

Original Article

UNRAVELING THE GENOMIC LANDSCAPE OF CCRF-CEM: A HIGH-RESOLUTION ANALYSIS OF A KEY LEUKAEMIA CELL LINE

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ABSTRACT

The CCRF-CEM cell line, a key model for T-cell acute lymphoblastic leukaemia (T-ALL), is widely used in research, but its complete genomic profile has not been fully characterised. This study used high-resolution array comparative genomic hybridisation (array CGH), validated with multiplex ligation-dependent probe amplification (MLPA), to comprehensively map copy number aberrations (CNAs) in CCRF-CEM. The Agilent Human Genome Microarray Kit 244K platform, containing ~240,000 oligonucleotide probes, was utilised. A total of 65 CNAs were identified, including 27 gains and 38 losses, spanning ~131 Mb. Chromosome 14 had the highest number of CNAs (13/65). Sixteen of the 38 losses were homozygous deletions (~37 Mb). Notable CNAs included trisomy 20, an unbalanced der(5) t(5;14)(q35;q32.2) translocation, deletions at 9p24.3- p21.1 (~28.4 Mb) and 9p21.3 (~0.2 Mb) and amplifications at 20p13-11.1 (~25.7 Mb) and Xp22.3 (~0.01 Mb), affecting key genes like JAK2, CDKN2A, CDKN2B, CRLF2, and SIRPA. MLPA confirmed these findings. Additionally, 25 CNAs < 100 kb were detected, underscoring the sensitivity of our approach, which were previously undetected by conventional methods. These results enhance the understanding of CCRF-CEM as a representative T-ALL model and aid in identifying potential therapeutic targets.

KEYWORDS: CCRF-CEM, T-cell acute lymphoblastic leukaemia (ALL), Array comparative genomic hybridisation (array CGH), Multiplex ligation-dependent probe amplification (MLPA) and Copy number aberrations (CNAs)

INTRODUCTION

T-cell acute lymphoblastic leukaemia (T-ALL) is recognised as an aggressive hematologic malignancy characterised by the uncontrolled proliferation of immature T lymphocytes. This disease constitutes approximately 15% of pediatric and 25% of adult cases of acute lymphoblastic leukaemia (ALL) (1). It is associated with a poor prognosis despite significant advancements in therapeutic strategies, particularly in high-risk patient populations (2). The complexity of T-ALL necessitates a comprehensive understanding of its genetic landscape to elucidate the molecular drivers of disease progression and to identify potential therapeutic targets that may improve patient outcomes (3, 4).

The CCRF-CEM cell line, established from a pediatric patient with T-ALL, has become a widely utilised *in vitro* model for investigating biological mechanisms and their response to therapeutic agents in T-ALL. This cell line has been used in research, including mitochondrial respiration, apoptosis, cell cycle arrest, and the DNA damage response (1, 4). Additionally, the CCRF-CEM cell line has been employed to evaluate the targeting and delivery of therapeutic agents, such as aptamer-tethered DNA and ferrocene derivatives, highlighting its value in the development of novel cancer treatments (1). These studies underscore the utility of this cell line for understanding the underlying mechanisms of leukemogenesis and identifying potential therapeutic targets (1, 4).

Despite its extensive application in research, the complete structural rearrangement of the CCRF-CEM genome remains inadequately characterised. Previous cytogenetic analyses have identified several chromosomal abnormalities within this cell line, including trisomy 20 and an unbalanced der(5)t(5;14)(q35;q32.2) translocation (5,6). However, a comprehensive high-resolution genomic analysis delineating the full spectrum of genomic alterations has yet to be conducted. Array comparative genomic hybridisation (array CGH) has emerged as a powerful technique for detecting genome-wide copy number aberrations (CNAs) with superior resolution compared to traditional karyotyping and fluorescence in situ hybridisation (FISH) (7). The integration of array CGH with multiplex ligation-dependent probe amplification (MLPA) provides a robust framework for validating specific genetic alterations and enhancing the resolution of genomic analyses. This combination allows for a more nuanced understanding of the genetic underpinnings of T-ALL, which is critical for developing targeted therapies (7, 8).

In this study, we aimed to refine the genomic profile of the CCRF-CEM cell line by applying high-resolution array CGH and validating it with MLPA techniques. By identifying and characterising CNAs at an unprecedented resolution, we seek to provide deeper insights into the complex karyotype of this leukaemia cell line. Our findings will not only enhance the utility of CCRF-CEM as a model for studying T-ALL pathogenesis and therapeutic responses but will also underscore the significance of advanced genomic tools in cancer biology. This study adds to our understanding of the genetic foundations of T-ALL and could guide future research related to T-ALL.

