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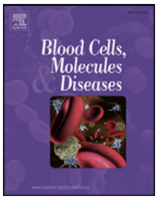

Gene expression profile of circulating CD34$^+$ cells and granulocytes in chronic myeloid leukemia

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ABSTRACT

Purpose: We compared the gene expression profile of peripheral blood CD34$^+$ cells and granulocytes in subjects with chronic myeloid leukemia (CML), with the accent on signaling pathways affected by *BCR–ABL* oncogene.
Methods: The microarray analyses have been performed in circulating CD34$^+$ cells and granulocytes from peripheral blood of 7 subjects with CML and 7 healthy donors. All studied *BCR–ABL* positive CML patients were in chronic phase, with a mean value of 2012 ± SD of CD34$^+$ cells/μl in peripheral blood.
Results: The gene expression profile was more prominent in CML CD34$^+$ cells (3553 genes) compared to granulocytes (2701 genes). The 41 and 39 genes were significantly upregulated in CML CD34$^+$ cells (*HINT1*, *TXN*, *SERBP1*) and granulocytes, respectively. *BCR–ABL* oncogene activated PI3K/AKT and MAPK signaling through significant upregulation of *PTPN11*, *CDK4/6*, and *MYC* and reduction of *E2F1*, *KRAS*, and *NFKBIA* gene expression in CD34$^+$ cells. Among genes linked to the inhibition of cellular proliferation by *BCR–ABL* inhibitor Imatinib, the *FOS* and *STAT1* demonstrated significantly decreased expression in CML.
Conclusion: The presence of *BCR–ABL* fusion gene doubled the expression quantity of genes involved in the regulation of cell cycle, proliferation and apoptosis of CD34$^+$ cells. These results determined the modified genes in PI3K/AKT and MAPK signaling of CML subjects.

1. Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder that originates from an abnormal pluripotent bone marrow hematopoietic stem cell, characterized by various biological and clinical features [1]. The main molecular marker of CML is the *BCR–ABL* fusion gene generation as a result of a t(9;22)(q34;q11) translocation [2]. It has been shown that distribution of malignant cells in CML is not induced by the neoplastic stem cell, but by the lineage-committed progenitor cells [3]. During the chronic phase CML, pool of circulated CD34$^+$ cells demonstrate an increase in the proportion of megakaryocyte–erythroid progenitors, whereas the proportion of hematopoietic stem cells and granulocyte–macrophage progenitors usually decrease [4]. The gene expression profiles of quiescent bone marrow leukemic and peripheral blood CD34$^+$ cells of untreated CML subjects demonstrate no significant difference compared to normal CD34$^+$ cells [4,5]. The sedentary CML CD34$^+$ cells are more similar to their dividing counterparts than quiescent normal cells are to theirs [6].

In patients with CML, mitogenic signaling pathways such as rat sarcoma viral oncogenes homolog (RAS)/mitogen-activated protein kinase (MAPK) pathway, the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway, phosphoinositide-3 kinase (PI3K)/AKT pathway and the MYC pathway are usually constitutively activated, in addition to the deregulation of proliferation, apoptosis and release of progenitors from the bone marrow [7]. The following cellular processes are dysregulated by the *BCR–ABL* oncoprotein: RAS/MAPK signaling that activates proliferation, and PI3K/AKT signaling that activates apoptosis. It has been shown that most components of the MAPK and PI3K/AKT pathways and some genes of the alternative JNK and p38 MAPK pathways are upregulated in primary CML CD34$^+$ cells [4]. A wide range of genes are identified as being dependent on *BCR–ABL1*-mediated signaling, including genes involved in signal transduction of JAK/STAT, MAPK, and transforming growth factor-beta (TGF-β). *BCR–ABL1* activates several genes involved in negative feedback regulation that indirectly suppress the tumor promoting effects exerted by *BCR–ABL1* [8].

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Previous microarray analyses of CML subjects have been performed on selected CD34$^+$ cells or mononuclear cells [9–12]. In our study we combined gene expression analyses of selected CD34$^+$ cells and granulocytes to determine persistent and transient gene expression in MAPK, PI3K/AKT and TGF-β pathways, influenced by *BCR–ABL*, during cell maturation. Gene expression patterns reflect *BCR–ABL*-induced functional modifications such as cell-cycle, apoptosis and proliferation. This observation highlights the difference in gene expression between CD34$^+$ cells of CML and control subjects, with the accent on genes that direct the pathogenic course of malignancy.

