in T-ALL. This hypermethylation prevents transcription factors from binding to the promoter regions, leading to the inhibition of gene expression and abnormal cellular function. At the same time, some regions of the genome exhibit hypomethylation, which can disturb cell proliferation and differentiation [20, 21]. Similarly, the promoter regions of genes like CDKN2A and Pax5 are also prone to hypermethylation in B-ALL [22]. Specifically, the hypermethylation of CDKN2A gene leads to the loss of p16INK4a protein, which is vital for regulating the cell cycle [23]. Pax5, an essential transcription factor for B cell development, is also impacted by hypermethylation in its promoter region, which disrupts the normal differentiation of B cells [24]. Furthermore, the distribution of hypomethylated regions in B-ALL is different from that in T-ALL. This variation may be linked to the unique cellular origins and different pathway characteristic of these two subtypes.

Oxford Nanopore Technologies (ONT) sequencing is the next generation of nanopore-based real-time electrical signal sequencing of single molecules [25]. The DNA/RNA double strands are bound to the nanopore proteins embedded in the biofilm and de-helicalized under the guidance of motor proteins. Under the effect of the voltage difference between the two sides of the biofilm, the DNA/RNA strand passes through the nanopore channel proteins at a certain rate, and due to the differences in chemical properties of the different bases on the DNA/RNA strand, it also causes different electrical signals to be changed [26]. The signals are detected and corresponded to each other by the nanopore channel proteins. By detecting and corresponding to these signals, the type of corresponding bases can be calculated, and real-time sequence determination can be accomplished [27]. The nanopore can be used to determine the sequence in real time [27]. Currently, nanopore uses a complex algorithm called"recurrent neural network"to interpret bases based on the

magnitude and variations of electrical current [28]. Additionally, compared to methylation sequencing and methylation microarray techniques, ONT has the advantage of comprehensively detecting four types of methylation—6 mA, CHG, CHH, and CpG—across the whole genome [29].

Here, we reported a pair of monozygotic twins in which the patient had ALL while his twin brother did not. Moreover, we analyzed four methylation modification types, 6 mA, CpG, CHG, and CHH, by ONT sequencing, and further characterized the significant genome-wide CpG hypomethylation and 6 mA, CHG, and CHH hypermethylation of the ALL patient compared to paired twins. Additionally, for CpG modification, DNA hypomethylation is more pronounced in the open sea, gene body, and 3'UTR regions of the genome compared to CGIs and promoter regions. For 6 mA modification and CHG modification, the gene body, TSS1500, TSS200, and the 3'UTR regions had hypermethylation. In contrast, in CHH modification, patients reflected significant hypermethylation in all regions. Finally, we summarized the relevant literature on leukemia and DNA methylation modification and comprehensively described the association of them. Collectively, our study will provide a reference for in-depth investigation of the role of DNA methylation in the pathogenesis of leukemia.

Case report

We admitted a 23-year-old male patient with abnormal blood picture on regular physical examination (WBC 14 $\times 10^9$/L, Hb 121 g/L, PLT 152 $\times 10^9$/L, and NEUT 3.37 $\times 10^9$/L). The patient had no fever, no cough and sputum, no abdominal pain and diarrhea and bloating, and occasional sweating without the previous history of chronic diseases such as hypertension, diabetes mellitus, infectious diseases, or blood transfusion trauma.

Upon admission, examination revealed a fusion gene BCR::ABL p190/internal reference gene ABL 110.83%. Peripheral blood smear suggested an increased leukocyte count. The percentage of lymphocytes was markedly increased and 46% primitive lymphocytes were seen. Bone marrow biopsy suggested diffuse proliferation of primitive cells with reticulofibrillar staining (MF-1 grade). Flow immunophenotyping showed 85.7% naive lymphocytes expressing HLA-DR, CD123, CD19, CD10, C79a, TDT, moderate CD34, CD22, and weakly CD38. Abdominal ultrasound demonstrated a mildly enlarged spleen. A comprehensive diagnosis of ph + ALL was made in the patient.

The patient's identical twin brother is in good daily health and has no abnormalities on examination.

Method and material

Sample collection and sequencing

We performed ONT sequencing on the above ALL patient and his identical twin brother, with the assistant of Biomarker Technologies (Beijing, China). The above studies were conducted under the approval of the Medical Ethics Committee of Shengjing Hospital of China Medical University (Shenyang, China). Written informed consent was obtained from each participant.

ONT sequencing was performed according to the standard protocol provided by the manufacturer. In brief, genomic DNA was extracted using the Tiangen® Genomic DNA Extraction Kit (DP348). The purity of the extracted DNA was checked using a NanoDrop™ One UV–Vis Spectrophotometer (Thermo Fisher Scientific, USA), then the DNA was accurately quantified using a Qubit® 3.0 Fluorometer (Invitrogen, USA) and checked for purity, concentration, and integrity using 0.35% agarose gel electrophoresis. Genomic DNA was fragmented to an average size of approximately 8 kb using Gtube, followed by end repair and adapter ligation using the SQK-LSK109 ligation kit and magnetic bead purification. Finally, we initiated the flow cell of the Nanopore GridION X5 sequencer and loaded the DNA libraries into the flow cell. All samples were sequenced with 1D R9.4.1 nanopores. The sequencing coverage depth for each genome was 10–20 ×.

Methylation site detection

CpG sites were detected using Nanopolish based on a Hidden Markov Model [30], while CHH (H = A/T/C), CHG, and 6 mA sites were detected using the alternative model of Tombo [31].

Since the methylation level of sites with high sequencing depth is more realistic, C sites with detection depth above 10 × depth and A sites with detection depth above 10 × depth were retained for subsequent analysis.

Based on the positions of the methylation sites on the reference genome and the gene location information on the reference genome, we plotted the average methylation levels for all regions, including 1 st Exon, 3'UTR, 5'UTR, body, TSS1500, and TSS200. Each region was divided into 50 bins, and the methylation level of each bin was calculated. (TSS200: 200 bp upstream of the transcription start site; TSS1500: 1500 bp upstream of the transcription start site).

CGIs are regions rich in CpG dinucleotides, and the genome is split into open sea, N shelf/shore, CGIs, and S shelf/shore according to the reference [5]: 2 kb upstream of a CpG island is the N shore, 2 kb-4 kb upstream is the N shelf, and similarly 2 kb downstream is the S shore, 2 kb-4 kb downstream is the N shelf. Open sea is the area

beyond the downstream 4 kb on CpG island. The bins were divided into 50 bins, and the average methylation level of each bin was counted.

Repeat regions were predicted using RepeatMasker software [32], and the repeat region, upstream 2 kb, and downstream 2 kb were divided into 50 bins, and the average methylation level of each bin was counted.

Differential methylation analysis

The DMC module of SMART2 software [33] was used to perform differential methylation loci (DML) and differential methylation region (DMR) analysis.

For each of the four methylation modifications, 6 mA, CpG, CHG, and CHH, the DML with a significance *p*-value less than 0.01 and methylation specificity (MS) greater than 0.5 was selected. The samples were subjected to a cluster analysis of the different samples according to the normalization of methylation levels by SMART2. The results were visualized in the heat map.

DMLs were annotated to different gene regions based on their positions using the ChIPseeker package [34]. If a DML was annotated to a non-intergenic region of a gene, the gene was considered to be associated with the DML. GO/KEGG functional annotation and enrichment analysis were performed for all DML-associated genes.

For 6 mA modification, DMRs with a significance P-value less than 0.05 and a difference level greater than 0.1 were selected; for CpG, CpG, CHG, and CHH modifications, DMRs with a significance P-value less than 0.05 and a difference level greater than 0.2 were selected. The samples were subjected to a cluster analysis of the different samples according to the normalization of methylation levels by SMART2. The results were visualized in the heat map.

DMRs were annotated to different gene regions based on their positions using the ChIPseeker package [34]. If a DMR was annotated to a non-intergenic region of a gene, the gene was considered to be associated with the DMR. The R package clusterProfiler was used to perform GO/KEGG enrichment analysis for the DMR-associated gene [35].

Literature search

We searched two online electronic repositories (PubMed, NCBI), without restrictions on English article types, to identify eligible studies that qualified for this literature (last search updated June 2024). The following keywords were used: leukemia, cancer, m6A RNA methylation, CpG methylation. The selected studies met the following inclusion criteria, involving genes with some association with leukemia or with other cancers. Among studies published by the same authors with identical or overlapping data, the most recent article was selected. The main reasons for exclusion were as follows: (1) lack of available information on the association of genes with leukemia or other cancers. (2) Duplication with earlier publications. If we needed to retrieve additional data not included in the original report, we contacted the study authors for more detailed information. The extracted information all included: gene name, gene biological function, association with cancer, and relevant clinical significance.

Result

ONT sequencing and methylation site detection

ONT methylation sequencing was performed on two monozygotic twins, and methylation sites were detected based on alignment results and raw electrical signals. The patient's sample detected A total of 4,585,572 CpG sites, 8,953,746 CHG sites, 26,106,623 CHH sites, and 13,385,983 6 mA sites were detected in the ALL patient; while a total of 4,509,547 CpG sites, 9,059,371 CHG sites, 26,418,109 CHH sites, and 13,015,359 6 mA sites were detected in his twin brother (Fig. 1).

Compared to the healthy twin sample, the patient's result showed a significant decrease in overall CpG modification levels ($p = 6.19E{-}242$), while 6 mA ($p < 0.0001$), CHG ($p < 0.0001$), and CHH ($p < 0.0001$) modification levels were significantly increased.

