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Genomic landscape of human erythroleukemia K562 cell line, as determined by next-generation sequencing and cytogenetics

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ABSTRACT

We have performed detailed analysis of the genomic landscape of commercially available K562 cells, employing targeted enrichment of nearly 1300 cancer-related genes followed by next-generation sequencing (NGS) and also classical cytogenetics. Deep sequencing revealed 88 variants of potentially biological significance. Among them we have detected alterations in genes already known to be mutated in K562, such as *TP53* but also in several other genes, which are implicated in tumorigenesis and drug resistance, such as *MLH1*, *ASXL1* and *BRCA1* as the most prominent examples. Fluorescence *in situ* hybridization (FISH) of interphases of K562 cells revealed multiplication of the *BCR* and *ABL1* gene copies, as well as the amplification of the *BCR-ABL1* fusion gene. Our results may help to better understand genomic instability of the blastic phase of CML represented by the K562 cell line and can help researchers who want to employ this cell line in various experimental settings.

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Introduction

Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder characterized by the increased and deregulated growth and maturation of myeloid cells in the bone marrow. Disease is caused by reciprocal chromosomal translocation t(9;22)(q34;q11) which results in the genetic abnormality called the Philadelphia chromosome (Ph), encoding fusion gene [1]. *BCR-ABL1* encodes a constitutively active oncogenic tyrosine kinase BCR-ABL1, which transforms hematopoietic stem cells by activating several proliferative and antiapoptotic pathways, but also by increasing genomic instability. One of the most frequently used CML cell line model is the K562 BCR-ABL1-positive human erythroleukemia cell line, which was derived in 1970 from a pleural effusion of a female patient with CML in blastic phase (CML-BP, also known as blast crisis) [2], decades before the era of targeted therapy with tyrosine kinase inhibitors. K562 cells do not express MHC molecules on their surface and serve also as one of the typical target cells for measuring activity of NK cells [3, 4]. Therefore, K562 represents an important tool for the studies of malignant hematopoiesis as well as for the studies on the molecular pathogenesis of leukemia and human cancer in general. This is reflected by the fact that a number of publications mentioning K562 available in PubMed exceeds 800 per year in recent years and totals in more than 16.000 publications since 1975 with K562 among the key words. The cell line was cytogenetically characterized many times giving different results. K562 karyotype was described as hypodiploid in short term cultures and near triploid in long time cultures[2] but also Ph-positive hyperdiploid karyotypes [5], Ph positive and near triploid[6] or Ph-negative and near triploid [7, 8]. Possible reasons for such discrepancy include genomic instability of K562 cell line, especially in the long-term culture, amplification *BCR-ABL1* oncogene but also new mutations in DNA repair genes (such as *MLH1*) described in this work. However, despite so numerous research employing K562 cells a detailed genomic analysis of this cell line is not available and so far there are no publications describing genomic landscape of K562 cell line. To accurately characterize genetic features of K562 cell line that is currently used in leukemia research, we performed next-generation sequencing (NGS) of K562 cell line DNA from the early passage. We also investigated the copy number of *BCR*, *ABL1* and fusion gene *BCR-ABL1* using fluorescence *in situ* hybridization (FISH).

Materials and methods

Cell line

The K562 cell line was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). Cells were grown in RPMI 1640 medium (Gibco, Life Technologies) with 10% fetal bovine serum (Invitrogen) at 37 °C in 5% CO_2. K562 cells were passaged 5 times before isolating genetic material with the use of Gentra Puregene Cell Kit (QIAGEN) for NGS sequencing and FISH experiments. The cell line was confirmed at each passage to be mycoplasma free (PCR-based test).

Next-generation and Sanger sequencing

Mutational analysis of K562 cell line was performed using SeqCap EZ Choice (Roche NimbleGen) custom enrichment. Coding sequences of almost 1300 genes, selected on the basis of literature review and major commercial cancer gene panels (Supplementary Table I), were sequenced on Illumina HiSeq 1500, as described previously [9, 10]. The mean depth of coverage was 146×, 96.5% of our target was covered at least 10× and 93.3% of the target was covered at least 20×. Paired, 100 bp reads were trimmed, quality-filtered and aligned to hg19 genome, followed by duplicate removal, variant calling and variant annotation (GATK, SnpEff [11]).