MATERIALS AND METHODS

Cell culture

CCRF-CEM cell line was purchased from CLS Cell Line Service GmbH (Eppelheim, Germany). Cells were cultured in RPMI-1640 complete medium supplemented with 10% heat-inactivated FBS and 1% penicillin-streptomycin (10,000 units/mL penicillin and 10,000 µg/mL streptomycin). Cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂. The cells were split constantly every 3-4 days. The cells were cryopreserved in FBS containing 5% DMSO and stored in liquid nitrogen for further use.

Array CGH experiments and data processing

DNA of the CCRF-CEM cell line was isolated using the QIAamp DNA kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. Female Human Genomic DNA (Promega, Madison, WI) was used as reference DNA. A total of 1-3 µg each of test DNA and reference DNA was labelled with Cy5 and Cy3, respectively, using the 244A Human Genome CGH Microarray Kit (Agilent Technologies, Inc., CA, USA). The yield, degree of labelling and specific activity of the samples were measured using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, USA). Hybridisation, post-hybridisation washing, and signal scanning were performed according to the protocol provided by Agilent Technologies.

The array CGH data were analysed and processed using Agilent Cytogenomics software version 5.4. In brief, array CGH raw data were analysed using the Aberration Detection Method 2 (ADM-2) algorithm and then processed by passing through a post-analysis aberration filter. The ADM-2 algorithm identified all aberrant intervals with consistently high or low log ratios based on the statistical score and quality information about each log ratio measurement. It automatically determines the optimal size of a statistically significant aberration. The post-analysis aberration filter defined the minimum number of consecutive probes with the exact copy number change, and the minimum absolute average log₂ ratio per region, to be called as having a copy number aberration (CNA).

Multiplex ligation-dependent probe amplification (MLPA)

MLPA probe mixes, P335 (MRC-Holland, Netherlands), were used to detect the amplification and deletion of genes in T-ALL. The DNA from the CCRF-CEM samples was subjected to MLPA following the manufacturer's protocol. Briefly, 50 ng of DNA was denatured at 98°C for 5 min, followed by hybridisation with probes for 18 h at 60°C. The reaction mixture was ligated at 54°C for 15 min and subjected to PCR. The PCR products were then separated using a capillary sequencer (ABI 3730XL). Sample data were analysed using the Coffalyser software (MRC-Holland, Netherlands) for copy number evaluation. Cut-off values of <0.75 and >1.3 were used to define deletion and amplification, respectively.

RESULTS

High-resolution array CGH analysis

The array CGH analysis of the CCRF-CEM cell line revealed a comprehensive genomic profile, encompassing 65 distinct copy number aberrations (CNAs) with a total size of 131.47 Mb. Of these, 27 were gains (approximately 72 Mb) and 38 were losses (approximately 58 Mb). Figure 1 visually represents these chromosomal aberrations, with blue bars indicating gains and red bars indicating losses, mapping their physical positions and sizes across the entire genome. The most prominent CNA observed was trisomy 20, characterised by a duplication (three copies) spanning both arms of chromosome 20. Specifically, a gain of approximately 25.7 Mb was detected at 20p13-p11.1 (genomic location: 96,296-25,873,124), and another gain of approximately 33.4 Mb was found at 20q11.21-q13.33 (genomic location: 29,467,309-62,908,674). These findings, clearly depicted in Figure 1, underscore the significant duplication of chromosome 20. Furthermore, the analysis identified an unbalanced der(5)t(5;14)(q35;q32.2) translocation from the detection of a heterozygous deletion of approximately 7.9 Mb at chromosome 5q35-q35.1 (genomic location: 172,755,974-180,712,263) and a concomitant duplication of approximately 7.8 Mb at chromosome 14q32.2-q32.33 (genomic location: 99,411,665-107,278,770). These alterations were observed at the telomeric regions of their respective chromosomes.