6 mA modification was similar to CHG modification, with an overall increase in DNA methylation levels across the genome ($p < 0.0001$). This included elevated DNA methylation levels in the gene body ($p = 2.83E{-}90/0$), TSS1500 ($p = 1.82E{-}08/2.38E{-}50$), TSS200 ($p = 0.0245/6.43E{-}15$), and 3'UTR ($p = 0.0102/1.61E{-}35$) regions, while the 1 st Exon ($p = 0.45/0.119$) and 5'UTR ($p = 0.973/0.205$) regions showed no significant differences (Fig. 2a, b). For CHH modification, DNA methylation levels were elevated across all regions of the genome (Fig. 2c). In the most common CpG modification, DNA methylation levels decreased in the gene body ($p = 1.28E{-}208$), 3'UTR ($p = 5.54E{-}05$), and the overall genome ($p = 6.19E{-}242$), but increased in the TSS200 ($p = 8.25E{-}04$) and 1 st Exon ($p = 1.36E{-}05$) regions, with no significant differences in the 5'UTR ($p = 0.87$) and TSS1500 ($p = 0.617$) regions (Fig. 2d).

Specifically, for CpG modifications, the methylation levels of regions, such as open sea ($p = < 0.0001$), N shelf ($p = 7.22E{-}22$)/shore ($p = 1.14E{-}16$), and S shelf ($p = 4.53E{-}15$)/shore ($p = 6.75E{-}15$), were significantly reduced, whereas CGIs methylation levels significantly changed not significantly ($p = 0.47$) (Fig. 2e).

Considering the high density of repetitive elements in the open sea regions, we compared the modification levels in the repeat regions. Compared to the control group, the patient's repeat regions showed significantly higher levels of 6 mA ($p = 1.74E{-}291$) (Fig. 2f), CHG ($p =$

3.85E−298) (Fig. 2g), and CHH ($p. = 0$) (Fig. 2h) modifications, while CpG ($p = 3.85E-298$) (Fig. 2i) modification levels were significantly lower.

DML and DMR analysis

DML and DMR analysis was performed using the DMC module of the SMART2 software [33]. Differences between the two samples were calculated using pairwise_t_test, and P-value correction was performed using the parameter *p*.adjust.method ="bonferroni."All the DMLs and their associated genes are shown in Additional file: sTable 1. 2, 3, 4. Cluster analysis of the samples was performed based on the DML and DMR levels of the genomes, and the results of the cluster analysis of the methylation levels of different samples after normalization are shown in the heatmaps Fig. 3a–d and Fig. 4a–d.

A total of 2245 DMLs for 6 mA (Fig. 3i), 1355 DMLs for CHG (Fig. 3j), 5938 DMLs for CHH (Fig. 3k), and 1773 DMLs for CpG (Fig. 3l) were detected, along with 684 DMRs for 6 mA (Fig. 4i), 22 DMRs for CHG (Fig. 4j), 677 DMRs for CHH (Fig. 4k), and 513 DMRs for CpG (Fig. 4l). We selected significant DMLs with P-value less than 0.01 and MS level greater than 0.5 for 6 mA (Fig. 3e), CHG (Fig. 3f), CHH (Fig. 3g), and CpG (Fig. 3h) modifications. For DMRs, we selected 6 mA (Fig. 4e) modifications with P-value less than 0.05 and MS level greater than 0.1, and CHG (Fig. 4f), CHH (Fig. 4g), and CpG (Fig. 4h) modifications with P-value less than 0.05 and MS level greater than 0.2. Volcano plots were generated to visualize these selections.

DMLs were annotated to different gene regions based on their positions via the ChIPseeker package [34]. 6 mA sites were annotated to 139 genes, CHG sites to 384 genes, CHH sites to 1238 genes, and CpG sites to 470 genes. The four types of DMLs for 6 mA, CpG, CHG, and CHH were commonly annotated to 9 common differential genes (Fig. 5a). Similarly, DMRs were annotated to different gene regions: 6 mA sites were annotated to 55 genes, CHG sites to 8 genes, CHH sites to 201 genes, and CpG sites to 252 genes. No common differential genes were found when annotating the four types of DMRs for 6 mA, CpG, CHG, and CHH (Fig. 5b).

The four types of DMLs (6 mA, CHG, CHH, and CpG) were commonly enriched in seven genes associated with tumorigenesis and progression: KIAA1217, CELF1, FANK1, NBPF1, TPTE, ANKRD36C, and ZDHHC11. The three types of DMLs (6 mA, CHG, and CpG) were commonly enriched in the FMN2 gene. The three types of DMLs (6 mA, CHH, and CpG) were commonly enriched in four genes associated with tumorigenesis and progression: CA10, USP39, PAWR, and NRXN1. The three types of DMLs (CHG, CHH, and CpG) were commonly enriched in twelve genes associated with tumorigenesis and progression: TSPAN9, HDAC4, RAP1GAP, CRIM1, CACNA1C, RBPJ, MAF, MXRA7, RFTN1, ADGRA3, SLFN13, and COL4A1. The three types of DMLs (6 mA, CHG, and CHH) were commonly enriched in fourteen genes associated with tumorigenesis and progression: AFG1L, ANKRD36, ARHGEF3, CROCC, DHX32, EXT2, LPA, RASA4, RIN2, SDK1, TPPP, USP17L17, USP17L20,

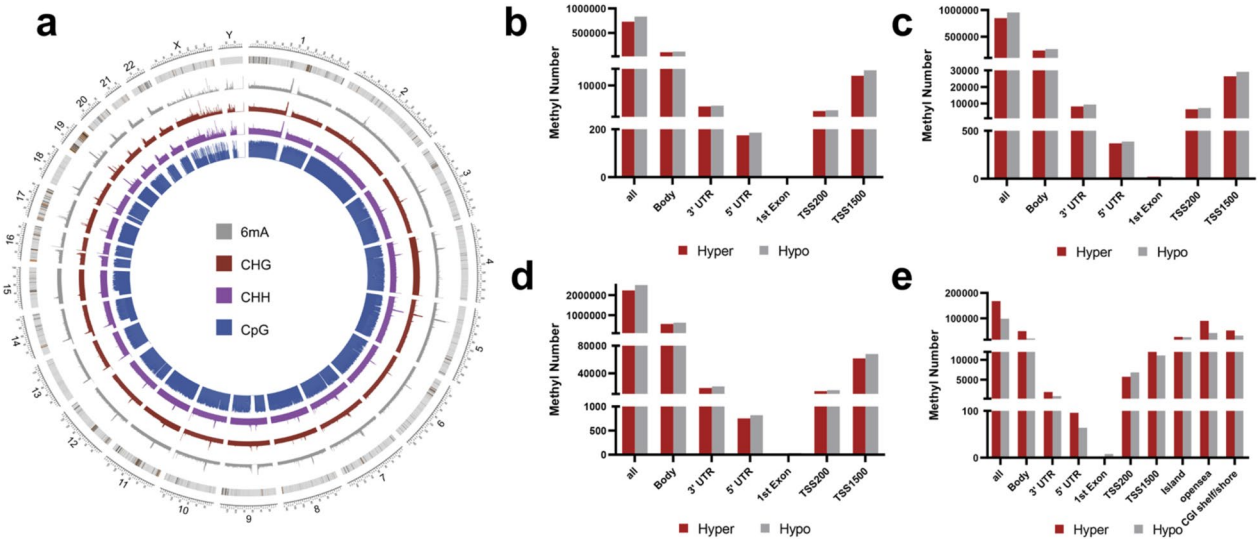


Fig. 1 Detection of methylation sites. **a** Chromosomal localization of detected methylation sites. **b** Distribution of 6 mA differential methylation loci in genomic regions. **c** Distribution of CHG differential methylation loci in genomic regions. **d** Distribution of CHH differential methylation loci in genomic regions. **e** Distribution of CpG differential methylation loci in genomic and CpG island regions

