Genetic heterogeneity of RPMI-8402, a T-acute lymphoblastic leukemia cell line

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Abstract. Thorough examination of genetic heterogeneity of cell lines is uncommon. In order to address this issue, the present study analyzed the genetic heterogeneity of RPMI-8402, a T-acute lymphoblastic leukemia (T-ALL) cell line. For this purpose, traditional techniques such as fluorescence *in situ* hybridization and immunocytochemistry were used, in addition to more advanced techniques, including cell sorting, Sanger sequencing and massive parallel sequencing. The results indicated that the RPMI-8402 cell line consists of several genetically different cell subpopulations. Furthermore, massive parallel sequencing of RPMI-8402 provided insight into the evolution of T-ALL carcinogenesis, since this cell line exhibited the genetic heterogeneity typical of T-ALL. Therefore, the use of cell lines for drug testing in future studies may aid the progress of anticancer drug research.

Introduction

In general, cell lines are considered to be genetically and phenotypically homogenous populations of cells. When cell lines are cultured *in vitro*, the most adaptable clone is usually the one that expands most rapidly during the stabilization process, and the cells derived from this clone become the most abundant in the culture. Generally, ≤20-30 passages are required in order to purify the cells derived from this clone from other clones that may also be present in the culture. This potential genetic heterogeneity displayed by cell lines cultured *in vitro* has led to certain opposition to the use of cancer cell lines in modern drug testing (1,2). The number of cell lines described as consisting of several genetically different cell populations has increased in the recent years (3,4). However, the number of publications based on genetically heterogeneous cell lines is limited. The analysis of genetically heterogenous cell cultures is more complicated than that of genetically homogenous cell populations, due to the flexibility and differences in adaptation exhibited by the different populations present in the culture, and typically requires the combination of well-established methodologies, including immunocytochemistry (ICC) and cell sorting, with novel technologies such as massive parallel sequencing. These types of genetic analyses are of importance, particularly for cancer, since the mutational analysis of the cellular heterogeneity of an specimen offers an evolutionary perspective of the carcinogenic process. In the present study, the genetic heterogeneity of RPMI-8402, a T-acute lymphoblastic leukemia (T-ALL) cell line, is analyzed in depth, and the usefulness of cell lines in anticancer drug research is debated (5-7).

Materials and methods

RPMI-8402 cell culture. The RPMI-8402 cell line (ACC 290 human, peripheral blood, leukaemia, acute lymphoblastic T cell) was purchased from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Brunswick, Germany). RPMI-8402 cells were cultured at low and high density in RPMI-1640 expansion medium supplemented with 10% FBS (both PAA, Linz, Austria) and penicillin/streptomycin/glutamin (Thermofisher Scientific, Inc., Waltham, MA< USA) in %% CO_2 for 24 h.

DNA and RNA extraction. DNA and RNA were isolated from RPMI-8402 cells at 48 and 72 h post-seeding, using AllPrep DNA/RNA Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. The concentration and purity of the nucleic acids were measured by NanoPhotometer® (Implen GmbH, Munich, Germany).

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TP53 mutation detection. The *TP53* gene was sequenced via the Sanger's method (also known as the dideoxy sequencing or chain termination method), using cDNA as template. Reverse transcription was performed with QuantiTect® Reverse Transcription Kit (Qiagen GmbH), according to the manufacturer's protocol. The exons 4-8 of the *TP53* gene on the cDNA template were amplified by polymerase chain reaction (PCR), using Q5® Hot Start High-Fidelity DNA Polymerase (New England BioLabs, Inc., Ipswich, MA, USA). The primer sequences used for PCR and sequencing of the *TP53* gene are indicated in Table I. The cycling conditions were as follows: 30 sec at 98˚C (polymerase activation), followed by 35 cycles of 10 sec at 98˚C (denaturation), 20 sec at 63˚C (annealing), 20 sec at 72˚C (extension), and 2 min at 72˚C (final extension). Next, samples were purified with NucleoSpin Gel and PCR Clean-up (Macherey-Nagel GmbH, Düren, Germany). cDNA sequencing was performed using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Thermo Fisher Scientific, Inc., Waltham, MA, USA) following the manufacturer's protocol. Upon ethanol/EDTA precipitation, the sequences were analysed with ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, Inc.), using the DNA sequencing analysis software provided with the instrument.