2. Material and methods

2.1. Isolation of CD34$^+$ cells and granulocytes from the peripheral blood of CML subjects

Informed consent was obtained from 7 de novo subjects with CML included in the study. All subjects had signed the consent form approved by the local ethical committee. All studied de novo CML subjects were subject to 10 ml of peripheral blood draw on one occasion, collected in 10% sodium citrate. The maximum time interval between venepuncture and arrival in the laboratory was 2 h. Each 20 ml of diluted blood (1:1 with Ca^{2+}/Mg^{2+}-free PBS) was then layered gently on the top of 10 ml lymphocyte separation medium (LSM, PAA Laboratories GmbH, Pasching, Austria). After centrifugation (400 g, 30 min, 20 °C), the interface containing mononuclear cells was collected and washed with PBS. The CD34$^+$ cells were isolated from the collected mononuclear cells using a positive immunomagnetic separation (Super Macs II, Miltenyi Biotec, Bergisch Gladbach, Germany). Control CD34$^+$ cells were also isolated by positive immunomagnetic separation from 7 leukapheresis products of healthy donors (4 females, 3 males). The pellet formed during centrifugation with LSM was comprised mostly of erythrocytes and granulocytes that migrated through the gradient. The erythrocytes were removed by using lysing solution (0.15 M NH_4Cl, 0.1 mM Na_2EDTA, 12 mM $NaHCO_3$). High quality of purified granulocytes was confirmed by cytospin preparations and Wright–Giemsa staining. The viable CD34$^+$ cell and granulocyte counts were performed by trypan-blue exclusion technique (BioWhittaker). The purity of recovered cells was determined by flow cytometry using PE-anti-CD34 mAb (BD Biosciences, San Jose, CA, USA) and was over 80% in samples used for microarray analysis. Karyotype analyses confirmed the Philadelphia chromosome aberrations t(9:22)(q34:q11) in all examined CML subjects.

2.2. Isolation of total RNA

We use the RNeasy protocol for isolation of total RNA from CD34$^+$ cells and granulocytes according to the manufacturer's instructions (Qiagen GmbH, Hilden, Germany). Concentration and integrity of total RNA were assessed using the NanoDrop spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, Delaware, USA) and Agilent 2100 Bioanalyzer Software (Agilent Technologies, Waldbronn, Germany) comparing the ratio of 28S and 18S RNA peaks to ensure that there is minimal degradation of the RNA sample.

2.3. Microarray analysis

The human oligo probe set is purchased from Operon Human genome Array-Ready Oligo Set Version 4.0 (Eurofins MWG Operon, Huntsville, AL, USA) which contains 35,035 oligonucleotide probes, representing approximately 25,100 unique genes. The human version 4.0 is constructed based on the Ensemble human database build (NCBI-35c), with a full coverage on NCBI human Refseq dataset. We have followed the MIAME (minimum information about a microarray experiment) guidelines for the data presentation. Oligonucleotides were diluted in 150 mM sodium phosphate, pH 8.5, at 20 μM concentration for printing. Also, our prior experience with these primary cell cultures includes quantitative PCR with housekeeping genes (S16 and HPRT) to establish similar efficiency of cDNA synthesis and PCR (data not shown). In microarray studies, for determination of broad gene expression in CD34$^+$ cells, we analyzed 7 CML subjects in chronic phase (1 female and 6 males, average age 60) and 7 healthy subjects (4 females and 3 males, average age 48). For determination of gene expression in granulocytes we used 2 CML subjects (1 female and 1 male, average age 58) in chronic phase and 4 healthy subjects (2 females and 2 males, average age 51), that matched subjects used for isolation of CD34$^+$ cells.

2.4. Amplification of mRNA

We isolated a low quantity of CD34$^+$ cells (~2 × 10^6 cells) that correspond to low mRNA levels insufficient for microarray analysis, so we performed the amplification of total RNA using the Amino Allyl MessageAmp™ II aRNA Amplification kit (Life Technologies Corp., Carlsbad, CA, US). This amplification protocol was performed both in CD34$^+$ cells and granulocytes, for parallel studies, according to the manufacturer's instructions. We used 0.5 μg of total RNA from CML subjects for amplification. Briefly, 11 μl of total RNA was mixed with 1 μl of T7 dT primer and incubated at 70 °C for 5 min and quickly chilled for 3 min. Then, 8 μl Reverse Transcription Master Mix (10× First Strand Buffer, dNTP Mix, Rnase inhibitor, ArrayScript) was added and incubated for 2 h at 42 °C and quickly chilled. We added 80 μl Second Strand Master Mix (10× Second Strand Buffer, dNTP Mix, DNA polymerase, Rnase H) and incubated for 2 h at 16 °C. cDNA was purified by 250 μl cDNA Binding buffer and the mixture applied to the cDNA filter cartridge. After discharging the flow-through, 500 μl of washing buffer was added to the column, and centrifuged for 1 min at 10,000 rpm. cDNA was diluted with 18 μl of 55 °C preheated nuclease free water, mixed with 26 μl of in vitro transcription (IVT) Master Mix (aaUTP, ATP, CTP, GTP mix, UTP solution, 10× reaction buffer, T7 enzyme mix) and after 14 h incubation at 37 °C we added 60 μl of nuclease free water. Amino allyl-modified antisense RNA (aRNA) was purified with aRNA Binding buffer and ethanol, applied to the cDNA filter column and quantified by the NanoDrop spectrophotometer (Thermo Fisher Scientific Inc.). Vacuum dried 3 μg of aRNA was resuspended in 9 μl Coupling buffer, mixed with 11 μl Cy5 dye resuspended in DMSO and incubated 45 min at room temperature (RT) in the dark. After incubation, labeled aRNA was purified and eluted with 10 μl preheated nuclease free water by centrifugation.