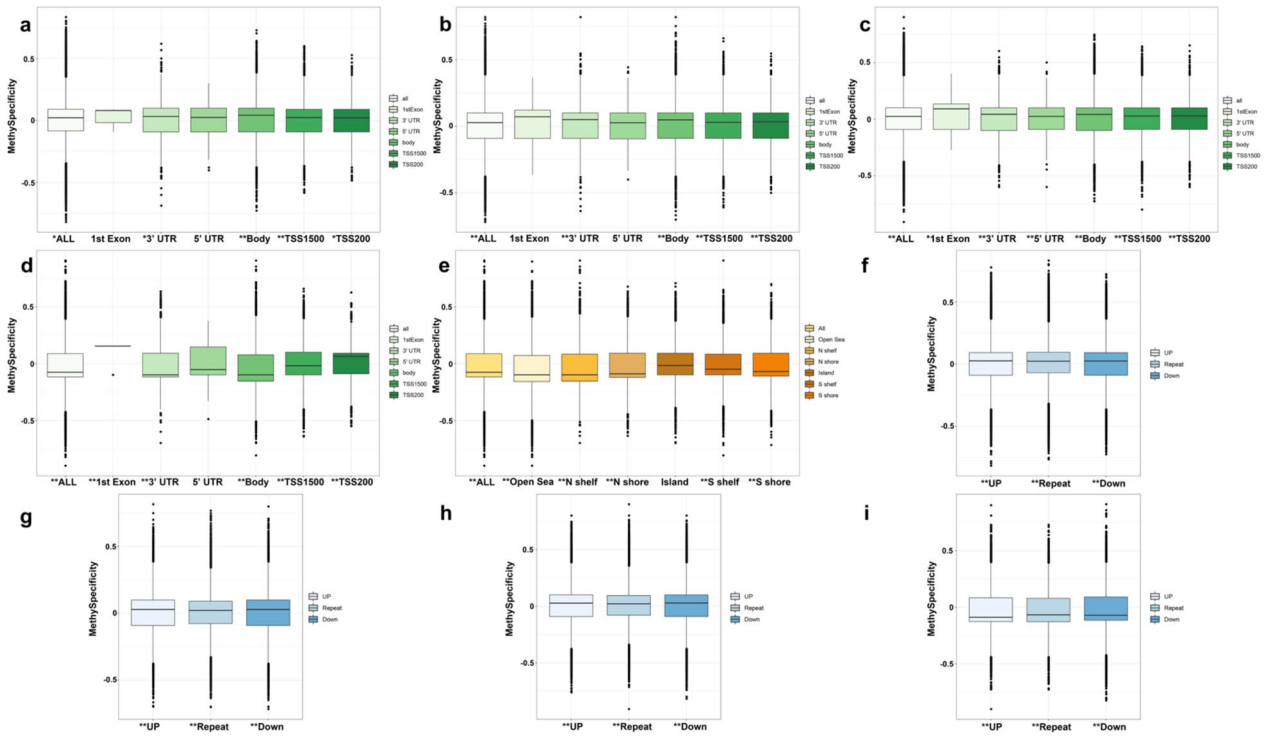


Fig. 2 Region-specific DNA methylation profiles in twin cases. **a** Differences in 6 mA methylation levels in genomic regions**. b** Differences in CHG methylation levels in genomic regions. **c** Differences in CHH methylation levels in genomic regions. **d** Differences in CpG methylation levels in genomic regions. **e** Differences in CpG methylation levels in CpG island regions. **f** Differences in 6 mA methylation levels in repeat regions. **g** Differences in CHG methylation levels in repeat regions. **h** Differences in CHH methylation levels in repeat regions. **i** Differences in CpG methylation levels in repeat regions

WWOX, and ZDHHC11B. All the detailed information of genes associated with tumorigenesis and progression based on the enrichment of DMLs is shown in Table 1.

Subsequently, GO/KEGG functional annotation and enrichment analysis (Additional file: sFig. 1–2) were performed for DML and DMR-associated genes, respectively, using the R package clusterProfiler [35].

Literature review

After literature searching, we collected a total of 136 articles that met the criteria, spanning from 2002 to 2024. A total of 4 types of leukemia were reported, including 22 articles of acute myeloid leukemia (AML) type, 6 articles of ALL type, 5 articles of multiple myeloma (MM) type, 3 articles of chronic myeloid leukemia (CML) type, 2 articles of Burkitt's lymphoma type and 1 article of juvenile myelomonocytic leukemia (JMML) type. A total of 109 genes were affected, of which the most involved genes were IGF2BP2, DNMT3A, BCL2, MYC, and so on. The signaling pathways involved were PI3K-AKT, JAK/STAT, KDM4C-ALKBH5-AXL, and so on.

Discussion

In this study, we reported a pair of identical twin cases in which the older brother had ALL and the twin brother did not have the disease. Meanwhile, we analyzed the four methylation modification types of 6 mA, CHG, CHH, and CpG in the peripheral blood of the two individuals by ONT sequencing, and further described the significant genome-wide CpG hypomethylation and 6 mA, CHG, and CHH hypermethylation features of the ALL patient compared with paired twins. In addition, for CpG methylation, DNA hypomethylation was higher in the high seas, gene body regions, and 3'UTR regions of the genome compared to CGIs and promoter regions. For 6 mA modification and CHG modification, the gene body, upstream of the promoter and the 3'UTR region had hypermethylation. While in CHH modification, the patients and their paired twin siblings reflected significant hypermethylation in all regions. As shown in Figs. 2, 3, 4, similar to the previous studies [36], it suggests that hypomethylation of CpG and hypermethylation of CpG, CHG, and CHH may contribute more to the risk of ALL.

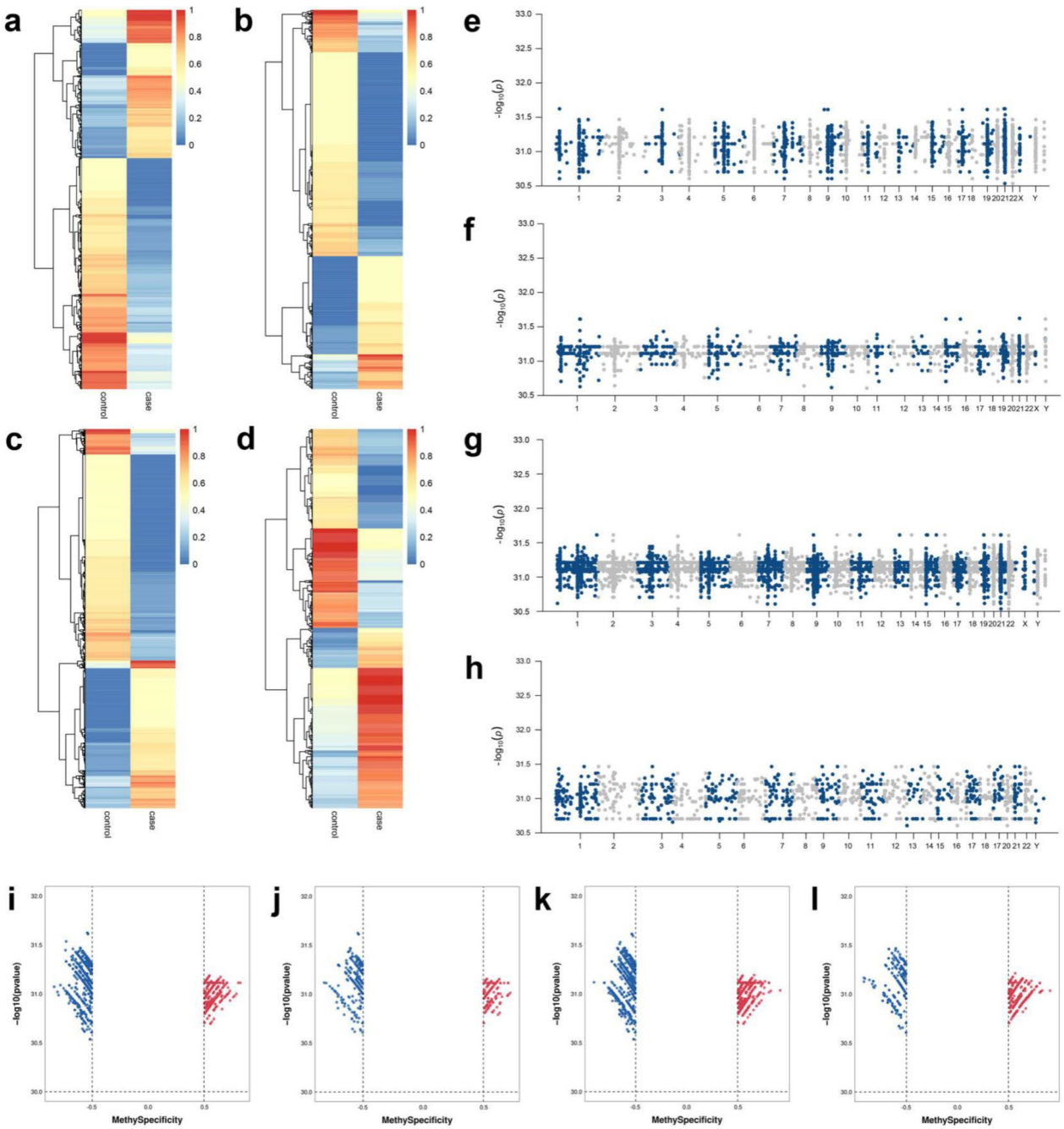


Fig. 3 Distribution of differential methylation loci in twin cases. **a** Cluster analysis of normalized 6 mA differential methylation loci. **b** Cluster analysis of normalized CHG differential methylation loci. **c** Cluster analysis of normalized CHH differential methylation loci. **d** Cluster analysis of normalized CpG differential methylation loci. **e** Distribution of 6 mA differential methylation loci on genomic chromosomes. **f** Distribution of CHG differential methylation loci on genomic chromosomes. **g** Distribution of CHH differential methylation loci on genomic chromosomes. **h** Distribution of CpG differential methylation loci on genomic chromosomes. **i** Volcano plot showing significance and specificity of 6 mA differential methylation loci. **j** Volcano plot showing significance and specificity of CHG differential methylation loci. **k** Volcano plot showing significance and specificity of CHH differential methylation loci. **l** Volcano plot showing significance and specificity of CpG differential methylation loci

The close association was observed between methylation and the occurrence and development of diseases, as well as the commonality across different diseases. In our study, among the three types of four types of DMLs (6 mA, CHG, CHH, and CpG), seven genes commonly enriched are associated with tumorigenesis and