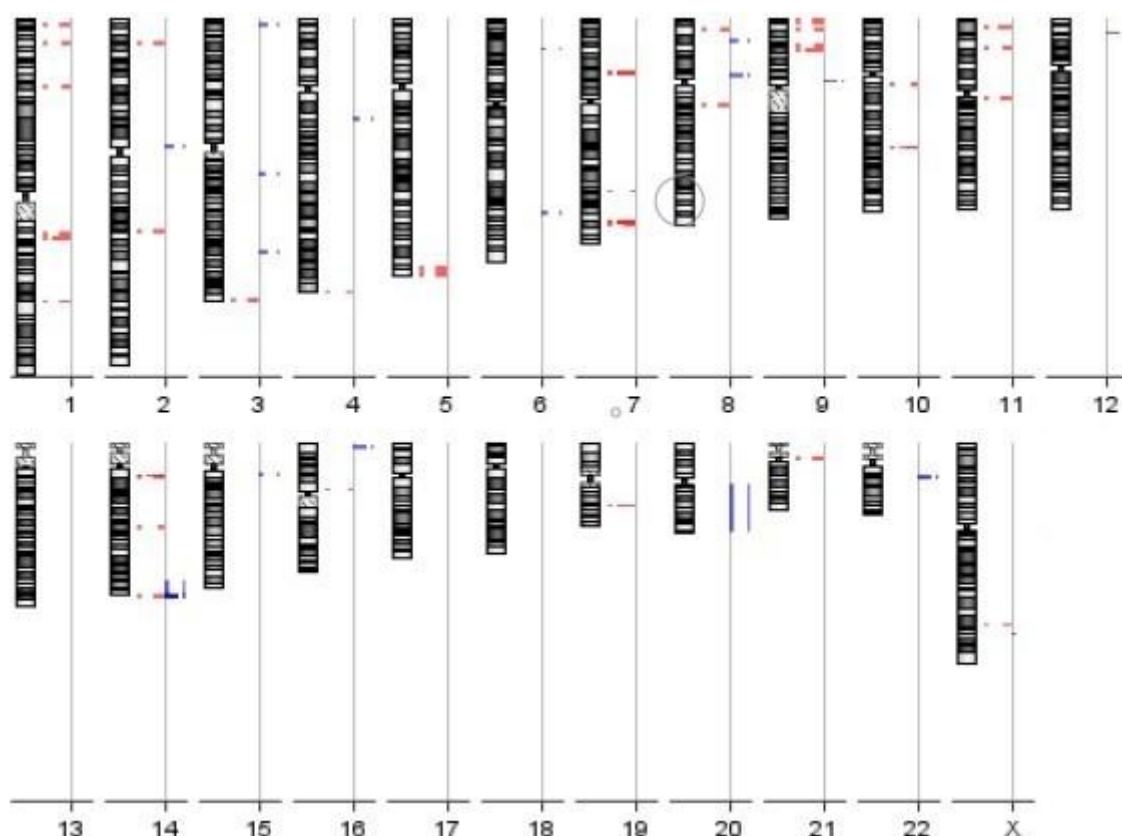


Figure 1. Karyoview of copy number aberrations (CNAs) detected in CCRF-CEM cells using high-resolution array-comparative genomic hybridisation. The bars depict the physical positions and the size of aberration: blue bars indicate chromosomal gains and red bars indicate losses.

A detailed summary of all identified CNAs, including their chromosomal locations, specific genomic coordinates, size in kilobase pairs (Kb), the type of CNAs detected (e.g., duplication, amplification, heterozygous deletion, complete deletion, homozygous deletion), and the gene names or loci affected, is given in Table 1. For instance, high amplifications were noted at 2p11.2 (176.12 Kb), 3q26.1 (104.61 Kb), 8p11.22 (148.72 Kb), 9p12-p11.2 (181.13 Kb), 14q32.33 (203.57 Kb, 35.98 Kb, 20.21 Kb, 48.70 Kb), and 22q11.22 (189.33 Kb). Significant heterozygous deletions were observed at 1p36.32-p36.31 (2005.76 Kb), 4q35.2 (110.34 Kb), 5q35.1-q35.3 (7956.29 Kb), 7q31.31 (488.57 Kb), 8p23.1 (731.09 Kb), 9p24.1-p23 (1516.22 Kb), 9p22.2-p21.3 (2943.85 Kb), 10q11.21 (151.34 Kb), 11p15.1-p14.3 (1405.44 Kb), 14q23.1 (105.45 Kb), 16p11.2-p11.1 (2450.48 Kb), and Xq25 (271.37 Kb). Complete deletions, indicative of homozygous loss, were identified at 7q34 (384.60 Kb and 225.11 Kb), 9p21.3 (157.85 Kb), and 14q11.2 (328.88 Kb).