ICC. RPMI-8402 cells, obtained from expansion of the culture, were fixed for 15 min in phosphate-buffered saline (PBS) containing 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA), and next permeabilised with 0.1% Triton™ X-100 (Sigma-Aldrich) for 10 min at room temperature (RT) while agitating. The fixed cells were subsequently blocked with 2% donkey serum (Sigma-Aldrich) dissolved in PBS for 1 h at RT while agitating, and then incubated with monoclonal rabbit antibody against human TP53 (dilution 1:200; cat no. sc-6243, Santa Cruz Biotechnology, Inc., Dallas, TX, USA). Next, the cells were incubated with anti-rabbit Alexa Fluor 488 secondary antibody (dilution 1:1,000; Invitrogen, Thermo Fisher Scientific, Inc.) for 1 h at RT in the dark, and the cell nuclei were counterstained with DAPI (Sigma-Aldrich). Slides were cover-slipped and examined under a fluorescence microscope (ECLIPSE Ci-S; Nikon, Corporation, Tokyo, Japan), using ACT-1 software (Nikon, Corporation).

Fluorescence in situ hybridization (FISH) analyses. For the simultaneous detection of the number of copies of the *TP53* gene and chromosome 17 centromere (CEP17) by FISH, TP53/CEP 17 FISH Probe Kit (Vysis, Abbott Molecular, Des Plaines, IL, USA) was used. The reaction was conducted according to the following protocol: Fixed samples were incubated in 2X standard saline citrate (SSC) buffer (Abbott Molecular) at 72˚C for 2 min, followed by 5-min incubation at 37˚C in a 0.5 mg/l protease solution (Abbott Molecular, Des Plaines, IL, USA), and then washed in PBS for 5 min at RT. Next, the specimens were fixed for 5 min at RT in 1% formaldehyde solution; washed in PBS for 5 min at RT; dehydrated in 70% ethanol for 1 min, followed by 1-min incubation in 85% ethanol, and 5-min incubation in 100% ethanol; and dried at RT, prior to be placed on a slide warmer at 50˚C for 2 min. The FISH probe mix was centrifuged and denatured at 73˚C for 5 min. Upon addition of the denaturated probe, the specimens were cover-slipped and incubated at 37˚C overnight in a humidified chamber, prior to be subjected to hybridization. Subsequently, the cell specimens were washed with 0.4X SSC buffer containing 0.3% Nonidet (N)P-40 (Abbott Molecular) at 73˚C for 2 min, followed by 1-min wash at RT with 2X SSC buffer containing 0.1% NP-40. Next, the specimens were dried in the dark at RT, stained with 10 μl 125 ng/ml DAPI solution (Abbott Molecular) and cover-slipped. The samples were analysed with ECLIPSE Ci-S fluorescence microscope, which was equipped with a specifically designed combination of filters for a range of green and orange light. The number of red signals, resulting from the binding of the *TP53*-specific probe, directly indicated the number of copies of *TP53*, while the number of green signals, caused by the binding of the CEP 17 probe, directly indicated the number of copies of the chromosome 17.

Fluorescence activated cell sorting (FACS). For FACS analysis, $5x10^6$ cells were suspended in 0.5 ml 0.5% bovine serum albumin (BSA) (Sigma-Aldrich) in PBS; fixed in PBS containing 4% paraformaldehyde for 10 min; permeabilised with 0.1% Triton™ X-100 for 10 min at RT; and washed three times with 0.5 ml 0.5% BSA dissolved in PBS. Nonspecific binding sites were blocked by incubation with 2% BSA dissolved in PBS for 15 min. Next, 25% cells were subjected to a preliminary analysis to evaluate their size and granularity, and 75% of them were then selected for immunolabeling with rabbit anti-TP53 antibody (diluted 1:200 in PBS; cat no. sc-6243; Santa Cruz Biotechnology, Inc.). For immunolabeling, the fixed cells were incubated with the aforementioned primary antibody for 15 min at RT, followed by 15-min incubation at RT with a goat anti-rabbit Alexa Fluor® 488 fluorochrome-conjugated secondary antibody (dilution 1:1,000 in PBS; cat no. , Invitrogen, Thermo Fisher Scientific, Inc.) for visualization of the labeled proteins. Cell sorting was performed with Amnis® Imaging Flow Cytometer (Amnis, EMD Millipore, Billerica, MA, USA). Side scatter and forward scatter gate parameters were selected to sort cells according to their cell size and granularity, which had been previously determined during the preliminary analysis. The selected population of cells was divided into two groups, $TP53^+$ and $TP53^-$, according to the intensity of the fluorescence signal arising from the secondary antibody. To ensure the precision of the selection process, the two cells detected following a $TP53^+$ cell, were excluded. Cell debris was collected in a separate tube. Sorted cells ($\leq 5x10^5$ cells) were subjected to DNA/RNA isolation for further sequencing analysis.