2.5. Probe preparation

Total human universal RNA (HuURNA) isolated from a collection of adult human tissues to represent a broad range of expressed genes from both male and female donors (BD Biosciences, Palo Alto, CA) served as a universal reference control in the competitive hybridization. All examined CML and healthy control samples are hybridized against HuURNA. Briefly, 5 μg of HuURNA was incubated at 70 °C for 5 min along with 1 μl of aminoallyl-oligo dT primer and quickly chilled for 3 min. Then, 2 μl 10× first strand buffer, 1.5 μl SSII enzyme (Stratagene, La Jolla, CA), 1.5 μl 20× aminoallyl dUTP and 2 μl of 0.1 M DTT were added and incubated for 90 min at 42 °C. After incubation, volume of the reaction mixture was raised to 60 μl with 40 μl of DEPC water. cDNA was purified by the MinElute column (Qiagen) where 300 μl of Binding buffer PB was added to the coupled cDNA, and the mixture applied to the MinElute column, and centrifuged for 1 min at 10,000 rpm. After discharging the flow-through, 500 μl of washing buffer PE was added to the column, and centrifuged for 1 min at 10,000 rpm. The flow-through was discharged and the washing repeated. Then the columns were placed into a fresh Eppendorf tube and 10 μl elution buffer added to the membrane, incubated for 1 min at RT, centrifuged for 1 min at 10,000 rpm and probe collected. The probe was dried in speed-vac for 20 min. Finally, cDNA diluted in 10 μl of 2× coupling buffer was mixed with 10 μl of Cy3 dye (GE Healthcare Bio-Sciences Corp., Piscataway, NJ), diluted in DMSO, and incubated at RT in the dark for 90 min. After incubation, the volume was raised to 60 μl by 40 μl DEPC water and then cDNA was purified by the MinElute column and eluted with 10 μl elution buffer by centrifugation. Eluted cDNA probe and aRNA were combined in the final volume of 20 μl for hybridization.

2.6. Hybridization

For hybridization, the mixture of cDNA probe and aRNA was preheated at 100 °C for 2 min and centrifuged for 1 min at 10,000 rpm. 20 μl of preheated (42 °C) Ambion hybridization buffer (20× SSC and 10% SDS) is mixed with hybridization mixture. Total volume of the hybridization mixture was added on the array in slide and covered with cover slip. Slides were placed in MAUI hybridization chamber (BioMicro Systems, Inc., Salt Lake City, UT, USA) and incubated overnight at 42 °C. Slides were then washed each in 1× SSC and 0.1× SSC and spin-dried.

2.7. Data filtration, normalization and analysis

Microarray slides were scanned in both Cy3 (532 nm) and Cy5 (635 nm) channels using an Axon GenePix 4000B scanner (Axon Instruments, Inc., Foster City, CA) with a 10-μm resolution. Scanned microarray images were exported as TIFF files to GenePix Pro 3.0 software for image analysis. The raw images were collected at 16-bit/pixel resolutions with 0 to 65,535 count dynamic range. The area surrounding each spot image was used to calculate a local background and subtracted from each spot before the Cy5:Cy3 ratio calculation. The average of the total Cy3 and Cy5 signal gave a ratio that was used to normalize the signals. Each microarray experiment was globally normalized to make the median value of the log2-ratio equal to zero. The Loess normalization process corrects for dye bias, photo multiplier tube voltage imbalance and variations between channels in the amounts of the hybridized labeled cDNA probes. The data files representing the differentially expressed genes were then created. For advanced data analysis, gpr and jpeg files were imported into microarray database and normalized by software tools provided by the NIH Center for Information Technology (http://nciarray.nci.nih.gov/). Spots with a confidence interval of 99% (≥2 fold) with a fluorescence intensity of at least 150 for both channels and 30 μm spot size were considered as good quality spots for analysis. The complete results of our microarray experiments are available in the gene expression omnibus database (http://www.ncbi.nlm.nih.gov/geo; accession no. GSE55976) according to MIAME standards.

2.8. Statistical analysis

For microarray data management and analysis, we used NCI/CIT microArray database (mAdb) system. The one way ANOVA was applied using mAdb software for measurement of statistical significance in gene expression in CML. For mAdb hierarchical clustering we used uncentered correlation that applies a modified Pearson correlation equation. It is basically the same as the standard Pearson correlation function, except that it assumes that the means are 0.