Table 1. Summary of array CGH analysis of CCRF-CEM

Chromosomal Locations		CNAs Detection	Size (Kb)	Key Genes
<i>Regions of chromosomal gains</i>				
2p11.2	Chr2: 89143858-89319978	Amplification (4++ copies)	176.12	NA
3q13.12 - q13.13	Chr3: 107745813-108002362	Duplication (3 copies)	256.55	CD47, LINC01215, IFT57
3q26.1	Chr3: 162514534-162619141	Amplification (4++ copies)	104.61	NA
4q13.2	Chr4: 69375140-69483277	Duplication (3 copies)	108.14	UGT2B17
6q23.3	Chr6: 135507017-135732665	Duplication (3 copies)	225.65	MYB, MIR548A2, AHI1
8p11.22	Chr8: 39237438-39386158	High Amplification	148.72	ADAM5, ADAM3A
9p12 - p11.2	Chr9: 43505843-43686974	Amplification (4++ copies)	181.13	FAM74A7, SPATA31A6, CNTNAP3B, CNTNAP3P2
14q32.2 - q32.33	Chr14: 99411665-107278770	Duplication (3 copies)	7867.11	BCL11B, YY1, DLK1, MEG3, TRAF3, AKT1, MTA1, CYP46A1, ...
14q32.33	Chr14: 104546943-106251205	Duplication (3 copies)	1704.26	ASPG, AKT1, INF2, ADSS1, MTA1, SIVA1, ...
14q32.33	Chr14: 106601570-106705307	Amplification (4++ copies)	103.74	NA
14q32.33	Chr14: 106334907-106538480	Amplification (6++ copies)	203.57	FAM30A, ADAM6
15q11.2	Chr15: 21272376-22698581	Duplication (3 copies)	1426.21	CXADRP2, POTE3, POTE2, POTE1, OR4N4C, ...
20p13 - p11.1	Chr20: 96296-25873124	Duplication (3 copies)	25776.83	TCF15, PTPRA, MAVS, PANK2, PRNP, RASSF2, BMP2, JAG1, OVOL2, FOXA2, CD93, ...
20q11.21 - q13.33	Chr20: 29467309-62908674	Duplication (3 copies)	33441.37	ID1, BCL2L1, TPX2, HCK, ASXL1, DNMT3B, E2F1, ITCH, SRC, TGM2, TOP1, MYBL2, HNF4A, ADA, UBE2C, MMP9, ZMYND8, NCOA3, PREX1, CSE1L, SNAI1, CEBPB, PTPN1,

				ADNP, SALL4, ZNF217, AURKA, BMP7, PCK1, GNAS, KCNQ2, PTK6, RTEL1, ...
22q11.22	Chr22: 23056562-23245888	High Amplification	189.33	MIR650, MIR5571, IGLL5
Xq26.2	ChrX: 132690333-133117765	Duplication (3 copies)	427.43	GPC3, GPC3-AS1
<i>Regions of chromosomal losses</i>				
1p36.32 - p36.31	Chr1: 4789122-6794876	Heterozygous Deletion	2005.76	AJAP1, NPHP4, KCNAB2, CHD5, RPL22, ...
4q35.2	Chr4: 190552246-190662588	Heterozygous Deletion	110.34	LINC01262, LOC105379514
5q35.1 - q35.3	Chr5: 172755974-180712263	Heterozygous Deletion	7956.29	STC2, FGFR4, NSD1, FLT4, SQSTM1, MAPK9, ...
7q31.31	Chr7: 120150782-120639353	Heterozygous Deletion	488.57	KCND2, TSPAN12, ING3, CPED1
7q34	Chr7: 142107488-142492085	Complete Deletion	384.60	MIR11400, MTRNR2L6, PRSS1, PRSS3P2
7q34	Chr7: 142115307-142340419	Complete Deletion	225.11	MIR11400
8p23.1	Chr8: 7371630-8102719	Heterozygous Deletion	731.09	PRR23D1, FAM90A7P, USP17L8, USP17L3, ...
9p24.1 - p23	Chr9: 7804748-9320965	Heterozygous Deletion	1516.22	PTPRD, PTPRD-AS1
9p22.2 - p21.3	Chr9: 18111168-21055016	Heterozygous Deletion	2943.85	ADAMTSL1, SAXO1, RPS6, SLC24A2, MLLT3, ...
9p21.3	Chr9: 21832728-21990581	Complete Deletion	157.85	MTAP, CDKN2A-DT, CDKN2A
10q11.21	Chr10: 45247685-45399020	Heterozygous Deletion	151.34	TMEM72-AS1
11p15.1 - p14.3	Chr11: 20620997-22026435	Heterozygous Deletion	1405.44	SLC6A5, NELL1
14q23.1	Chr14: 58680272-58785717	Heterozygous Deletion	105.45	ACTR10, PSMA3, PSMA3-AS1, LINC00216, ARID4A
14q11.2	Chr14: 22636039-22964922	Complete Deletion	328.88	LOC105370401
16p11.2 - p11.1	Chr16: 32197603-34648078	Heterozygous Deletion	2450.48	HERC2P4, TP53TG3D/3B/3F/G3, ...

Xq25	ChrX: 126633808-126905177	Heterozygous Deletion	271.37	NA
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*Genes highlighted in bold have a reported association with leukaemia

Specifically, our analysis focused on homozygous deletions and CNAs with a size of less than 100 kilobase pairs (Kb), as shown in Table 2. We detected 11 CNAs gains under 100 Kb in size, predominantly located on chromosomes 3, 8, 12, 14, 16, 22, and X. Some of these affected genes or loci, such as *MSR1* (on Chr8:p22, 63.44 Kb), *FAM30A* (on Chr14:q32.33, 20.21 Kb), *LINC00221* (on Chr14:q32.33, 35.32 Kb), *GSTT4* (on Chr22:q11.23, 38.66 Kb), and *CRLF2* (on ChrX:p22.33, 14.10 Kb), while others were intergenic or unannotated. Notably, five small gains were clustered on chromosome 14q32.33, a region frequently altered in haematological malignancies.