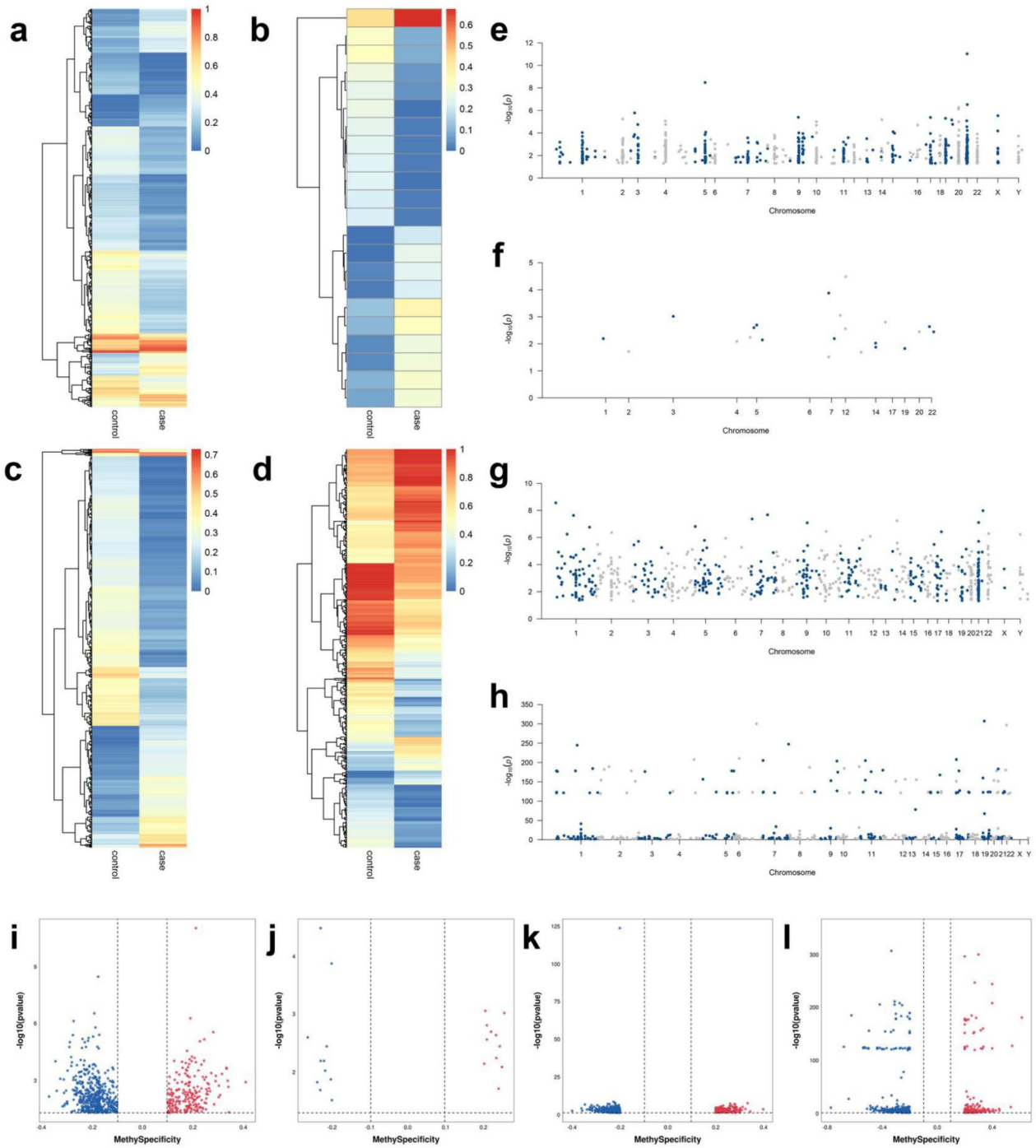


Fig. 4 Distribution of differential methylation regions in twin cases. **a** Cluster analysis of normalized 6 mA differential methylation regions. **b**. Cluster analysis of normalized CHG differential methylation regions. **c** Cluster analysis of normalized CHH differential methylation regions. **d** Cluster analysis of normalized CpG differential methylation regions. **e** Distribution of 6 mA differential methylation regions on genomic chromosomes. **f** Distribution of CHG differential methylation regions on genomic chromosomes. **g** Distribution of CHH differential methylation regions on genomic chromosomes. **h** Distribution of CpG differential methylation regions on genomic chromosomes. **i** Volcano plot showing significance and specificity of 6 mA differential methylation regions. **j** Volcano plot showing significance and specificity of CHG differential methylation regions. **k** Volcano plot showing significance and specificity of CHH differential methylation regions. **l** Volcano plot showing significance and specificity of CpG differential methylation regions

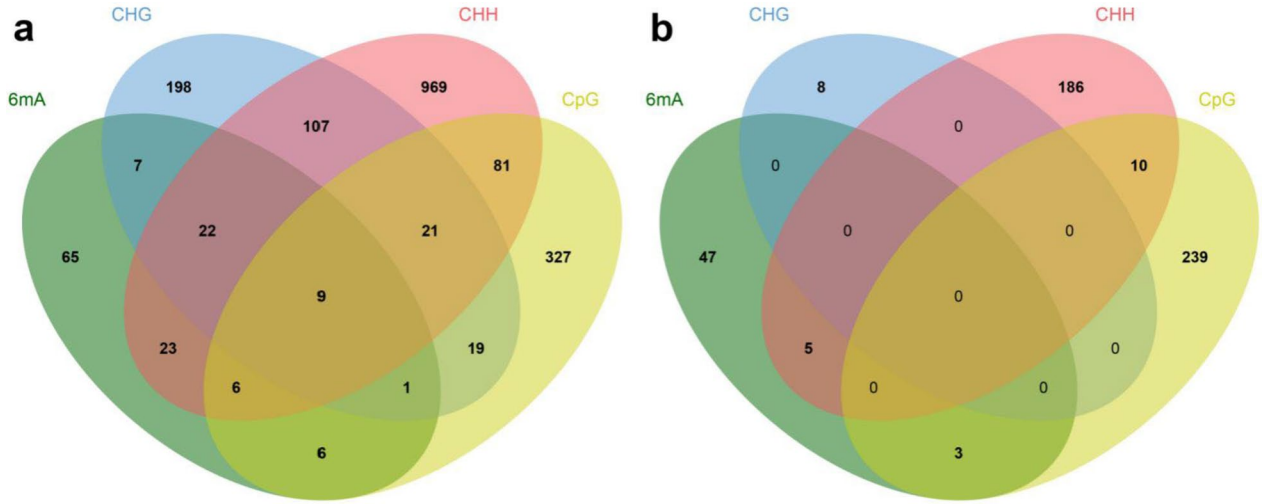


Fig. 5 Venn diagrams of gene enrichment for differential methylation loci and regions. **a** Gene enrichment results for differential methylation loci annotations. **b** Gene enrichment results for differential methylation region annotations

progression. Specifically, the following genes are also related to the development of hematological malignancies. The ZDHHC11 gene is involved in regulating the growth of Burkitt's lymphoma [37]. NBPF1, as a member of the neuroblastoma breakpoint family (NBPF), not only promotes the early progression of follicular lymphoma, but also has a pan-cancer role [38]. TPTE is involved in encoding PTEN-associated tyrosine phosphatase and plays a role in the development and drug resistance of various cancers, including AML [39] and ALL [40].

The three types of DMLs (6 mA, CHG, and CpG) are commonly enriched in the FMN2 gene, which has been reported to regulate the proliferation and cell cycle transition of ALL cells [41]. Among the three types of DMLs (6mA, CHH, and CpG), four genes commonly enriched are associated with tumorigenesis and progression. Specifically, the following genes are also related to the development of hematological malignancies. USP39 acts as a deubiquitinating enzyme [42], and PAWR, as a proapoptotic WT1 regulatory factor [43, 44], regulates the occurrence and progression of various malignancies, including leukemia.

Among the three types of DMLs (CHG, CHH, and CpG), twelve genes commonly enriched are associated with tumorigenesis and progression. Specifically, the following genes are also related to the development of hematological malignancies. HDAC4 has histone deacetylase activity and is involved in regulating biological processes such as the cell cycle, playing a crucial role in the development of MM and AML [45–47]. RAP1GAP, as a ras subfamily protein, acts as a tumor suppressor gene in various malignancies [48, 49]. CRIM1, which includes an insulin-like growth factor domain, regulates the proliferation and adhesion of malignancies, indicating a poor prognosis [50, 51]. CACNA1C is involved in regulating the cellular matrix and adhesion and negatively regulates cell proliferation [52, 53]. RBPJ is an important transcriptional regulator in the Notch signaling pathway and plays a key role in inhibiting tumor growth [54–56]. MAF is a leucine zipper-containing transcription factor that regulates various cellular processes and has been reported in MM [57, 58] and breast cancer [59]. MXRA7 plays a key role in blocking the differentiation of acute promyelocytic cell [60].

Moreover, fourteen genes commonly enriched are associated with tumorigenesis and progression among the three types of DMLs (6 mA, CHG, and CHH). Specifically, the following genes are also related to the development of hematological malignancies. ANKRD36 may serve as a marker for disease progression in CML [61, 62]. ARHGEF3 regulates AML cell differentiation by activating Rho GTPase [63] and promotes nasopharyngeal carcinoma progression through antiapoptotic effects [64]. RASA4, as an activator of RAS p21 protein, is involved in the development and progression of various malignancies [65–68] and has been reported to have a tendency for DNA hypermethylation in drug-resistant JMML [69]. DHX32 is an RNA helicase involved in many cellular processes that change RNA secondary structure and regulates the development and progression of various malignancies, including childhood AML [70–75]. The ZDHHC11B gene is involved in the growth regulatory network of Burkitt's lymphoma [76].