3. Results

3.1. Comparison of gene expression between CML subjects and controls in CD34$^+$ cells and granulocytes

The t(9;22)(q34;q11) translocation was present in all CML subjects, with an average of 2012 CD34$^+$ cells/μl (SD ± 3158) and 56 × 10^9/l granulocytes (SD ± 44) in peripheral blood. In controls, the average number was 3.1 ± 1.4 CD34$^+$ cells/μl, but through leukapheresis we separated 5.7 × 10^5 CD34$^+$ cells per control subject. Within CML and control subjects the average correlation was high: CML — 0.89 and controls — 0.88 (Supplemental Table 1). Also, the average correlation coefficient was even higher among granulocytes: Controls — 0.93 and CML — 0.94. Using the Venn diagram we compared the total gene expression in CD34$^+$ cells from control and CML subjects before and after 50% filtration (Fig. 1A, B). The total gene expression in CD34$^+$ CML cells revealed 6457 genes, while after filtration of 50% the total gene expression was reduced to 3553 genes determined by microarray analysis (Fig. 1A, B). Before filtration, the total gene expression in granulocyte revealed 3947 genes, while after 50% filtration this number declined to 2701 genes (Fig. 1C, D). Therefore, the total gene expression was almost doubled in CD34$^+$ CML cells compared to granulocytes after 50% filtration (Fig. 1). The 64 genes overexpressed, more than 2-fold, exclusively in CML CD34$^+$ cells are presented in Table 1.

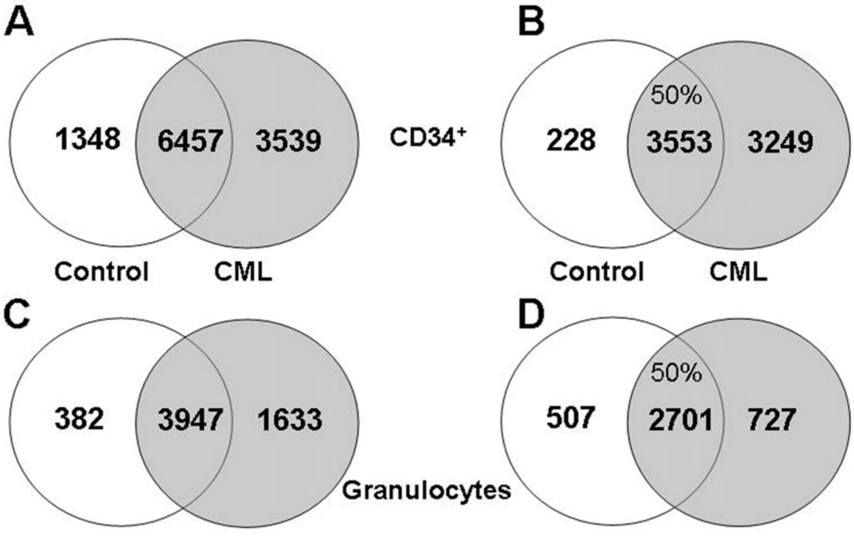


Fig. 1. Microarray study of gene expression in CD34$^+$ cells and granulocytes from peripheral blood. (A) The Venn diagram shows similarity of total gene expression between CML (N = 7) and control (N = 7) CD34$^+$ cells. (B) The Venn diagram shows similarity of gene expression between CML and control CD34$^+$ cells after 50% filtration. (C) The Venn diagram shows similarity of gene expression between CML and control granulocytes. (D) The Venn diagram shows similarity of gene expression between CML and control granulocytes after 50% filtration.

Among them, the genes with most prominent expression were *DEFA1/3* and *MPO* (Table 1). Genes overexpressed exclusively in CML granulocytes more than 2 fold were lectin galactoside-binding soluble 16 (*LGALS16*), hyperpolarization activated cyclic nucleotide gated potassium channel 3 (*HCN3*), chemokine (C–C motif) ligand 13 (*CCL13*), hect domain and RLD 2 pseudogene 4 (*HERC2P4*), SRSF protein kinase 1 (*SRPK1*), tripartite motif containing 69 (*TRIM69*), G-protein signaling modulator 1 (*GPSM1*) and natriuretic peptide receptor 2 (*NPR2*).

Table 1
Genes overexpressed exclusively in CML CD34$^+$ cells more than 2 fold.