Regions of chromosomal losses (<100 Kb) were located on chromosomes 1, 7, and 10, which affected genes including *CROCC* (on Chr1:p36.13, 34.32 Kb), *STIL* (on Chr1:p33, 76.28 Kb), *LCE3C* (on Chr1:q21.3, 25.50 Kb), *TARP* (on Chr7:p14.1, 85.18 Kb), *TRG-AS1* (on Chr7:p14.1, 85.18 Kb), and *PTEN* (on Chr10:q23.31, 64.39 Kb). Eight CNAs (<100 Kb) were homozygous deletions mapped to chromosomes 1, 3, 8, 11, 14, and 21 affecting several coding and non-coding genes, including *CFHR3* and *CFHR1* (on Chr1:q31.3, 46.97 Kb). The CNAs with a size of less than 100 Kb in CCRF-CEM have not been reported due to limitations of previous methods of CNA detection.

Of particular note is the homozygous deletion observed on chromosome 9 at position p24.3 - p21.1, spanning from 204,193 to 28,648,396. This 28.5 Mb deletion encompasses the *CDKN2A* (p16) and *CDKN2B* (p15) tumour suppressor genes, a characteristic and frequently reported aberration in T-cell acute lymphoblastic leukaemia (T-ALL). These findings detail the specific genomic microdeletions in the CCRF-CEM cell line, confirming the well-established homozygous loss of the *CDKN2A/B* locus and revealing additional smaller CNAs that may contribute to the leukemic phenotype.

MLPA Verification of array CGH-identified CNAs in CCRF-CEM

To validate the CNAs detected by array CGH in the CCRF-CEM cell line, we performed multiplex ligation-dependent probe amplification (MLPA) focusing on candidate genes of interest. The MLPA results corroborated the array CGH findings, confirming the deletions and amplifications at specific loci.

As shown in Figure 2a, the MLPA analysis revealed significant copy number loss (ratio < 0.7) in *CDKN2A* and *CDKN2B*, located at chromosome 9p21.3 and *JAK2* on 9p24.1. These deletions were also clearly evident in the array CGH data (Figure 2b, left panel, chromosome 9), further supporting a hemizygous or homozygous deletion in this region. The consistent loss detected by both platforms strengthens the evidence of tumour suppressor gene disruption in CCRF-CEM.

Conversely, gene amplification (ratio > 1.3) was detected for *CRLF2* at Xp22.33 and *SIRPA* at 20p13. The amplification signals were consistently detected in MLPA (Figure 2a) and aCGH (Figure 2b, chromosomes X and 20, respectively). This finding suggests potential oncogenic roles of these genes in leukemic progression or survival. Overall, the concordance between aCGH and MLPA data supports the reliability of the identified CNAs and highlights specific genes with potential functional relevance in T-ALL pathogenesis.

Table 2. CNAs with <100kb and homozygous deletions in CCRF-CEM

Chromosomal Location		Size (Kb)	Genes/ locus
<u>Gains</u>			
Chr3: 4070291-4144113	p26.1	+73.82	NA
Chr8: 15952011-16015454	p22	+63.44	MSR1
Chr12: 9637323-9693948	p13.31	+56.63	LOC408186
Chr14: 106334907-106370883	q32.33	+35.98	NA
Chr14: 106376324-106396531	q32.33	+20.21	FAM30A
Chr14: 106474500-106523203	q32.33	+48.70	NA
Chr14: 106786148-106810278	q32.33	+24.13	NA
Chr14: 106931134-106966454	q32.33	+35.32	LINC00221
Chr16: 2698580-2705529	p13.3	+6.95	NA
Chr22: 24356690-24395353	q11.23	+38.66	GSTT4, LOC391322, GSTT1-AS1, GSTT1, GSTTP2
ChrX: 1317536-1331637	p22.33	+14.10	CRLF2
<u>Losses</u>			
Chr1: 17241750-17276064	p36.13	-34.32	CROCC
Chr1: 47703106-47779386	p33	-76.28	STIL
Chr1: 152556449-152581944	q21.3	-25.50	LCE3C
Chr7: 38296176-38381355	p14.1	-85.18	TARP, TRG-AS1