Ion Torrent™ Personal Genome Machine (PGM) sequencing and sequencing data analysis. Library preparation and sequencing were performed with Ion Torrent™ PGM (Thermo Fisher Scientific, Inc.), following the manufacturer's protocol, using whole genomic DNA isolated from RPMI-8402 cells.

The high-quality DNA required for sequencing was isolated from RPMI-8402 cells using AllPrep DNA/RNA Mini Kit, and purified with NucleoSpin Gel and PCR Clean-up. The concentration and purity of the extracted DNA were estimated by NanoPhotometer® and Qubit 2.0® Fluorometer (Thermo Fisher Scientific, Inc).

Table I. Primer sequences used for sequencing the *TP53* gene.

TP53 sequencing primer	Exon	Sequence
TP53_Forward1	4-5	5'-CCAGAGGCTGCTCCCCCCGT-3'
TP53_Reverse1	4-5	5'-TCATCCAAATACTCCACACG-3'
TP53_Forward2	5-8	5'-GCCATCTACAAGCAGTCACAGC-3'
TP53_Reverse2	5-8	5'-ATCCAGTGGTTTCTTCTT-3'

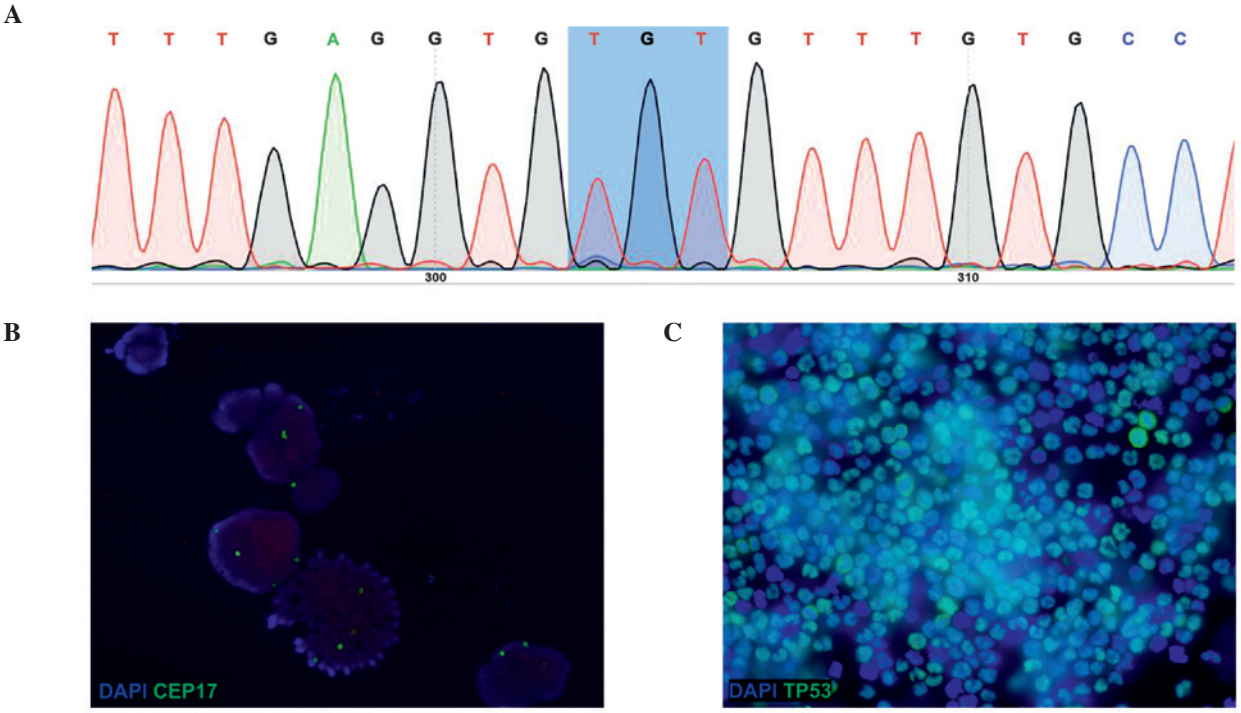

Figure 1. Analysis of the RPMI-8402 cell line. (A) Sanger sequencing results confirmed the presence of the mutation CGT-TGT (Arg-Cys) in the codon 273 of the *TP53* gene. (B) Fluorescence *in situ* hybridization analysis of CEP17 identified a variable number of copies (3-7 copies/cell) of the chromosome 17. (C) Immunocytochemical analysis revealed two different cell populations, one exhibiting nuclear accumulation of TP53, and the other one displaying low protein expression levels of TP53. CEP17, chromosome 17 centromere.

Ion AmpliSeq™ Libraries were constructed using Ion AmpliSeq 2.0 Library Kit (Thermo Fisher Scientific, Inc.), and purified with Agencourt AMPure XP (Beckman Coulter, Inc., Brea, CA, USA). Target amplification was performed using four pools of 4,000 primers (Ion AmpliSeq™ Comprehensive Cancer Panel; Thermo Fisher Scientific, Inc.). The quantification of libraries was estimated with Qubit 2.0 Fluorometer, using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Inc.). The size of the DNA fragments in each pool and the concentration of the final libraries were evaluated by 2100 BioAnalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA) with High Sensitivity DNA Analysis Kit (Agilent Technologies, Inc.).

Upon dilution, the libraries were mixed and used to prepare Ion Sphere™ particles (ISPs), using Ion OneTouch™ 2 System (Thermo Fisher Scientific, Inc.) and Ion PGM™ Template OT2 200 Kit (Thermo Fisher Scientific, Inc.). The quality of the unenriched, template-positive ISPs was assessed with Qubit 2.0 Fluorometer, using Alexa Fluor® 488 and 647 fluorophores (Invitrogen, Thermo Fisher Scientific, Inc.). Following emulsion PCR and recovery, the template-positive ISPs were enriched with Ion OneTouch™ ES (Thermo Fisher Scientific, Inc.), using Dynabeads® MyOne™ Streptavidin C1 (Thermo Fisher Scientific, Inc.), and subjected to sequencing.