Through KEGG enrichment analysis, we identified key pathways such as the PI3K-AKT signaling pathway and the JAK/STAT signaling pathway. The PI3K-AKT

Table 1 Enrichment of genes associated with tumorigenesis and progression based on DMLs

	Gene name	Official full name	Gene function	Association with cancer
6 mA CHG CHH CpG	NBPF1	NBPF member 1	Member of the neuroblastoma breakpoint family (NBPF), involved in cell cycle control	①Promotes occurrence: follicular lymphoma ②Inhibits occurrence: neuroblastoma, prostate cancer, squamous cell carcinoma of the skin, cervical cancer ③Prognostic biomarker: adrenal cortex carcinoma
	TPTE	Transmembrane phosphatase with tensin homology	Encodes phosphatase from the PTEN tumor suppressor gene family	①Promotes occurrence: acute myeloid leukemia, acute lymphoblastic leukemia, melanoma ②Upregulated in: prostate cancer ③Therapeutic target and prognostic biomarker: prostate cancer
	KIAA1217	KIAA1217	Involved in embryonic skeletal system development, acts as a partner gene in various cancers	①Promotes metastasis: liver cancer ②Partner gene: anaplastic lymphoma kinase (ALK-RCC)
	CELF1	CUGBP Elav-like family member 1	Regulates alternative splicing of pre-mRNA, involved in mRNA editing and translation	①Promotes growth: oral squamous cell carcinoma, esophageal gastrointestinal stromal tumor, lung adenocarcinoma
	FANK1	Fibronectin type III and ankyrin repeat domains 1	Involved in the regulation of apoptosis and transcription	①Promotes growth: ductal carcinoma, breast cancer ②Promotes apoptosis
	ANKRD36C	Ankyrin repeat domain 36C	Ankyrin repeat domain	①Promotes occurrence: lung adenocarcinoma
	ZDHHC11	Zinc finger DHHC-type containing 11	Member of the ZDHHC family, involved in posttranslational modification through S-acyltransferase activity	①Promotes occurrence: Burkitt lymphoma, prostate cancer
6 mA CHG CpG	FMN2	Formin 2	Involved in cell cycle transition	①Promotes occurrence: colon cancer ②Upregulated in: acute myeloid leukemia, acute lymphoblastic leukemia, melanoma, brain cancer ③Downregulated in: prostate cancer
6 mA CHH CpG	USP39	Ubiquitin specific peptidase 39	Deubiquitinase, regulates cell cycle transition	①Promotes occurrence and metastasis: acute myeloid leukemia, liver cancer, breast cancer, cervical cancer, ovarian cancer, colorectal cancer, esophageal squamous cell carcinoma, gastric adenocarcinoma, non-small cell lung cancer, bladder cancer, endometrial cancer
	PAWR	Proapoptotic WT1 regulator	Regulates WT1 proapoptotic factor	①Promotes occurrence: acute myeloid leukemia, prostate cancer, breast cancer, bladder cancer, endometrial cancer
	CA10	Carbonic anhydrase 10	Encodes carbonic anhydrase	①High expression suggests poor prognosis: breast cancer, renal cell carcinoma, bladder cancer
	NRXN1	Neurexin 1	Encodes single-pass transmembrane protein I-type neurexin	①Therapeutic target and prognostic biomarker: prostate cancer, endometrial cancer

Table 1 (continued)

	Gene name	Official full name	Gene function	Association with cancer
6 mA CHG CHH	HDAC4	Histone deacetylase 4	Histone deacetylase	①Promotes occurrence: acute myeloid leukemia, multiple myeloma, gastric cancer, nasal cancer ②Upregulated in: esophageal squamous cell carcinoma, gastric cancer, non-small cell lung cancer, bladder cancer, endometrial cancer ③Therapeutic target: prostate cancer
	RAP1GAP	RAP1 GTPase activating protein	Ras family protein, key tumor suppressor gene	①Inhibits occurrence: thyroid carcinoma, breast cancer, renal cell carcinoma
	CRIM1	Cysteine rich transmembrane BMP regulator 1	Contains insulin-like growth factor domains, regulates angiogenesis and cell proliferation	①Promotes metastasis: melanoma, gastric adenocarcinoma, endometrial cancer ②Therapeutic target and prognostic biomarker: breast cancer, renal cell carcinoma
	CACNA1C	Calcium voltage-gated channel subunit alpha1 C	Regulates cellular substrate adhesion, cell adhesion, cell response to calcium ion stimulus and cell growth	①Upregulated in: acute myeloid leukemia, brain tumor, breast cancer, ovarian cancer ②Inhibits occurrence: colorectal cancer
	RBPJ	Recombination signal binding protein for immunoglobulin kappa J region	Key transcription factor in Notch signaling pathway, critical tumor suppressor gene	①Promotes growth: acute myeloid leukemia, acute lymphoblastic leukemia, acute myeloid leukemia, neuroblastoma, soft tissue sarcoma, colorectal cancer, ovarian cancer ②Downregulated in: breast cancer, prostate cancer ③Prognostic biomarker: liver cancer
	MAF	MAF bZIP transcription factor	Leucine zipper transcription factor, key tumor suppressor gene	①Promotes occurrence: multiple myeloma, breast cancer, prostate cancer, soft tissue sarcoma, colorectal cancer, ovarian cancer ②Inhibits occurrence: nasopharyngeal carcinoma, cervical cancer ③Prognostic biomarker: neuroblastoma
	MXRA7	Matrix remodeling associated 7	Related to matrix remodeling	①Upregulated in: acute myeloid leukemia ②Prognostic biomarker: multiple myeloma
	TSPAN9	Tetraspanin 9	Encodes tetraspanin family protein, involved in cell growth and signal transduction	①Inhibits occurrence: gastric cancer
	RFTN1	Raftlin, lipid raft linker 1	Activates double-stranded RNA, enhances B cell receptor signaling and lipid rafts formation	①Promotes growth: melanoma
	ADGRA3	Adhesion G protein-coupled receptor A3	Encodes G protein-coupled receptor family, involved in cell adhesion and signal transduction	①Therapeutic target and prognostic biomarker: endometrial cancer
	SLFN13	Schlafen family member 13	Nuclease activity, involved in rRNA degradation	①Prognostic biomarker: gastric cancer ②Predicts poor prognosis: lung cancer
	COL4A1	Collagen type IV alpha 1 chain	Encodes type IV collagen protein, ECM component	①Prognostic biomarker: liver cancer ②Prognostic biomarker: colorectal cancer

Table 1 (continued)

	Gene name	Official full name	Gene function	Association with cancer
6 mA CHG CHH	ANKRD36	Ankyrin repeat domain 36	Possibly involved in intracellular signaling, associated with bone marrow and cartilage	①Highly expressed in: acute myeloid leukemia, myeloproliferative diseases, kidney cancer ②Inhibits occurrence: colorectal cancer
	ARHGEF3	Rho guanine nucleotide exchange factor 3	Activates Rho GTPase, involved in cell morphology, movement, and adhesion	①Upregulated in: chronic myeloid leukemia, acute myeloid leukemia, nasal cancer
	RASA4	RAS p21 protein activator 4	Regulates Ras-MAPK pathway signaling, acts as a tumor suppressor	①Highly expressed in: juvenile myelomonocytic leukemia with DNA hypermethylation trend ②Inhibits occurrence: ovarian cancer
	DHX32	DEAH-box helicase 32	RNA helicase, involved in RNA secondary structure changes and chromatin remodeling	①Inhibits occurrence: colorectal cancer, neuroblastoma, ovarian cancer ②Promotes occurrence: gastric cancer, pancreatic cancer
	ZDHHC11B	Zinc finger DHHC-type containing 11B	Member of the ZDHHC family, involved in posttranslational modification through S-acyltransferase activity	①Promotes occurrence: Burkitt lymphoma, prostate cancer ②Inhibits occurrence: colorectal cancer
	AFG1L	AFG1 like ATPase	Induces cell apoptosis	①Inhibits occurrence
	CROCC	Ciliary rootlet coiled-coil, rootletin	Induces cell apoptosis	①Promotes occurrence: rhabdomyosarcoma, colorectal cancer (RCT)
	EXT2	Exostosin glycosyltransferase 2	Involved in cell signaling and cell–cell interaction	①Promotes occurrence: head and neck squamous cell carcinoma, multiple bone and soft tissue tumors ②Prognostic biomarker: head and neck squamous cell carcinoma
	RIN2	Ras and Rab interactor 2	Involved in early endocytic pathway membrane transport	①Promotes occurrence: triple-negative breast cancer (TNBC)
	SDK1	Sidekick cell adhesion molecule 1	Cell adhesion molecule, regulates cell cycle, apoptosis	①Promotes occurrence: lung adenocarcinoma, prostate cancer
	TP55	Tubulin polymerization promoting protein	Regulates microtubule assembly and cytoskeletal organization	①Promotes occurrence: bladder cancer ②Indicates poor prognosis: bladder cancer, liver cancer
	USP17L17	Ubiquitin specific peptidase 17 like family member 17	Deubiquitinase, regulates protein deubiquitination and apoptosis	①Highly expressed in various tumors
	USP17L20	Ubiquitin specific peptidase 17 like family member 20	Deubiquitinase, regulates protein deubiquitination and apoptosis	①Highly expressed in various tumors
	WWOX	WW domain containing oxidoreductase	Encodes short chain dehydrogenase/reductase (SDR) family protein, tumor suppressor gene	①Inhibits occurrence: breast cancer, prostate cancer

signaling pathway plays a critical role in processes including cell growth, proliferation, survival, and metabolism. In the development of leukemia, aberrant activation of this pathway facilitates cancer cells'evasion of apoptosis, allows continuous proliferation, and enhances their invasive capabilities [77]. The JAK/STAT signaling pathway, which is involved in cytokine-mediated signal transduction, is closely associated with uncontrolled proliferation and impaired differentiation of leukemia cells [78]. When over-activated, this pathway continuously transmits pro-proliferative signals, disrupting normal hematopoietic stem cell differentiation and leading to the accumulation of leukemia cells [79]. These pathways are intricately involved in the development of various cancers, including leukemia. Through regulation of the expression of relevant genes, they play a deep role in cell proliferation, apoptosis regulation, signal transduction and so on. Additionally, we identified several significant overlap genes, such as TPTE, FMN2, and RBPJ. Furthermore, we also screened a number of genes linked to pan-cancer, such as NBPF1, USP39, CELF1, FANK1, HDAC4, RAP1GAP, CRIM1, CACNA1C, MAF, ANKRD36, and ZDHHC11B.