Chr7: 38315240-38376560	p14.1	-61.32	NA
Chr10: 89625664- 89690055	q23.31	-64.39	PTEN
<u>Homozygous deletions</u>			
Chr1: 149041013-149232481	q21.2	191.47	NBPF25P, RNVU1-17, RNVU1-18, RNU1-3, RNU1-4, RNU1-2, RNU1-1
Chr1: 196748493-196795460	q31.3	46.97	CFHR3, CFHR1
Chr2: 17288413-19679108	p24.2 - p24.1	2390.70	RAD51AP2, VSNL1, SMC6, GEN1, MSGN1, KCNS3, NT5C1B-RDH14, RDH14, NT5C1B, LINC01376, MIR4757, OSR1
Chr2: 148285397-148616732	q22.3	331.34	ACVR2A
Chr3: 195419168-195472855	q29	53.69	MIR570HG, MIR570, MUC20
Chr7: 143951931-144075320	q35	123.39	ARHGEF35-AS1, OR2A1-AS1, OR2A7, ARHGEF34P, CTAGE8, CTAGE4, OR2A20P, OR2A9P, OR2A1, OR2A42, ARHGEF5
Chr8: 60036833-60099836	q12.1	63.00	NA
Chr9: 204193-28648396	p24.3 - p21.1	28444.20	DOCK8-AS1, DOCK8, KANK1, DMRT1, DMRT3, LINC01230, DMRT2, SMARCA2, VLDLR-1, VLDLR, KCNV2, PUM3, LINC01231, RFX3, RFX3-AS1, GLIS3, GLIS3-AS1, SLC1A1, SPATA6L, PLPP6, CDC37L1-DT, CDC37L1, AK3, RCL1, MIR101-2, JAK2, INSL6, INSL4, RLN2, RLN1, PLGRKT, CD274, PDCD1LG2, RIC1, ERMP1, MLANA, KIAA2026, MIR4665, RANBP6, IL33, TPD52L3, UHRF2, GLDC, KDM4C, DMAC1, PTPRD, PTPRD-AS1, LOC105375972, PTPRD-AS2, TYRP1, LURAP1L-AS1, LURAP1L, SNORD137, MPDZ, LINC01235, LINC00583, NFIB, ZDHHC21, CER1, FREM1, LOC389705, TTC39B, SNAPC3, PSIP1, CCDC171, C9orf92, BNC2, BNC2-AS1, CNTLN, SH3GL2, ADAMTSL1, MIR3152, SAXO1, RRAGA, HAUS6, SCARNA8, PLIN2, DENND4C, RPS6, ACER2, SLC24A2, MLLT3, MIR4473, MIR4474, FOCAD, FOCAD-AS1, MIR491, SNORA30B, HACD4, IFNB1, IFNW1, IFNA21, IFNA4, IFNA7, IFNA10, IFNA16, IFNA17, IFNA14, IFNA22P, IFNA5, KLHL9, IFNA6, IFNA13, IFNA2, IFNA8, IFNA1, MIR31HG, IFNE, MIR31, MTAP, CDKN2A-DT, CDKN2A, CDKN2B-AS1, CDKN2B, DMRTA1, LINC01239, LOC101929563, ELAVL2, IZUMO3,

			TUSC1, LINC01241, LOC100506422, CAAP1, PLAA, IFT74, IFT74-AS1, LRRC19, TEK, LINC00032, EQTN, MOB3B, IFNK, C9orf72, LINGO2
Chr9: 902248-5825142	p24.3 - p24.1	4922.90	DMRT1, DMRT3, LINC01230, DMRT2, SMARCA2, VLDLR-AS1, VLDLR, KCNV2, PUM3, LINC01231, RFX3, RFX3-AS1, GLIS3, GLIS3-AS1, SLC1A1, SPATA6L, PLPP6, CDC37L1-DT, CDC37L1, AK3, RCL1, MIR101-2, JAK2, INSL6, INSL4, RLN2, RLN1, PLGRKT, CD274, PDCD1LG2, RIC1, ERMP1
Chr10: 89698253-89851332	q23.31	153.08	PTEN
Chr11: 5785900-5799891	p15.4	13.99	OR52N5
Chr11: 55368154-55438473	q11	70.32	OR4C11, OR4P4, OR4S2, OR4C6
Chr14: 22976257-23001999	q11.2	25.74	NA
Chr14: 107046730-107094058	q32.33	47.33	NA
Chr19: 43354259-43545271	q13.2 - q13.31	191.01	PSG10P, PSG1, PSG6, PSG7, PSG11
Chr21: 10874759-10942079	p11.2 - p11.1	67.32	TPTE