All variants that did not pass quality check were excluded from further analysis. Protein sequence-changing variants, splicing regions variants and start or stop codon-gain variants were then subject to filtering based on their frequencies in 1000 genomes [12] and Exome Sequencing Projects [Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL:http://evs.gs.washington.edu/EVS/)]. All variants more common than 1% in those databases (also considering frequencies in European population) were excluded. Variants more common than 10% in our internal database (more than 2000 sequenced samples, representing various diseases, including cancer, inherited genetic disorders and normal tissue samples from affected individuals) were also removed. This filtering step was applied to remove frequent non-pathogenic variants unique to the Polish population as well as sequencing artifacts (false positives) generated during sequencing process, which cannot be eliminated using publicly available databases. The existence of selected mutations/variants identified by the NGS was confirmed by Sanger sequencing. Briefly, DNA of a particular coding region surrounding the mutation was amplified in PCR reaction using HotStarTaq Plus DNA Polymerase (QIAGEN). The PCR primer sequences were as follows: TP53-F: tgttcacttgtgccctgact, TP53-R: ttaacccctcctcccagaga, ASXL1e12F: tgtatgccatgacccttaagct, ASXL1e12R: cctcaccaccatcaccactg. The PCR products were purified using Agencourt AMPure XP (Beckman Coulter), labeled with BigDye Terminator v.3.1 (Applied Biosystems) according to the manufacturer's instructions and sequenced by an external sequencing facility.

CADD [13], PolyPhen 2 [14], SIFT [15], fathmm [16], MutationTaster [17] and CHASM [18, 19] methods were used to predict functional consequences of identified variants. CHASM was run on default parameters using acute myeloid leukemia passenger mutation rate table.

Fluorescence in situ hybridization (FISH)

K562 cells were harvested according to standard cytogenetical procedures. After cell synchronization by colcemid for 20 min at 37 °C (10 μg/ml, Biosera, France), pelleted cells underwent a hypotonic treatment using 0.075 M KCl solution (Merck, Germany) for 20 min at 37 °C to swell the cells. The cells were then fixed in cold Carnoy's fixative solution composed of 3:1 methanol and 100% acetic acid (Merck) and

Table I – Newly identified mutations in K562 cells in genes commonly mutated in human malignancies (such as tumor suppressors and oncogenes)

Gene name	Mutation description Nucleotide change/ AA change(NM number)	Mutation	Disease related to the gene	Protein function (GO terms)
BRCA1	c.1618A>G/p.Ile540Val (NM_007297.3)	New	Familial breast-ovarian cancer, neoplastic syndromes	Tumor suppressor, maintaining genomic stability, RNA binding and ligase activity
ASXL1	c.1773C>A/p.Tyr591* (NM_015338.5)	Described (in MDS [34] and in AML (1 patient) [35])		transcriptional regulator; retinoic acid receptor binding activity
MLH1	c.523delA/p.Lys175fs (NM_001258271.1)	New	Lynch syndrome	ATPase activity and post-replicative DNA mismatch repair
BIRC6	c.10865C>T/p.Ala3622Val (NM_016252.3)	New		Ligase activity, the protein inhibits apoptosis by facilitating the degradation of apoptotic proteins by ubiquitination
AKT3	c.109G>T/p.Gly37* (NM_001206729.1)	New		Cell signaling regulator; transferase activity and protein tyrosine kinase activity
TERT	c.2663G>A/p.Arg888Gln (NM_001193376.1)	Described (in glioblastoma (1 patient) [36])		The protein maintains telomere ends by addition of the telomere repeat TTAGGG; protein homodimerization activity and tRNA binding
FANCC	c.178G>A/p.Val60Ile (NM_001243744.1)	New	Hereditary neoplastic syndromes	DNA repair protein that may operate in a postreplication repair or a cell cycle checkpoint function

washed three times to ensure complete removal of cytoplasmic debris. The resulting suspension of metaphase and interphase cells was applied to microscopic slides. FISH was performed with the commercially available LSI BCR-ABL Dual Color, Dual Fusion Translocation Probe (Vysis, Abbott Molecular Inc., USA), dedicated to identification of BCR-ABL1/ABL1-BCR fusion genes. The procedure was applied according to the manufacturer's protocol with modification of denaturation and post hybridization washing time to 8 and 3 min, respectively. Results were analyzed using an epifluorescence microscope Imager.Z2 (Carl Zeiss, Germany) and documented using an ISIS (Metasystems, Germany) Imaging System.