Gene symbol	Description	Mean	SD
DEFA3	Defensin, alpha 3, neutrophil-specific	6.38	0.30
DEFA1	Defensin, alpha 1	6.35	0.30
MPO	Myeloperoxidase	4.63	1.39
HSH2D	Hematopoietic SH2 domain containing	3.96	0.24
FBXO4	F-box protein 4	3.65	0.47
MLLT3	Myeloid/lymphoid or mixed-lineage leukemia	3.47	0.42
SRSF7	Serine/arginine-rich splicing factor 7	3.47	0.40
CHST13	Carbohydrate (chondroitin 4) sulfotransferase 13	3.18	0.43
ZNF180	Zinc finger protein 180	3.10	0.38
LIMS1	LIM and senescent cell antigen-like domains 1	2.96	0.34
KCNH2	Potassium voltage-gated channel, subfamily H	2.94	1.12
PLAC8	Placenta-specific 8	2.90	0.79
TRMT2B	tRNA methyltransferase 2 homolog B	2.86	0.39
HTRA3	HtrA serine peptidase 3	2.84	0.95
GFI1	Growth factor independent 1 transcription repressor	2.83	1.10
PHF14	PHD finger protein 14	2.80	0.25
USP38	Ubiquitin specific peptidase 38	2.79	0.30
CASP6	Caspase 6, apoptosis-related cysteine peptidase	2.73	0.25
SLC39A8	Solute carrier family 39 (zinc transporter), member 8	2.72	0.57
ERLIN1	ER lipid raft associated 1	2.61	0.20
CD33	CD33 molecule	2.59	0.56
N4BP2L2	NEDD4 binding protein 2-like 2	2.57	0.41
CREB3L4	cAMP responsive element binding protein 3-like 4	2.55	0.34
RARRES3	Retinoic acid receptor responder	2.52	0.44
CASP3	Caspase 3, apoptosis-related cysteine peptidase	2.52	0.59
ASB8	Ankyrin repeat and SOCS box containing 8	2.51	0.33
CPT2	Carnitine palmitoyltransferase 2	2.49	0.43
MAGEH1	Melanoma antigen family H, 1	2.48	0.77
SBNO1	Strawberry notch homolog 1	2.48	0.37
CRBN	Cereblon	2.47	0.47
TMEM69	Transmembrane protein 69	2.45	0.33
KLF6	Kruppel-like factor 6	2.43	0.35
LSM10	LSM10, U7 small nuclear RNA associated	2.43	1.57
DCUN1D1	DCN1, defective in cullin neddylation 1, domain cont 1	2.37	0.33
FAM175A	Family with sequence similarity 175, member A	2.36	0.36
FBXW9	F-box and WD repeat domain containing 9	2.34	0.79
EEF1A1	Eukaryotic translation elongation factor 1 alpha 1	2.32	0.45
EED	Embryonic ectoderm development	2.32	0.42
AFF3	AF4/FMR2 family, member 3	2.28	0.39
OIP5	Opa interacting protein 5	2.26	0.60
THEM4	Thioesterase superfamily member 4	2.23	0.29
UBE2V2	Ubiquitin-conjugating enzyme E2 variant 2	2.19	0.27
MRPL19	Mitochondrial ribosomal protein L19	2.18	0.12
XK	X-linked Kx blood group (McLeod syndrome)	2.18	0.95
ISY1	ISY1 splicing factor homolog	2.17	0.21
MINPP1	Multiple inositol-polyphosphate phosphatase 1	2.16	0.53
USP48	Ubiquitin specific peptidase 48	2.16	0.33
PXMP2	Peroxisomal membrane protein 2, 22 kDa	2.14	0.39
BCCIP	BRCA2 and CDKN1A interacting protein	2.11	0.31
ARMCX1	Armadillo repeat containing, X-linked 1	2.10	0.39
CHEK1	Checkpoint kinase 1	2.08	0.53
BDH2	3-Hydroxybutyrate dehydrogenase, type 2	2.06	0.13
SP100	SP100 nuclear antigen	2.06	0.07
RNFT1	Ring finger protein, transmembrane 1	2.04	0.31
SFXN4	Sideroflexin 4	2.04	0.23
WDR5	WD repeat domain 5	2.03	0.39
WDR18	WD repeat domain 18	2.03	0.35
RNPC3	RNA-binding region	2.03	0.22
CD3EAP	CD3e molecule, epsilon associated protein	2.03	0.47
BTN3A2	Butyrophilin, subfamily 3, member A2	2.02	0.60
TMEM216	Transmembrane protein 216	2.02	0.49
ETNK1	Ethanolamine kinase 1	2.01	0.30
RPL34	Ribosomal protein L34	2.01	0.58
ALKBH2	alkB, alkylation repair homolog 2	2.00	0.23

3.2. Determination of significantly expressed genes in CD34$^+$ cells and granulocytes of CML compared to control subjects

We previously mentioned that CML and control CD34$^+$ cells shared 3553 common genes using the Venn diagram (Fig. 1 B). We compared these common genes by Student's t-test, and defined the significantly upregulated 41 genes in CML versus control CD34$^+$ cells ($p < 0.05$) (Table 2). The most significantly upregulated genes, in favor of CD34$^+$ CML cells, were *HINT1*, *TXN*, *SERBP1* and *RPL6* (Table 2). On the other hand, the most significantly downregulated genes in CML versus control CD34$^+$ cells, with more than 2.5 fold difference in gene expression, were *KCNQ1OT1*, *FREM2*, *PPP1R3F* and *MLLT4* (Table 3). Also, Student's t-test determined significant genes, presented by hierarchical clustering to describe their relation (Fig. 2). We also showed that granulocytes of CML subjects significantly expressed 39 genes in comparison to control subjects (Supplemental Table 2).