The methylation characteristics observed in our study of ALL patients are highly consistent with the commonalities of different types of methylation presented in numerous previous studies on diseases, including cancer, cardiovascular diseases, and neurodegenerative diseases [80, 81]. For example, changes in the methylation of specific genes (such as PR/SET domain 6 and ZNF714 genes) are closely related to the occurrence of ovarian cancer [82]. By comparing the methylation patterns of cancer patients and healthy individuals in monozygotic twins, researchers are able to identify specific methylation sites associated with cancer. Twin studies provide important evidence for revealing the roles of environmental and genetic factors in disease development by comparing the methylation differences between monozygotic and dizygotic twins.

Nickels et al. focused on the relationship between DNA methylation at birth and specific gene expression in ALL. Through whole-genome methylation sequencing, they identified several important methylated sites and associated genes, including RUNDC3B, ABCB1, and MARVELD3. In contrast, our study employed ONT sequencing, which enabled the detection of various types of methylation modifications, including 6 mA, CHG, CHH, and CpG. We identified several genes linked to ALL, including TPTE, FMN2, and RBPJ, which enhance the understanding of postnatal environmental factors for DNA methylation in ALL. Additionally, the pathways enriched through GO/KEGG analysis, such as PI3K-AKT and JAK/STAT, align with the findings of Nickels et al., further reinforcing the critical role these signaling pathways play in ALL [5].

The previous studies have shown that in subgroups of ALL cells with significant variations in DNA methylation levels, a quantitative correlation can be observed between the methylation levels of CpG sites in promoter regions and bidirectional allele-specific gene expression (ASE). This further indicates that methylation of CpG sites in promoter regions is associated with gene expression in primary ALL cells [10]. Methylation of CGIs can inhibit gene expression directly by preventing the binding of transcription factors or RNA polymerase, or indirectly by affecting histone modifications or chromatin remodeling proteins [83]. Numerous studies have shown that CpG methylation of different genes is associated with the risk of leukemia [84–86]. Genes associated with DNA repair, cell cycle regulation, apoptosis, or detoxification (such as CEBPα, SHP1, DNMT3A, TET2, the HOX gene family, MEIS1, and FOX) are involved in the aberrant methylation of CGIs and can initiate oncogenic processes, including leukemia [87–89]. Thus, epigenetic abnormalities, especially DNA methylation, can serve as biomarkers for stratifying patients with ALL and predicting relapse [90, 91].

M6A methylation regulates processes such as RNA stability, splicing, transport, and translation by influencing chromatin structure and transcription factor binding [95]. It has been found that METTL3 and METTL14, as m6A methyltransferase complexes, can mediate 6mA methylation modification to promote cell proliferation, inhibit cell differentiation, and play an oncogenic role [96, 97]. IGF2BP2 and IGF2BP3 are able to participate in the 6mA methylation of AML by regulating the expression of some genes (e.g., PRMT6, YBX1 etc.) expression, which are involved in the regulation of m6A-tagged mRNA stability and thus maintain the survival of leukemia stem cell [98–100]. Moreover, high expression of IGF2BP2 as a"reading protein"of m6A in AML is associated with poor prognosis of patients, and its absence promotes apoptosis, impairs the function of leukemic stem cells, and inhibits the development of AML [101]. In addition, ALKBH5, a m6A demethylase, was also found to regulate the stability of AXL mRNA in a m6A-dependent manner, which in turn affects AML cell function [102].

In eukaryotes, 6 mA methylation can regulate gene expression and cell proliferation by affecting the binding of chromatin to transcription factors [95]. 6 mA methylation is associated with mitochondrial oxidative phosphorylation and ATP metabolism, and promotes the occurrence and progression of various cancers by affecting the metabolic pathway of tumor cells [103]. In this

study, 6 mA hypermethylation in the relevant region of ALL patients is likely to affect the transcriptional activity of key genes through this mechanism. Dysregulation of its related components can induce the expression of oncogenes, promoting the progression of various cancers and tumorigenesis [92–94]. CHG methylation is established through the RNA-directed DNA methylation pathway. In this pathway, small interfering RNA forms a complex with AGO4 protein [104] and guides methyltransferase DRM2 to recognize and methylate CHG sites [105, 106]. This methylation pattern is involved in transposon silencing and maintaining genome stability in plants, though its mechanistic role in animal studies, especially in the context of cancer, remains unclear [107]. CHH methylation, a relatively novel non-CpG methylation type, is similar to CHG methylation. While it has been widely studied in plants, research on its role in animal cancer studies is still in the early stages.

In studies on leukemia and DNA methylation, no shared genes or loci were identified. This indicates a high degree of heterogeneity in the various leukemia types and the DNA methylation level, as well as individual variability and heterogeneity among tumor cells. Additionally, acquired factors such as the patient's gender and lifestyle influence the onset of the disease and affect the degree of DNA methylation as an epigenetic modification. Moreover, the type of leukemia and the use of medication also impact the type and extent of DNA methylation modifications in patients.

However, the present study has some shortcomings. First, we reported only one pair of monozygotic twins, whereas Nickels et al. investigated multiple samples [5], which is not enough to provide insight into DNA methylation differences considering the heterogeneity of genetic background among individuals. Second, in this study, we aimed to consider environmental factors. Therefore, we specifically chose peripheral blood samples obtained before the onset of the disease, which leads to the peripheral blood sample would contain nearly 50% lymphoblasts. Third, methylation can be affected by acquired factors, and even monozygotic twins may have different DNA methylation profiles depending on their life habits, and the present study could not completely exclude the influence of these acquired factors. Finally, DNA methylation is usually involved in the development and progression of diseases by affecting the transcriptional activity of gene promoter regions to regulate gene expression. Combined high-throughput sequencing technology to detect changes in transcriptional levels will be more helpful in revealing the characteristics of leukemia. Therefore, the interpretation of the differential loci needs to be carried out in conjunction with other studies.

Conclusion

In conclusion, we report on a pair of monozygotic twins, one of whom had ALL and whose twin brother did not have the disease. Four methylation modifications (6 mA, CpG, CHG, and CHH) were analyzed by ONT sequencing, and it was found that ALL patients exhibited significant genome-wide CpG hypomethylation and 6 mA, CHG, and CHH hypermethylation characteristics. In particular, CpG hypomethylation was more pronounced in the open sea, gene body, and 3'UTR regions, while 6 mA and CHG exhibited hypermethylation in the gene body, TSS1500, TSS200, and the 3'UTR regions. CHH modification exhibits significant hypermethylation across all regions of the genome. We also reviewed the literature related to leukemia and DNA methylation modification for further studies.

Abbreviations

ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
ASE	Allele-specific gene expression
CGIs	CpG islands
CML	Chronic myeloid leukemia
CoRSIV	Correlated interindividual variant loci
DMRs	Differential methylation regions
DML	Differential methylation loci
JMML	Juvenile myelomonocytic leukemia
MM	Multiple myeloma
MS	Methylation specificity
NBPF	Neuroblastoma breakpoint family
ONT	Oxford nanopore technologies

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13148-025-01906-z.

Additional file 1.

Additional file 2.

Additional file 3.

Additional file 4.

Additional file 5.

Author contributions

YQP conceived and designed the experiments. YL and MLS collected the sample. MLS and YY performed the experiments. RXM, SWW, and RG screened literature and analyzed the data. MLS and YQP wrote the paper. All authors reviewed and approved the final manuscript.

Funding

This study was supported by Student Innovation and Entrepreneurship Program of China Medical University.

Data availability

No datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Shengjing Hospital of China Medical University. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Pediatrics, Shengjing Hospital of China Medical University, No. 36, Sanhao Street, Heping District, Shenyang 110004, People's Republic of China. [2]Department of Blood Transfusion, Shengjing Hospital of China Medical University, No. 36, Sanhao Street, Heping District, Shenyang 110004, People's Republic of China. [3]Department of Gastroenterology, Shengjing Hospital of China Medical University, No. 36, Sanhao Street, Heping District, Shenyang 110004, People's Republic of China.