Results

Targeted enrichment and deep sequencing revealed 88 variants with potential biological significance (listed in Supplementary Table II). First, we selected mutations in genes involved in hematological malignancies and in tumor suppressors and oncogenes (shown in Table I). Additionally, we present new mutations in other genes implicated in human diseases, which have been detected in K562 cells (Table II). Literature and databases review followed by manual variant inspection allowed for prioritizing several of them as biologically significant mutations. Noteworthy, according to

Table II – New mutations in other genes with potential biological effects in K562 cells

Gene name	Mutation description Nucleotide change/ AA change (NM number)	Mutation	Protein function (GO terms)
BAZ2B	c.390T>G/p.Phe130Leu (NM_013450.3)	New	Potential role in transcriptional activation as it is a component of chromatin remodeling complex
PCLO	c.15253C>T/p.Arg5085* (NM_033026.5)	Described (in colon cancer [37])	Calcium ion binding and transporter activity; variations in this gene have been associated with bipolar disorder and major depressive disorder
WHSC1	c.1798C>T/p.Arg600* (NM_001042424.2)	New	Sequence-specific DNA binding and histone-lysine N-methyltransferase activity
PTPRN2	c.680C>T/p.Ala227Val (NM_130842.2)	Described (in lung adenocarcinoma [38])	Phosphatase activity and transmembrane receptor protein tyrosine phosphatase activity
KAT6A	c.5629C>T/p.Arg1877Cys (NM_001099412.1)	Described (in diffuse large B-cell lymphoma [39])	Histone acetyltransferase, chromatin binding and transcription coactivator activity
CIC	c.2782G>A/p.Ala928Thr (NM_015125.3)	New	Chromatin binding activity
PTPRZ1	c.1406G>A/p.Arg469His (NM_001206839.1)	Described (in natural killer/ T-cell lymphoma [40])	Phosphatase activity; the protein may be involved in the regulation of specific developmental processes in the central nervous system
KRTAP9-9	c.482C>A/p.Ser161Tyr (NM_030975.2)	New	Keratin-associated protein which forms a matrix of keratin intermediate filaments which contribute to the structure of hair fibers

American Type Culture Collection (ATCC) characteristics of K562 cell line, described in Leukemia Cell Line Panel documentation, it harbors homozygous mutations in *TP53* (c.406_407insC, p.Q136fs*13) and *CDKN2A* (c.1_471del471, p.0?) genes [https://www.lgcstandards-atcc.org/~/media/BF7C43065F5B49C8A93E71C4F830529B.ashx]. We have identified the same c.406_407insC, p.Q136fs*13 mutation in *TP53* in our K562 cell line. However, we have not identified the *CDKN2A* variant in our data because we did not get *CDKN2A* sequence, most probably due to deletion of the whole gene in our K562 cells. We have found that only few of our newly identified variants were reported in COSMIC or CCLE (Cancer Cell Line Encyclopedia [20]) databases, but with no literature annotation. Thus we decided to mark such variants here as new ones. Among all variants listed in Table I, we prioritized six genes on the basis of our analysis of mutations' significance. Mutations in these genes were identified as significant/pathogenic by at least 3 out of 6 used predictors. The top scoring mutations include *BRCA1* (missense mutation), *TP53* (frameshift mutation, as mentioned above this mutation has been described in K562 cell line from ATCC), *ASXL1* (stop gain variant), *MLH1* (frameshift mutation), *BIRC6* (missense variant), *AKT3* (stop gain mutation) as shown in Table I and Fig. 1. We have additionally confirmed two of these mutations by classical Sanger sequencing (Supplementary Fig. 1).