3.3. Signaling pathways and related gene expression affected by CML

Significantly upregulated expression of *E2F1*, *NFKBIA*, *TGFBR2* and *KRAS* in control subjects versus CML subjects was determined (Fig. 3A, Table 4), while significantly upregulated genes in CML subjects were *PTPN11*, *CTBP2*, *CDK4*, *CDK6* and *MYC* in CD34$^+$ cells (Fig. 3B, Table 4). The genes expressed only in CML subjects, in comparison to control (absent or sporadic), were *E2F3*, *NFKB1* and *MAPK1* (Fig. 3C, Table 4). Regarding Imatinib inhibition related genes, the *FOS* and *STAT1* genes were significantly decreased ($p < 0.01$) in CML compared to control subjects (Fig. 3D). PI3K/AKT and MAPK signaling pathways, affected by *BCR–ABL* mutation, promoted the CD34$^+$ cells proliferation and survival, while TGF-β signaling affected a growth of CD34$^+$ cells (Fig. 4). Significantly upregulated genes were *PTPN11*, *CDK4*/6 and *MYC*, while *KRAS*, *NFKBIA*, and *FOS* were downregulated in CD34$^+$ cells (Fig. 4, Table 4). *RUNX1* gene expression was upregulated both in CD34$^+$ cells and granulocytes of CML and controls (Table 4, Supplemental Table 2). *RAF1* gene expression, as part of MAPK signaling pathway, was upregulated both in CD34$^+$ cells and granulocytes of CML subjects (Table 4, Supplemental Table 2).

4. Discussion

The results of microarray study showed that the total gene expression of CD34$^+$ cells and granulocytes revealed 3553 and 2701 genes, respectively in CML. The genes with the most prominent expression in CD34$^+$ cells were *DEFA1/3*, *MPO*, *HSH2D*, *FBXO4*, *MLLT3*, *SRSF7*, *CHST13* and *ZNF180*, with induction more than 3 times. The genes overexpressed exclusively in CML granulocytes were *LGALS16*, *HCN3*, *CCL13*, *HERC2P4*, *SRPK1*, *TRIM69*, *GPSM1* and *NPR2*. Significantly downregulated genes in CML CD34$^+$ cells, with more than 2.5 fold difference in gene expression, were *KCNQ1OT1*, *FREM2*, *PPP1R3F* and *MLLT4*. PI3K/AKT and MAPK signaling pathway related genes were affected by *BCR–ABL* mutation, as well as TGF-β signaling. The significant difference was observed for *NFKBIA* and *CDK4/6* genes in PI3K/AKT activated signaling for cell proliferation, for *PTPN11*, *KRAS* and *FOS* genes in MAPK signaling, and for *TGFβR1/2* and *CTBP2* genes within TGF-β signaling in CD34$^+$ cells of CML subjects.