*Genes highlighted in bold have a reported association with leukaemia

*+/- indicates gains/losses

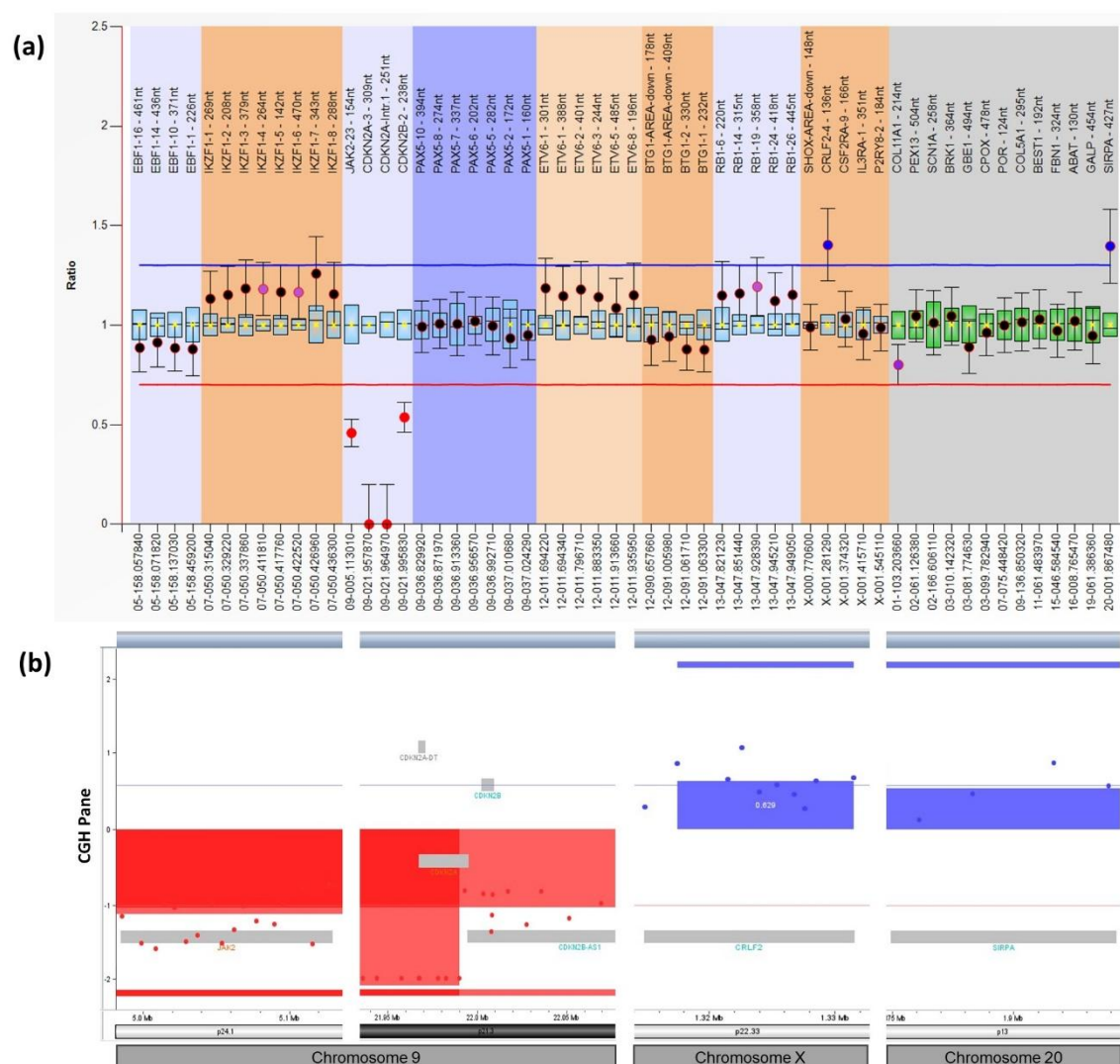


Figure 2. MLPA validation of aCGH-identified CNAs in the CCRF-CEM cell line. (a) MLPA analysis showing relative copy number ratios for selected genes across multiple chromosomes. Deletions (ratio < 0.7) were observed for CDKN2A, CDKN2B, and JAK2 (highlighted in red), while amplifications (ratio > 1.3) were observed for CRLF2 and SIRPA (highlighted in blue). (b) aCGH log2 ratio plots showing concordant CNAs in chromosomes 9, X, and 20. Red segments represent deletions, and blue segments represent amplifications. Both platforms confirm focal deletions at 9p21.3 and 9p24.1, and gains at Xp22.33 (CRLF2) and 20p13 (SIRPA).

DISCUSSION

The current research significantly enhances our understanding of the genomic landscape of the CCRF-CEM cell line, a widely utilised model for T-cell acute lymphoblastic leukaemia (T-ALL) studies. Through high-resolution array comparative genomic hybridisation (array CGH), we identified 65 copy number aberrations (CNAs), including 27 gains and 38 losses, collectively spanning approximately 131 Mb. This extensive genomic profiling not only corroborates previously reported chromosomal abnormalities, such as trisomy 20 and the unbalanced der(5)t(5;14)(q35;q32.2) translocation, but also uncovers

numerous novel CNAs, particularly those smaller than 100 Kb and homozygous deletions, which were previously undetectable by conventional methods.