The sequencing process, which included 500 flows and >5x10^6 total counts with 99.5% aligned bases, was performed on Ion 318™ Chip (Thermo Fisher Scientific, Inc.), using Ion PGM™ Sequencing 200 Kit v2 (Thermo Fisher Scientific, Inc.). Ion Torrent™ PGM sequencing of RPMI-8402 cells was performed using Ion AmpliSeq™ Comprehensive Cancer Panel (Thermo Fisher Scientific, Inc.), which included 16,000 pairs of primers (4,000 in each pool) that enabled to cover exons in ~400 genes involved in tumorigenesis.

The data obtained from the Ion Torrent™ PGM sequencing reaction were directly processed to generate sequence reads, and loaded onto Ion Torrent Server™ (Thermo Fisher Scientific, Inc.).

Table II. Sequencing results of the sorted cell populations.

Gene/population	$TP53^+$ population	$TP53^-$ population
TP53	Heterozygous	Trace or absence of mutated template
PTEN	Heterozygous	Trace or absence of mutated template
FBXW7	Homozygous	Homozygous

PTEN, phosphatase and tensin homolog; *FBXW7*, F-Box and WD repeat domain containing 7.

Table III. Mutations detected by Ion Torrent™ Personal Genome Machine sequencing in the RPMI-8402 cell line, which had been previously described in the Cancer Cell Line Encyclopedia.

Gene ID	Allele source	Type	Allele name	Frequency (%)	Quality
FBXW7	Hotspot	SNP	COSM22965 COSM117310 COSM117309 COSM117308	100	3759.76
NOTCH4	Novel	DEL	-	100	1037.50
TSC2	Novel	SNP	-	73.1	616.35
WT1	Novel	SNP	-	51.5	901.63
TP53	Hotspot	SNP	COSM99933	51.2	892.21
WHSC1	Novel	SNP	-	51.0	882.84
PKHD1	Novel	SNP	-	48.5	787.38
PPARG	Novel	SNP	-	45.5	687.62
PTEN	Hotspot	SNP	COSM5287	36.0	146.91
HRAS	Novel	SNP	-	30.7	251.17
LRP1B	Novel	SNP	-	24.0	91.27

ID, identity; *FBXW7*, F-Box and WD repeat domain containing 7; *NOTCH4*, neurogenic locus notch homolog 4; *TSC2*, tuberous sclerosis complex 2; *WT1*, Wilms tumor 1; *WHSC1*, Wolf-Hirschhorn syndrome candidate 1; *PKHD1*, polycystic kidney and hepatic disease 1; *PPARG*, peroxisome proliferator-activated receptor γ; *PTEN*, phosphatase and tensin homolog; *HRAS*, Harvey rat sarcoma viral oncogene homolog; *LRP1B*, low-density lipoprotein receptor-related protein 1B; SNP, single nucleotide polymorphism; DEL, deletion; COSM, catalogue of somatic mutations.