BRCA1, widely known as a gene which (in mutated form) is associated with familial breast cancer, encodes one of the major DNA repair nuclear protein that plays a role in homologous recombination and in maintaining genomic stability [21]. *TP53* encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains [22]. The encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair or changes in metabolism [22]. Mutations in this gene are associated with a variety of human cancers, including hereditary cancers and Li-Fraumeni syndrome [23]. *ASXL1* encodes the protein which functions as a ligand-dependent co-activator for retinoic acid receptor cooperating with nuclear receptor coactivator 1. Mutations in *ASXL1* are common in myelodysplastic syndromes, acute myeloid leukemia and chronic myelomonocytic leukemia [24]. *MLH1* was identified as a *locus* frequently mutated in hereditary non-polyposis colon cancer (HNPCC). The protein is one of key mismatch repair proteins that works coordinately in

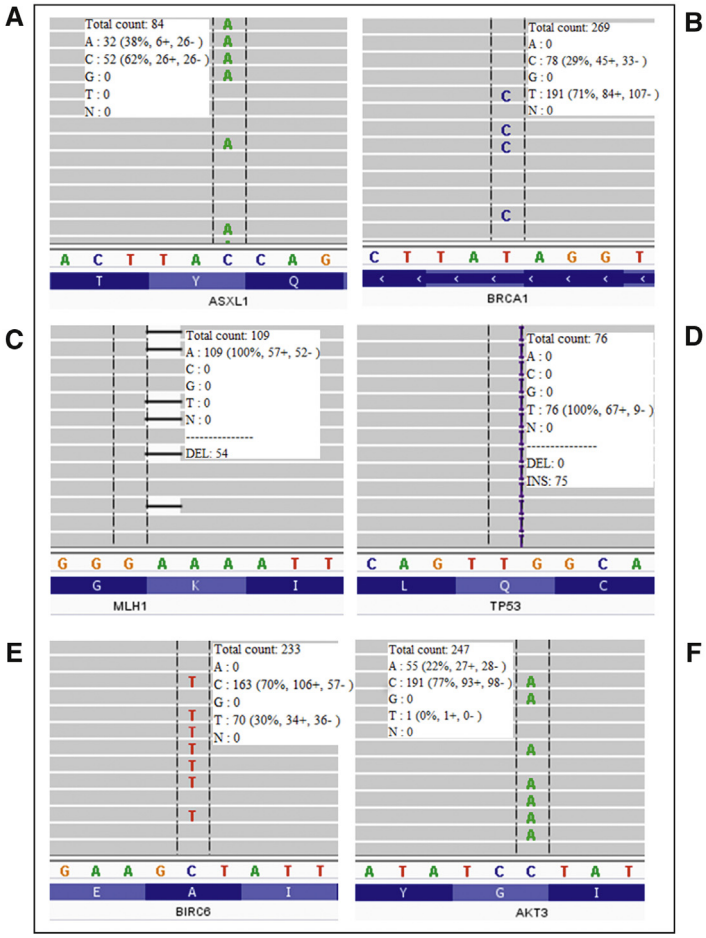

Fig. 1 – Newly identified and known mutations in K562 cell line. Mutation and counted allele frequencies are indicated on the IGV printscreens. Panels stay for known variant in *ASXL1* (A) and *TP53* (D), and new variants in *BRCA1* (B), *MLH1* (C), *BIRC6* (E), *AKT3* (F) genes