Received: 10 March 2025 Accepted: 22 May 2025
Published online: 06 June 2025

References

1. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA Cancer J Clin. 2024;74(1):12–49.
2. Rahmani M, et al. Aberrant DNA methylation of key genes and Acute Lymphoblastic Leukemia. Biomed Pharmacother. 2018;97:1493–500.
3. Singh SK, et al. A childhood acute lymphoblastic leukemia genome-wide association study identifies novel sex-specific risk variants. Medicine (Baltimore). 2016;95(46): e5300.
4. Ford AM, Colman S, Greaves M. Covert pre-leukaemic clones in healthy co-twins of patients with childhood acute lymphoblastic leukaemia. Leukemia. 2023;37(1):47–52.
5. Nickels EM, et al. DNA methylation at birth in monozygotic twins discordant for pediatric acute lymphoblastic leukemia. Nat Commun. 2022;13(1):6077.
6. Ford AM, Greaves M. ETV6-RUNX1 (+) acute lymphoblastic leukaemia in identical twins. Adv Exp Med Biol. 2017;962:217–28.
7. Timms JA, et al. DNA methylation as a potential mediator of environmental risks in the development of childhood acute lymphoblastic leukemia. Epigenomics. 2016;8(4):519–36.
8. Sanchez R, Mackenzie SA. Integrative network analysis of differentially methylated and expressed genes for biomarker identification in leukemia. Sci Rep. 2020;10(1):2123.
9. Chaber R, et al. Whole-genome DNA methylation characteristics in pediatric precursor B cell acute lymphoblastic leukemia (BCP ALL). PLoS ONE. 2017;12(11): e0187422.
10. Nordlund J, Syvanen AC. Epigenetics in pediatric acute lymphoblastic leukemia. Semin Cancer Biol. 2018;51:129–38.
11. Kessler NJ, et al. Establishment of environmentally sensitive DNA methylation states in the very early human embryo. Sci Adv. 2018. https://doi.org/10.1126/sciadv.aat2624.
12. Van Baak TE, et al. Epigenetic supersimilarity of monozygotic twin pairs. Genome Biol. 2018;19(1):2.
13. Hannon E, et al. Characterizing genetic and environmental influences on variable DNA methylation using monozygotic and dizygotic twins. PLoS Genet. 2018;14(8): e1007544.
14. Klutstein M, et al. DNA methylation in cancer and aging. Cancer Res. 2016;76(12):3446–50.
15. Hetzel S, et al. Acute lymphoblastic leukemia displays a distinct highly methylated genome. Nat Cancer. 2022;3(6):768–82.
16. Choi SJ, et al. Alteration of DNA methylation in gastric cancer with chemotherapy. J Microbiol Biotechnol. 2017;27(8):1367–78.
17. Kelly AD, et al. A CpG island methylator phenotype in acute myeloid leukemia independent of IDH mutations and associated with a favorable outcome. Leukemia. 2017;31(10):2011–9.
18. Cao LL, et al. The clinical values of dysregulated DNA methylation and demethylation intermediates in acute lymphoblastic leukemia. Hematology. 2019;24(1):567–76.
19. Mackowska N, et al. DNA methylation in T-cell acute lymphoblastic leukemia: in search for clinical and biological meaning. Int J Mol Sci. 2021;22(3):1388.
20. Paganin M, et al. The presence of mutated and deleted PTEN is associated with an increased risk of relapse in childhood T cell acute lymphoblastic leukaemia treated with AIEOP-BFM ALL protocols. Br J Haematol. 2018;182(5):705–11.
21. Zhang Y, Lee S, Xu W. Miltefosine suppression of Pten null T-ALL leukemia via beta-catenin degradation through inhibition of pT308-Akt and TGFbeta1/Smad3. Biochem Biophys Res Commun. 2020;524(4):1018–24.
22. Ou Z, et al. The genomic landscape of PAX5, IKZF1, and CDKN2A/B alterations in B-Cell precursor acute lymphoblastic leukemia. Cytogenet Genome Res. 2016;150(3–4):242–52.
23. Wang Y, et al. Clinical characteristics and prognosis of ALL in children with CDKN2A/B gene deletion. Exp Biol Med (Maywood). 2025;250:10447.
24. Jia Z, Gu Z. PAX5 alterations in B-cell acute lymphoblastic leukemia. Front Oncol. 2022;12:1023606.
25. Deamer D, Akeson M, Branton D. Three decades of nanopore sequencing. Nat Biotechnol. 2016;34(5):518–24.
26. Magi A, et al. Nanopore sequencing data analysis: state of the art, applications and challenges. Brief Bioinform. 2018;19(6):1256–72.
27. Jain M, et al. The Oxford Nanopore MinION: delivery of nanopore sequencing to the genomics community. Genome Biol. 2016;17(1):239.
28. Krishnakumar R, et al. Systematic and stochastic influences on the performance of the MinION nanopore sequencer across a range of nucleotide bias. Sci Rep. 2018;8(1):3159.
29. Yue X, et al. Simultaneous profiling of histone modifications and DNA methylation via nanopore sequencing. Nat Commun. 2022;13(1):7939.
30. Simpson JT, et al. Detecting DNA cytosine methylation using nanopore sequencing. Nat Methods. 2017;14(4):407–10.
31. Stoiber M, et al. De novo identification of DNA modifications enabled by genome-guided nanopore signal processing. bioRxiv; 2017. 2016;94672.
32. Tarailo-Graovac, M., Chen, N. *Using RepeatMasker to identify repetitive elements in genomic sequences.* Curr Protoc Bioinform; 2009. Chapter 4: 4 10 1–4 10 14
33. Liu H, et al. Systematic identification and annotation of human methylation marks based on bisulfite sequencing methylomes reveals distinct roles of cell type-specific hypomethylation in the regulation of cell identity genes. Nucleic Acids Res. 2016;44(1):75–94.
34. Yu G, Wang LG, He QY. ChIPseeker: an R/Bioconductor package for ChIP peak annotation, comparison and visualization. Bioinformatics. 2015;31(14):2382–3.
35. Yu G, et al. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012;16(5):284–7.
36. Gaal Z, et al. Strong correlation between the expression levels of HDAC4 and SIRT6 in hematological malignancies of the adults. Pathol Oncol Res. 2017;23(3):493–504.
37. Liu Y, et al. Circular ZDHHC11 supports Burkitt lymphoma growth independent of its miR-150 binding capacity. Sci Rep. 2024;14(1):8730.
38. Li L, et al. Oncogene or tumor suppressor gene: an integrated pan-cancer analysis of NBPF1. Front Endocrinol (Lausanne). 2022;13: 950326.
39. Li L, Zhao W. The mutual regulatory loop between TPTEP1 and miR-1303 in leukemogenesis of acute myeloid leukemia. Cancer Cell Int. 2021;21(1):260.
40. Hales EC, Taub JW, Matherly LH. New insights into Notch1 regulation of the PI3K-AKT-mTOR1 signaling axis: targeted therapy of gamma-secretase inhibitor resistant T-cell acute lymphoblastic leukemia. Cell Signal. 2014;26(1):149–61.
41. Jin J, et al. MicroRNA-144 regulates cancer cell proliferation and cell-cycle transition in acute lymphoblastic leukemia through the interaction of FMN2. J Gene Med. 2017;19(6–7):e2898.
42. Liu C, et al. USP39 regulates the cell cycle, survival, and growth of human leukemia cells. 2019. Biosci Rep. https://doi.org/10.1042/BSR20190040.
43. Lavallee VP, et al. EVI1-rearranged acute myeloid leukemias are characterized by distinct molecular alterations. Blood. 2015;125(1):140–3.
44. Mahadevan D, et al. Transcriptosome and serum cytokine profiling of an atypical case of myelodysplastic syndrome with progression to acute myelogenous leukemia. Am J Hematol. 2006;81(10):779–86.
45. Cuttini E, et al. HDAC4 in cancer: a multitasking platform to drive not only epigenetic modifications. Front Mol Biosci. 2023;10:1116660.