Initial variant calling from the Ion AmpliSeq™ sequencing data were generated by Torrent Suite™ Software version 4.0.2 (Thermo Fisher Scientific, Inc.) with Torrent Variant Caller plug-in version 4.0 (Thermo Fisher Scientific, Inc.). The analysis was performed using *Homo sapien*s hg19 genome as reference.

In order to ensure the specificity and sensitivity of the assay, the following restrictions were applied during the generation of the final results: i) The frequency of the candidate sites with potential mutations was analysed, and those mutations presenting a frequency in the range of 20-100 were used for further analysis; ii) for the filters, 'total coverage depth >100' and 'coverage ± >20' settings were used to eliminate potential false positives arising from low read depth; and iii) a subsequent filtering step was applied to eliminate those mutations that were detected in one strand, in order to reduce possible strand-specific errors. The data fulfilling all the aforementioned filtering criteria were used for further analysis, leading to the identification of single nucleotide polymorphisms (SNPs), insertions and deletions. The variant calls were visualised with Integrative Genomics Viewer software, version 8 (http://www.broadinstitute.org/software/igv), and the detected mutations were compared to mutations in RPMI-8402 cells that had been previously reported in the Broad-Novartis Cancer Cell Line Encyclopedia (CCLE) (http://www.broadinstitute.org/ccle) (8).

Results

The ratio of mutated to wild-type (WT) TP53 DNA. Sequencing analysis demonstrated the presence of the heterozygous mutation CGT-TGT (Arg-Cys) in codon 273 of the *TP53* gene (Fig. 1A). In cells cultured at low density, the ratio of mutated to WT *TP53* detected by sequencing was 0.9, whereas in high density cultures this ratio reduced to 0.4.

Genetic heterogeneity visualized at the single cell level. FISH analysis with CEP17 demonstrated an heterogeneous number of copies of the chromosome 17 (3-7 copies/cell, 4 copies/cell on average) (Fig. 1B). In addition, ICC analysis identified two distinct populations of cells, one of them displaying

Table IV. Novel mutations detected by Ion Torrent™ Personal Genome Machine sequencing in the RPMI-8402 cell line, which had not been previously published in the Cancer Cell Line Encyclopedia.

Gene ID	Type	Frequency (%)	Allele call	Chromosome	Position	Reference	Variant	Coverage
PDE4DIP	DEL	20.4	Heterozygous	1	$1.45x10^8$	C	-	397
LPP	INS	25.3	Heterozygous	3	$1.88x10^8$	-	T	292
IGF1R	DEL	27.7	Heterozygous	15	99467943	GT	-	267
BLM	INS	34.6	Heterozygous	15	91358272	-	GAA	396
LTF	INS	37.0	Heterozygous	3	46501285	-	CTT	392
ROS1	INS	43.5	Heterozygous	6	$1.18x10^8$	-	TAA	168
TCF7L1	DEL	45.6	Heterozygous	2	85536129	TCTT	-	386
HSP90AB1	DEL	46.9	Heterozygous	6	44221146	GAGTTTGT	-	326
FN1	DEL	52.3	Heterozygous	2	$2.16x10^8$	AA	-	327
FN1	DEL	55.2	Heterozygous	2	$2.16x10^8$	AC	-	223
FN1	DEL	59.2	Heterozygous	2	$2.16x10^8$	AC	-	343
SF3B1	INS	52.4	Heterozygous	2	$1.98x10^8$	-	AA	246
CRBN	INS	52.9	Heterozygous	3	3192525	-	TAAC	363
NUP98	DEL	58.1	Heterozygous	11	3789983	AAAAAGAAAAA	-	327
PTEN	INS	63.3	Heterozygous	10	89717681	-	CCCCCGGCCC	294
CDK6	DEL	70.2	Heterozygous	7	92244631	ATACA	-	161
LIFR	DEL	78.9	Heterozygous	5	38528952	AC	-	209
MLL3	DEL	85.6	Heterozygous	7	$1.52x10^8$	T	-	209
MTR	INS	90.7	Homozygous	1	$2.37x10^8$	-	TCTG	396
MTR	INS	100	Homozygous	1	$2.37x10^8$	-	T	127
HLF	INS	92.1	Homozygous	17	53342797	-	TTTC	151
AFF1	DEL	98.7	Homozygous	4	88056720	T	-	311
PIK3C2B	INS	100	Homozygous	1	$2.04x10^8$	-	C	291
PDGFRA	INS	100	Homozygous	4	55151959	-	A	242
SYNE1	DEL	100	Homozygous	6	$1.52x10^8$	TGTT	-	217
WRN	INS	100	Homozygous	8	31005018	-	C	392
NUMA1	DEL	100	Homozygous	11	71728909	GTCAAC	-	142
PLEKHG5	DEL	33.4	Heterozygous	1	6529183	TCC	-	356
AKAP9	INS	89.7	Heterozygous	7	91669961	-	T	87