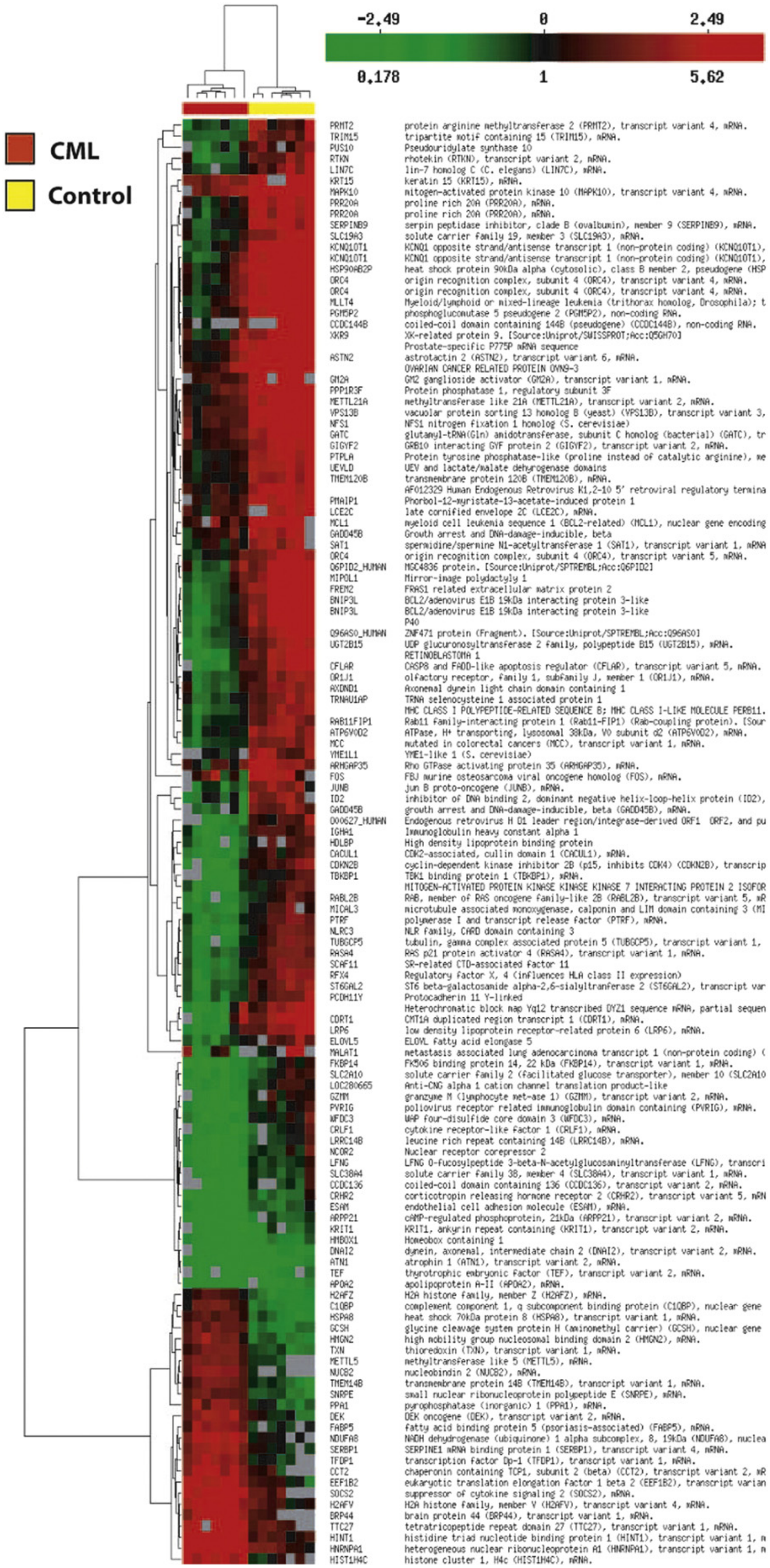
Previous microarray studies of chronic phase CML subjects analyzed mononuclear cells in the bone marrow [1,11,13] and peripheral blood [14], as well as CD34$^+$ progenitors in the bone marrow [4,5,15] and peripheral blood [6,9,10]. Also, comparative microarray analyses were performed in blast phase CML subjects [9–11]. We combined simultaneous microarray analyses of CD34$^+$ cells and granulocytes from

Table 2
Significantly upregulated genes in CML versus control CD34^{+} cells, with more than 2 fold difference in gene expression.