46. Hu Z, et al. Histone deacetylase inhibitors promote breast cancer metastasis by elevating NEDD9 expression. Signal Transduct Target Ther. 2023;8(1):11.
47. Wu H, et al. MIR145-3p promotes autophagy and enhances bortezomib sensitivity in multiple myeloma by targeting HDAC4. Autophagy. 2020;16(4):683–97.
48. Qiu T, et al. Rap1GAP alters leukemia cell differentiation, apoptosis and invasion in vitro. Oncol Rep. 2012;28(2):622–8.
49. Faam B, et al. RAP1GAP functions as a tumor suppressor gene and is regulated by DNA methylation in differentiated thyroid cancer. Cytogenet Genome Res. 2021;161(5):227–35.
50. Shi H, Xu B, Yang M. NF-kappaB signaling pathway promotes proliferation and inhibits apoptosis of childhood acute lymphoblastic leukemia cells via regulating CRIM1. Minerva Med. 2020. https://doi.org/10.1186/1476-4598-13-5.
51. Wen W, et al. Low CRIM1 levels predict poor prognosis in breast cancer patients. Front Oncol. 2022;12: 882328.
52. Wang W, et al. Identification of potential signatures and their functions for acute lymphoblastic leukemia: a study based on the cancer genome atlas. Front Genet. 2021;12: 656042.
53. Chang X, Dong Y. CACNA1C is a prognostic predictor for patients with ovarian cancer. J Ovarian Res. 2021;14(1):88.
54. Shi Y, et al. Interaction between BEND5 and RBPJ suppresses breast cancer growth and metastasis via inhibiting Notch signaling. Int J Biol Sci. 2022;18(10):4233–44.
55. Liu M, et al. Colon cancer cells secreted CXCL11 via RBP-Jkappa to facilitated tumour-associated macrophage-induced cancer metastasis. J Cell Mol Med. 2021;25(22):10575–90.
56. Kato T, et al. Hes1 suppresses acute myeloid leukemia development through FLT3 repression. Leukemia. 2015;29(3):576–85.
57. Rana S, et al. 16q23/MAF gene deletion is a frequent cytogenetic abnormality in multiple myeloma associated With IgH deletion but significantly lower incidence of high-risk translocations. Clin Lymphoma Myeloma Leuk. 2021;21(4):e398–401.
58. Qiang YW, et al. MAF protein mediates innate resistance to proteasome inhibition therapy in multiple myeloma. Blood. 2016;128(25):2919–30.
59. Pavlovic M, et al. Enhanced MAF oncogene expression and breast cancer bone metastasis. J Natl Cancer Inst. 2015. https://doi.org/10.1093/jnci/djv256.
60. Sun Z, et al. Critical role of MXRA7 in differentiation blockade in human acute promyelocytic leukemia cells. Exp Hematol. 2023;125–126:45–54.
61. Iqbal Z, et al. Integrated Genomic analysis identifies ANKRD36 gene as a novel and common biomarker of disease progression in chronic myeloid Leukemia. Biology (Basel). 2021;10(11):1182.
62. Absar M, et al. Clinical validation of Ankrd36 mutations as a novel biomarker for monitoring early progression and timely clinical interventions in blast crisis CML. J Popul Ther Clin Pharmacol. 2022;29(2):311–20.
63. D'Amato L, et al. ARHGEF3 controls HDACi-induced differentiation via RhoA-dependent pathways in acute myeloid leukemias. Epigenetics. 2015;10(1):6–18.
64. Liu TH, et al. The putative tumor activator ARHGEF3 promotes nasopharyngeal carcinoma cell pathogenesis by inhibiting cellular apoptosis. Oncotarget. 2016;7(18):25836–48.
65. Chen J, et al. RASA4 inhibits the HIFalpha signaling pathway to suppress proliferation of cervical cancer cells. Bioengineered. 2021;12(2):10723–33.
66. Wang Y, et al. TRPC3 regulates the proliferation and apoptosis resistance of triple negative breast cancer cells through the TRPC3/RASA4/MAPK pathway. Cancers (Basel). 2019;11(4):558.
67. Li L, et al. Tumor suppression of Ras GTPase-activating protein RASA5 through antagonizing Ras signaling perturbation in carcinomas. iScience. 2019;21:1–18.
68. Roy D, et al. Tumor suppressor genes FHIT and WWOX are deleted in primary effusion lymphoma (PEL) cell lines. Blood. 2011;118(7):e32–9.
69. Poetsch AR, et al. RASA4 undergoes DNA hypermethylation in resistant juvenile myelomonocytic leukemia. Epigenetics. 2014;9(9):1252–60.
70. McNeer NA, et al. Genetic mechanisms of primary chemotherapy resistance in pediatric acute myeloid leukemia. Leukemia. 2019;33(8):1934–43.
71. Lin H, et al. DHX32 promotes angiogenesis in colorectal cancer through augmenting beta-catenin signaling to induce expression of VEGFA. EBioMedicine. 2017;18:62–72.
72. Huang C, et al. Up-regulation and clinical relevance of novel helicase homologue DHX32 in colorectal cancer. J Exp Clin Cancer Res. 2009;28(1):11.
73. Hu X, et al. DEAH-box polypeptide 32 promotes hepatocellular carcinoma progression via activating the beta-catenin pathway. Ann Med. 2021;53(1):437–47.
74. Pant D, et al. Hypoxia-induced changes in intragenic DNA methylation correlate with alternative splicing in breast cancer. J Biosci. 2020;45:1. https://doi.org/10.1007/s12038-019-9977-0.
75. Lin H, et al. Overexpression of DHX32 contributes to the growth and metastasis of colorectal cancer. Sci Rep. 2015;5:9247.
76. Dzikiewicz-Krawczyk A, et al. ZDHHC11 and ZDHHC11B are critical novel components of the oncogenic MYC-miR-150-MYB network in Burkitt lymphoma. Leukemia. 2017;31(6):1470–3.
77. Yu L, Wei J, Liu P. Attacking the PI3K/Akt/mTOR signaling pathway for targeted therapeutic treatment in human cancer. Semin Cancer Biol. 2022;85:69–94.
78. Rizwi FA, et al. Janus Kinase-signal transducer and activator of transcription inhibitors for the treatment and management of cancer. J Environ Pathol Toxicol Oncol. 2023;42(4):15–29.
79. Liang D, et al. JAK/STAT in leukemia: a clinical update. Mol Cancer. 2024;23(1):25.
80. Kaur G, et al. DNA methylation: a promising approach in management of Alzheimer's disease and other neurodegenerative disorders. Biology (Basel). 2022. https://doi.org/10.3390/biology11010090.
81. Smith ZD, Hetzel S, Meissner A. DNA methylation in mammalian development and disease. Nat Rev Genet. 2025;26(1):7–30.
82. Alves VRG, et al. A systematic review of LINE-1 methylation profile in psychiatric disorders. Complex Psychiatry. 2023;9(1–4):119–29.
83. Papin C, et al. CpG Islands shape the epigenome landscape. J Mol Biol. 2021;433(6): 166659.
84. Giacopelli B, et al. DNA methylation epitypes highlight underlying developmental and disease pathways in acute myeloid leukemia. Genome Res. 2021;31(5):747–61.
85. Yang X, Wong MPM, Ng RK. Aberrant DNA methylation in acute myeloid leukemia and its clinical implications. Int J Mol Sci. 2019;20(18):4576.
86. Kalinkova L, et al. Targeting DNA methylation in leukemia, myelodysplastic syndrome, and lymphoma: a potential diagnostic, prognostic, and therapeutic tool. Int J Mol Sci. 2022;24(1):633.
87. WHO. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368(22):2059–74.
88. Sato H, et al. Multi-step aberrant CpG island hyper-methylation is associated with the progression of adult T-cell leukemia/lymphoma. Am J Pathol. 2010;176(1):402–15.
89. Ogunleye AJ, Romanova E, Medvedeva YA. Genome-wide regulation of CpG methylation by ecCEBPalpha in acute myeloid leukemia. F1000Res. 2021;10:204.
90. Jian J, et al. DNA methylation-based subtypes of acute myeloid leukemia with distinct prognosis and clinical features. Clin Exp Med. 2023;23(6):2639–49.
91. Jiang H, et al. DNA methylation markers in the diagnosis and prognosis of common leukemias. Signal Transduct Target Ther. 2020;5(1):3.
92. Liu J, et al. m(6)A mRNA methylation regulates AKT activity to promote the proliferation and tumorigenicity of endometrial cancer. Nat Cell Biol. 2018;20(9):1074–83.
93. Chen J, et al. Reducing N6AMT1-mediated 6mA DNA modification promotes breast tumor progression via transcriptional repressing cell cycle inhibitors. Cell Death Dis. 2022;13(3):216.
94. Wang X, et al. The N(6)-methyladenine DNA demethylase ALKBH1 promotes gastric carcinogenesis by disrupting NRF1 binding capacity. Cell Rep. 2023;42(3): 112279.
95. Li X, et al. The exploration of N6-deoxyadenosine methylation in mammalian genomes. Protein Cell. 2021;12(10):756–68.
96. Vu LP, et al. The N(6)-methyladenosine (m(6)A)-forming enzyme METTL3 controls myeloid differentiation of normal hematopoietic and leukemia cells. Nat Med. 2017;23(11):1369–76.

97. Weng H, et al. METTL14 inhibits hematopoietic stem/progenitor differentiation and promotes leukemogenesis via mRNA m(6)A modification. Cell Stem Cell. 2018;22(2):191e9-205e9.
98. Cheng Y, et al. Decoding m(6)A RNA methylome identifies PRMT6-regulated lipid transport promoting AML stem cell maintenance. Cell Stem Cell. 2023;30(1):69e7-85e7.
99. Feng M, et al. YBX1 is required for maintaining myeloid leukemia cell survival by regulating BCL2 stability in an m6A-dependent manner. Blood. 2021;138(1):71–85.
100. Zhao Y, et al. m(6)A-dependent upregulation of DDX21 by super-enhancer-driven IGF2BP2 and IGF2BP3 facilitates progression of acute myeloid leukaemia. Clin Transl Med. 2024;14(4): e1628.
101. Zhang N, et al. The m6A reader IGF2BP3 promotes acute myeloid leukemia progression by enhancing RCC2 stability. Exp Mol Med. 2022;54(2):194–205.
102. Wang J, et al. Leukemogenic chromatin alterations promote AML leukemia stem cells via a KDM4C-ALKBH5-AXL signaling axis. Cell Stem Cell. 2020;27(1):81e8-97e8.
103. Cui H, et al. DNA N6-Adenine methylation in HBV-related hepatocellular carcinoma. Gene. 2022;822: 146353.
104. Dinh TT, et al. Generation of a luciferase-based reporter for CHH and CG DNA methylation in *Arabidopsis thaliana*. Silence. 2013;4(1):1.
105. Fang J, et al. Mechanistic basis for maintenance of CHG DNA methylation in plants. Nat Commun. 2022;13(1):3877.
106. Wu X, et al. Turnip mosaic virus manipulates DRM2 expression to regulate host CHH and CHG methylation for robust infection. Stress Biol. 2022;2(1):29.
107. Bartels A, et al. Dynamic DNA methylation in plant growth and development. Int J Mol Sci. 2018;19(7):2144.

Publisher's Note