ID, identity; *PDE4DIP*, phosphodiesterase 4D-interacting protein; *LPP*, lipoma-preferred partner; *IGF1R*, insulin-like growth factor 1 receptor; *BLM*, Bloom syndrome; *LTF*, lactotransferrin; *TCF7L1*, transcription factor 7-like 1; *HSP90AB1*, heat shock protein 90 kDa α (cytosolic), class B member 1; *FN1*, fibronectin 1; *SF3B1*, splicing factor 3B subunit 1; *CRBN*, cereblon; *NUP98*, nuclear pore complex protein 98; *PTEN*, phosphatase and tensin homolog; *CDK6*, cyclin-dependent kinase 6; *LIFR*, leukemia inhibitory factor receptor; *MLL3*, mixed-lineage leukemia protein 3; *MTR*, 5-methyltetrahydrofolate-homocysteine methyltransferase; *HLF*, hepatic leukemia factor; *AFF1*, AF4/FMR2 family, member 1; *AF4*, acute lymphocytic leukemia-1 fused gene on chromosome 4; *FMR2*, fragile mental retardation 2; *PIK3C2B*, phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 β; *PDGFRA*, platelet-derived growth factor receptor α; *SYNE1*, synaptic nuclear envelope protein 1; *WRN*, Werner syndrome; *NUMA1*, nuclear mitotic apparatus protein 1; DEL, deletion; INS, insertion.

nuclear accumulation of TP53 ($TP53^+$), and the other one presenting low expression levels of TP53 ($TP53^-$) (Fig. 1C).

Cell sorting. Two cell populations were sorted by FACS, based on their expression levels of TP53, and subsequently subjected to sequencing analysis. $TP53^+$ cells exhibited a higher proportion of the mutated template, compared with the $TP53^-$ population (Table II). Further analyses revealed a heterozygous mutation in the phosphatase and tensin homolog (*PTEN)* gene in $TP53^+$ cells, which was absent in the $TP53^-$ population. By contrast, a homozygous mutation in the F-Box and WD repeat domain containing 7 (*FBXW7)* gene was observed in both cell populations.

Massive parallel sequencing and database analysis. The results of massive parallel sequencing were consistent with the data previously published in CCLE, confirming the reliability of the analyses performed on the present study. Furhermore, massive parallel sequencing demonstrated that the RPMI-8402 cell line consists of several genetically different cell populations. Consistently with the sorting analysis and Sanger's sequencing, massive parallel sequencing detected the aforementioned R159S mutation in the *PTEN* gene in 36% of the templates. In addition, mutations in Harvey rat sarcoma viral oncogene homolog *(HRAS)* (A134S), low-density lipoprotein receptor-related protein 1B (*LPRB1)* (R3239G) and tuberous sclerosis complex 2 (*TSC2)* (A614T) were detected in 30,

24 and 73% of the templates, respectively (Table III). These mutations, as well as mutations in Wolf-Hirschhorn syndrome candidate 1 (WHSC1) and peroxisome proliferator-activated receptor γ (PPARγ) (Table III), represent an additional indication of genetic heterogeneity in the RPMI-8402 cell line, which may be used to reconstruct the carcinogenic process leading to the development of T-ALL (Tables III and IV).