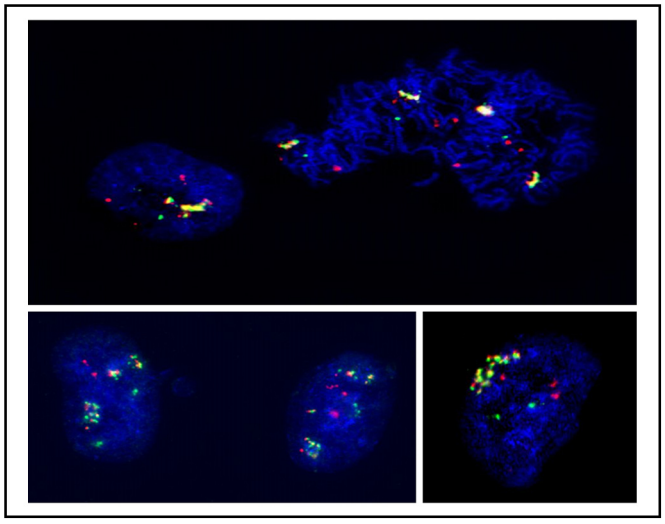

Fig. 2 – Multiplication of the BCR and ABL1 gene copies and the high amplification of the *BCR-ABL1* fusion gene in the K562 interphase cells. The result of FISH analysis with BCR (green) and ABL1 (red) translocation probes on the interphase K562 cells. An accumulations of the fusion genes *BCR-ABL1/ABL1*-BCR are presented in yellow. Nuclei were stained with Vectrashield mounting medium with DAPI (blue)

sequential steps to initiate repair of DNA mismatches in humans [25]. Defects in MLH1 are associated with the microsatellite instability (MSI) observed in HNPCC [26]. BIRC6 (Baculoviral IAP repeat-containing 6) belongs to family of endogenous inhibitors of apoptosis. Mutations in *BIRC6* are associated with leukemia, melanoma, breast cancer, lung cancer, and other cancers [27]. BIRC6 has been shown to interact with KIF23 [27] which is a plus-end directed motor protein expressed in mitosis, involved in the formation of the cleavage furrow (pinch) in late anaphase and in cytokinesis [28–30]. AKT3 is a serine/threonine kinase involved in a wide variety of biological processes including cell proliferation, differentiation, apoptosis and/or tumorigenesis. AKT kinases are known to be regulators of cell signaling in response to insulin and growth factors [31]. All of the proteins described above are related to tumorigenesis.

According to the DSMZ webpage, the cytogenetic characteristics of K562 cell line is given as follows: "human hypotriploid karyotype – 61–68<3n>XX, −X, −3, +7, −13, −18, +3mar, del(9)(p11/13), der(14)t(14;?)(p11;?), der(17)t(17;?)(p11/13;?), der(?18)t(15;?18)(q21;?q12), del(X)(p22) – two markers appear from FISH to have arisen from Ph".

We analyzed the interphase K562 cells by FISH using BCR-ABL1 translocation probes and observed a slightly different pattern of cytogenetic abnormalities in our K562 cell line (purchased from DSMZ). Our analysis of the K562 metaphase and interphase cells shows multiplication of the *BCR* and *ABL1* gene copies, as well as the amplification of the *BCR-ABL1* fusion gene, which is consistent with the previously published results describing 8- to 24-fold amplification of the *BCR-ABL1* fusion gene [32, 33]. However the most relevant alteration on the cytogenetic level is the presence of at least four additional copies of Ph chromosomes (Fig. 2).

Discussion

It is widely accepted that cell lines accumulate with time cytogenetic abnormalities. Our results confirm the existence of major chromosomal aberrations in K562 cell line but we describe new mutations in several oncogenes other than *BCR-ABL1* fusion gene. This confirms a high level of genomic instability in the blastic phase of CML represented by the K562 cell line. Some of the mutations identified in our study may have profound impact on the DNA repair mechanisms and genomic instability of K562 cells. Mutations in *MLH1* gene are frequent in Lynch syndrome and are associated with mismatch repair defect and microsatellite instability [23, 24]. *BRCA1* mutations, on the other hand, may affect another crucial element of DNA repair, homologous recombination, which is involved in the repair of DNA double strand breaks. The awareness of the mutations and karyotype aberrations of the human leukemia cell line K562 is important for further studies of the normal and pathological hematopoiesis or acquisition of the drug resistance as they could have an impact on the observed results. Our results point at the high level of genomic instability in the blastic phase of CML represented by the K562 cell line. The data presented here can further help researchers and hematologists who employ K562 cell line in their experimental procedures by broadening up their knowledge of genomic defects present in this model cell line.

Conflict of interest/Konflikt interesu

The Authors declare that there are no relevant conflicts of interest.

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Ethics/Etyka

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

Appendix A. Supplementary data/Dodatkowe informacje

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.achaem.2017.06.002.

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