The detection of 16 homozygous deletions, covering approximately 37.1 Mb, is particularly noteworthy as these aberrations can lead to the complete loss of tumour suppressor gene function, thereby promoting leukemogenesis. Specifically, the observed homozygous deletions at 9p24.3 and 9p21.3, encompassing the *CDKN2A* (p16) and *CDKN2B* (p15) tumour suppressor genes, are consistent with frequently reported aberrations in T-ALL and are known to play a crucial role in disease progression and drug resistance (9). This alteration is strongly associated with aggressive disease and relapse. Critically, recent studies demonstrate that the loss of *CDKN2A/B* creates a synthetic lethal dependency on *CDK4/6*, rendering these leukaemic cells highly sensitive to targeted inhibitors like palbociclib and ribociclib, thus opening a direct therapeutic avenue (10, 11).

Furthermore, our findings highlight less frequently observed but potentially significant amplifications at 20p13-11.1 and Xp22.3. The gain at chromosome 20p is notable as this region contains the *BLCAP*, *GNAS*, *NNAT*, *GNAS-AS1*, *HM13*, *MIR296*, *MIR298* and *PSIMCT-1*, whose overexpression has been linked to poor prognosis due to resistance to multiple antitumor agents in cancer cell lines (12). The amplification at Xp22.3, which includes the steroid sulfatase (*STS*) gene, may serve as a novel biomarker influencing steroid metabolism and resistance, a cornerstone of T-ALL therapy (13). Taken together, these genomic alterations delineate not only the drivers of this patient's disease but also potential biomarkers and personalised therapeutic strategies.

The comprehensive detection of CNAs smaller than 100 Kb, including those affecting genes like *MSR1*, *FAM30A*, *LINC00221*, *GSTT4*, and *CRLF2* as gains, and *CROCC*, *STIL*, *LCE3C*, *TARP*, *TRG-AS1*, and *PTEN* as losses, underscores the superior resolution of array CGH compared to traditional cytogenetic methods (8). For instance, the gain in *CRLF2* at Xp22.3 has been increasingly recognised as a key oncogenic driver in T-ALL, often associated with adverse prognosis and resistance to conventional therapies (2). Similarly, the homozygous deletion of *PTEN* at 10q23.31, also detected in this study, is a well-established tumour suppressor gene whose loss contributes to unregulated cell growth and survival pathways in various cancers, including T-ALL (14).

While array CGH excels at identifying segmental genomic changes and CNAs, it does have limitations in detecting balanced translocations and inversions (15). Multiplex ligation-dependent probe amplification (MLPA) was employed to address this, specifically utilising P335 kits for leukaemia-associated genes (16). The strong concordance between MLPA and array CGH results, particularly for critical genes like *CDKN2A*, *CDKN2B*, *JAK2*, *CRLF2*, and *SIRPA*, validates the reliability of our CNA findings. The consistent loss of *JAK2* at 9p24.1, confirmed by both methods, is particularly relevant given its role in the JAK-STAT signalling pathway, which is frequently dysregulated in haematological malignancies and linked to poor prognosis and therapeutic resistance (17). The amplification of *SIRPA* at 20p13, also validated by MLPA, suggests its potential oncogenic role in leukemic progression or survival, warranting further investigation.

The integration of array CGH with MLPA thus offers a robust and complementary approach for pinpointing and confirming CNAs, aiding in the discovery of novel genomic changes that may contribute to T-ALL development (18, 19). These findings extend beyond merely characterising the CCRF-CEM cell line; they reinforce its relevance as a model for studying T-ALL pathogenesis and developing targeted therapies. Recent literature underscores the importance of understanding genomic alterations in T-ALL for improving patient outcomes and tailoring therapeutic strategies (20).

The insights derived from this research contribute significantly to the expanding body of knowledge regarding the genetic factors of T-ALL, potentially guiding future studies to identify new therapeutic targets and enhance treatment effectiveness. The interplay of these diverse genetic changes contributes to the aggressive characteristics of CCRF-CEM, illustrating the intricate genetic architecture of this cell line and its significance as a model for T-ALL research. Further functional studies are essential to elucidate the precise contributions of these identified genetic alterations in leukemogenesis.

CONCLUSION

This study provides a comprehensive genomic profile of the CCRF-CEM cell line, a crucial model for T-cell acute lymphoblastic leukaemia (T-ALL). Using high-resolution array CGH validated by MLPA, we accurately identified numerous copy number aberrations (CNAs), including novel, smaller alterations previously undetected. These findings confirm the significant role of specific CNAs, such as homozygous *CDKN2A/B* loss and alterations in *JAK2*, *CRLF2*, and *SIRPA*, in T-ALL pathogenesis. This research enhances the utility of the CCRF-CEM cell line for studying T-ALL and highlights the power of advanced genomic tools in unravelling cancer complexities, paving the way for targeted therapeutic strategies.

Author Contributions: This work was carried out in collaboration with all the authors. NO carried out most of the experiments and data analysis and drafted the manuscript. NSMD and AI performed microarray data analysis, and NAA performed MLPA experiments and data analysis. IPNB manuscript review and editing. All authors have read and approved the final manuscript.

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