Discussion

RPMI-8402 is a genetically heterogenous T-ALL cell line (9). Using DNA sequencing, the present study identified a heterozygous Arg-Cys mutation at codon 273 of the *TP53* gene in RPMI-8402 cells, with varying proportions of the templates (50-75% of WT allele), depending on cell confluence. Additionally, FISH analysis detected a variable number of copies of the chromosome 17 in RPMI-8402 cells, while ICC analysis revealed two different cell populations, exhibiting high and low protein expression levels of TP53, respectively, which suggests genetic heterogeneity of the RPMI-8402 cell line. Cell sorting analysis identified heterogeneity of *PTEN* and *TP53* mutations, but an homozygous mutation in the *FBXW7* gene was detected in both cell populations. Furthermore, massive parallel sequencing confirmed the genetic heterogeneity of RPMI-8402 cells, and excluded the possibility of cross-contamination with another cell line. The comparison between the results obtained with Ion Torrent™ and Sanger sequencing indicates that the former method provides high sensitivity and specificity and, therefore, may be the a suitable technique for mutational analysis of cancer samples (10-12). However, strict parameters should be employed when using Ion Torrent™ sequencing, in order to ensure a correct analysis of the data. The results of the present study demonstrated that the RPMI-8402 cell line consists of several genetically different subpopulations. Furthermore, the proportion of cells with different mutations was observed to vary during cell culturing. Thus, in low density cultures, a significant proportion of the cells exhibited a mutated version of *TP53*, while at high density, cells carrying mainly the WT *TP53* template constituted the majority of the culture.

From the perspective of new drug development, the heterogeneity of RPMI-8402 cells may be regarded at the same time as a difficulty and an opportunity. Thus, the results of antineoplastic drug testing may be influenced by the culture confluence, since different cell subpopulations may display different drug resistance, which may complicate the interpretation of the data. However, the findings of the present study, which identified genetic heterogeneity in the cell line RPMI-8402, may support the use of cell lines in future anticancer drug studies, since the lack of genetic heterogeneity (contrarily to what is typically observed in neoplasms) was one of the arguments against the use of cell lines in drug research (13,14).

Additionally, the results of the analyses of the RPMI-8402 cell line conducted in the present study may be used to elucidate the evolution of mutational alterations that occur during carcinogenesis. The results of the present study suggest that mutations in genes such as *FBXW7,* neurogenic locus notch homolog 4, 5-methyltetrahydrofolate-homocysteine methyltransferase (*MTR;* Table IV), nuclear mitotic apparatus protein 1, platelet-derived growth factor receptor α, synaptic nuclear envelope protein 1 and Werner syndrome occurred early in the process of carcinogenesis, since all the cells analysed contained the mutated templates. These alterations were possibly followed by mutations in *TP53,* Wilms tumor 1, *PTEN,* cyclin-dependent kinase 6, leukemia inhibitory factor receptor, mixed-lineage leukemia protein 3, A-kinase anchor protein 9, hepatic leukemia factor and AF4/FMR2 family, member 1; while the mutations detected in lactotransferrin, *ROS1,* transcription factor 7-like 1, heat shock protein 90 kDa α (cytosolic), class B member 1, fibronectin 1, splicing factor 3B subunit 1, cereblon and nuclear pore complex protein 98 possibly occurred at later stages of the carcinogenic process. In addition, mutations in genes such as low-density lipoprotein receptor-related protein 1B, phosphodiesterase 4D-interacting protein, lipoma-preferred partner, insulin-like growth factor 1 receptor and pleckstrin homology domain containing, family G member 5 and Bloom syndrome genes were observed in minor subpopulations of cells, where the mutated template constituted ~20%, suggesting their generation at the final stages of carcinogenesis (Table IV). Previous studies have demonstrated that certain mutations, particularly of oncogenes, do not require an homo-/hemizygous status to efficiently promote neoplastic transformation (15). In the case of RPMI-8402 cells, previous studies have demonstrated hypertetraploidy in this cell line, which further complicates the interpretation of data (16). However, massive parallel sequencing seems to be a useful tool in the identification of driver genes in carcinogenesis (17,18). The observations of the present study are in agreement with previous analyses of clinical T-ALL samples, which revealed that individual patients with T-ALL contained various clones that shared a common genetic stem origin but responded differently to the therapy (5,19).

In conclusion, the present study has demonstrated that the RPMI-8402 cell line exhibits the genetic heterogeneity typical of T-ALL, and therefore, it may be useful in anticancer drug research.

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References

1. Gillet JP, Calcagno AM, Varma S, Marino M, Green LJ, Vora MI, Patel C, Orina JN, Eliseeva TA, Singal V, *et al*: Redefining the relevance of established cancer cell lines to the study of mechanisms of clinical anti-cancer drug resistance. Proc Natl Acad Sci USA 108: 18708-18713, 2011.
2. Gazdar AF, Gao B and Minna JD: Lung cancer cell lines: Useless artifacts or invaluable tools for medical science? Lung Cancer 68: 309-318, 2010.
3. Nugoli M, Chuchana P, Vendrell J, Orsetti B, Ursule L, Nguyen C, Birnbaum D, Douzery EJ, Cohen P and Theillet C: Genetic variability in MCF-7 sublines: Evidence of rapid genomic and RNA expression profile modifications. BMC Cancer 3: 13, 2003.
4. Inoue A, Yokomori K, Tanabe H, Mizusawa H, Sofuni T, Hayashi Y, Tsuchida Y and Shimatake H: Extensive genetic heterogeneity in the neuroblastoma cell line NB(TU)1. Int J Cancer 72: 1070-1077, 1997.

5. Treanor LM, Zhou S, Janke L, Churchman ML, Ma Z, Lu T, Chen SC, Mullighan CG and Sorrentino BP: Interleukin-7 receptor mutants initiate early T cell precursor leukemia in murine thymocyte progenitors with multipotent potential. J Exp Med 211: 701-713, 2014.
6. Hait WN: Anticancer drug development: The grand challenges. Nat Rev Drug Discov 9: 253-254, 2010.
7. Hutchinson L and Kirk R: High drug attrition rates - where are we going wrong? Nat Rev Clin Oncol 8: 189-190, 2011.
8. Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, Getz G and Mesirov JP: Integrative genomics viewer. Nat Biotechnol 29: 24-26, 2011.
9. Srivastava BI, Minowada J and Moore GE: High terminal deoxynucleotidyl transferase activity in a new T-cell line (RPMI 8402) of acute lymphoblastic leukemia origin. J Natl Cancer Inst 55: 11-14, 1975.
10. Gu Y, Lu K, Yang G, Cen Z, Yu L, Lin L, Hao J, Yang Z, Peng J, Cui S and Huang J: Mutation spectrum of six genes in Chinese phenylketonuria patients obtained through next-generation sequencing. PLoS One 9: e94100, 2014.
11. Cai X, Sheng J, Tang C, Nandakumar V, Ye H, Ji H, Tang H, Qin Y, Guan H, Lou F, *et al*: Frequent mutations in EGFR, KRAS and TP53 genes in human lung cancer tumors detected by ion torrent DNA sequencing. PLoS One 9: e95228, 2014.
12. Bai X, Zhang E, Ye H, Nandakumar V, Wang Z, Chen L, Tang C, Li J, Li H, Zhang W, *et al*: PIK3CA and TP53 gene mutations in human breast cancer tumors frequently detected by ion torrent DNA sequencing. PLoS One 9: e99306, 2014.
13. Mitra A, Mishra L and Li S: Technologies for deriving primary tumor cells for use in personalized cancer therapy. Trends Biotechnol 31: 347-354, 2013.
14. Marjanovic ND, Weinberg RA and Chaffer CL: Cell plasticity and heterogeneity in cancer. Clin Chem 59: 168-179, 2013.
15. Vogelstein B and Kinzler KW: Cancer genes and the pathways they control. Nat Med 10: 789-799, 2004.
16. Gebhart E, Thoma K, Verdorfer I, Drexler HG and Efferth T: Genomic imbalances in T-cell acute lymphoblastic leukemia cell lines. Int J Oncol 21: 887-894, 2002.
17. Gerlinger M, Horswell S, Larkin J, Rowan AJ, Salm MP, Varela I, Fisher R, McGranahan N, Matthews N, Santos CR, *et al*: Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. Nat Genet 46: 225-233, 2014.
18. Sottoriva A, Spiteri I, Piccirillo SG, Touloumis A, Collins VP, Marioni JC, Curtis C, Watts C and Tavaré S: Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. Proc Natl Acad Sci USA 110: 4009-4014, 2013.
19. Mullighan CG: The molecular genetic makeup of acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program 2012: 389-396, 2012.