Symbol	Gene description	p value	Mean differ	Mean contr	SD	Mean CML	SD
HINT1	Histidine triad nucleotide binding protein 1	2.5E−07	2.25	0.68	0.39	2.93	0.32
TXN	Thioredoxin	2.1E−06	2.06	−0.84	0.38	1.22	0.44
SERBP1	SERPINE1 mRNA binding protein 1	4.5E−06	2.10	−0.09	0.34	1.93	0.39
RPL6	Ribosomal protein L6	6.5E−06	2.15	−1.08	0.44	1.07	0.42
RPLP0	Ribosomal protein, large, P0	1.1E−05	2.49	−1.72	0.49	0.77	0.46
HSPA8	Heat shock 70 kDa protein 8	1.1E−05	2.14	−1.26	0.45	0.88	0.32
RPS18	Ribosomal protein S18	1.2E−05	2.60	−0.05	0.46	2.54	0.49
GCSH	Glycine cleavage system protein H	1.4E−05	2.38	−1.19	0.25	1.19	0.30
H2AFZ	H2A histone family, member Z	1.7E−05	2.03	−1.47	0.42	0.56	0.40
H2AFV	H2A histone family, member V	1.8E−05	2.21	0.56	0.54	2.77	0.58
C1QBP	Complement component 1, q subcomp. binding protein	1.8E−05	2.54	−1.56	0.40	0.98	0.53
RPS12	Ribosomal protein S12	2.0E−05	2.13	−1.09	0.36	1.04	0.48
HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1	3.4E−05	2.21	0.94	0.58	3.16	0.34
RPLP1	Ribosomal protein, large, P1	5.5E−05	2.20	−1.12	0.44	1.08	0.48
HIST1H4C	Histone cluster 1, H4	6.2E−05	2.47	0.06	0.49	2.78	0.69
PPA1	Pyrophosphatase (inorganic) 1	6.5E−05	2.16	−0.66	0.46	1.50	0.53
NDUFA8	NADH dehydrogenase 1α subcomplex, 8, 19 kDa	6.5E−05	2.41	0.01	0.17	2.27	0.39
METTL5	Methyltransferase like 5	7.1E−05	2.05	−0.50	0.18	1.36	0.32
TFDP1	Transcription factor Dp-1	7.3E−05	2.19	−0.31	0.24	1.94	0.49
EEF1B2	Eukaryotic translation elongation factor 1β2	7.6E−05	2.27	0.12	0.65	2.39	0.35
HMGN2	High mobility group nucleosomal bind domain 2	8.8E−05	2.41	−1.06	0.29	1.35	0.58
RPL6	Ribosomal protein L6	0.0002	2.00	0.39	0.69	2.39	0.56
RPL7	Ribosomal protein L7	0.0003	2.00	0.13	0.60	2.13	0.55
DEK	DEK oncogene	0.0003	2.06	−0.30	0.60	1.76	0.51
SNRPE	Small nuclear ribonucleoprotein polypept E	0.0003	2.10	−0.99	0.54	1.11	0.25
RPL26	Ribosomal protein L26	0.0004	2.04	−1.09	0.69	0.95	0.30
NUCB2	Nucleobindin 2	0.0004	2.27	−0.38	0.10	1.63	0.42
RPSAP58	Ribosomal protein SA pseudogene 58	0.0005	2.06	−1.23	0.63	0.83	0.20
TMEM14B	Transmembrane protein 14B	0.0006	2.14	−0.89	0.68	1.25	0.41
MRPL1	Mitochondrial ribosomal protein L1	0.0006	2.25	0.97	0.12	2.90	0.41
RPS3A	Ribosomal protein S3A	0.0009	2.11	−0.78	0.96	1.33	0.28
TTC27	Tetratricopeptide repeat domain 27	0.0016	2.19	0.97	0.28	3.08	0.52
CCT2	Chaperonin containing TCP1, subunit 2	0.0017	2.13	0.23	0.62	2.17	0.46
FABP5	Fatty acid binding protein 5	0.0017	2.14	−0.37	0.22	1.69	0.53
RPS2	Ribosomal protein S2	0.0026	2.40	−0.32	1.25	2.08	0.43
RPS3A	Ribosomal protein S3A	0.0030	2.08	−0.07	0.94	2.01	0.50
SOCS2	Suppressor of cytokine signaling 2	0.0030	2.07	1.10	0.34	2.96	0.58
MCM5	Minichromosome maintenance complex comp 5	0.0032	2.04	0.37	0.11	2.55	0.29
RPS3A	Ribosomal protein S3A	0.0047	2.79	0.68	1.67	3.47	0.70
BRP44	Brain protein 44	0.0060	2.12	0.71	0.14	2.52	0.59
RPL7	Ribosomal protein L7	0.0065	2.04	0.25	1.16	2.29	0.40

Table 3
Significantly downregulated genes in CML versus control CD34^{+} cells, with more than 2.5 fold difference in gene expression.

Symbol	Gene description	p value	Mean differ	Mean contr	SD	Mean CML	SD
KCNQ1OT1	KCNQ1 opposite strand/antisense	5.3E−05	3.71	3.63	0.42	−0.08	0.61
FREM2	FRAS1 related extracellular matrix protein 2	0.0002	3.66	3.22	0.79	−0.43	0.88
PPP1R3F	Protein phosphatase 1, regul. subunit 3F	0.0003	3.60	4.35	0.67	0.75	0.67
MLLT4	Myeloid/lymphoid mixed-lineage leukemia	0.0004	3.56	3.88	0.73	0.32	0.96
ASTN2	Astrotactin 2	0.0001	3.45	4.05	0.61	0.60	0.79
BNIP3L	BCL2/adenovirus E1B interact protein 3-like	0.0002	3.41	2.85	0.88	−0.56	0.72
CDRT1	CMT1A duplicated region	0.0055	3.40	2.51	1.04	−0.94	1.79
HSP90AB2P	Heat shock protein 90 kDa alpha	0.0002	3.24	3.70	0.61	0.46	0.85
PGM5P2	Phosphoglucomutase 5 pseudogene 2	0.0001	3.23	3.39	0.66	0.16	0.65
ORC4	Origin recognition complex, sub 4	0.0002	3.21	3.55	0.68	0.34	0.80
MIPOL1	Mirror-image polydactyly 1	0.0004	3.17	2.90	0.98	−0.27	0.87
LRP6	Low density lipoprotein receptor-related protein 6	0.0010	3.16	1.98	0.78	−1.18	1.14
SERPINB9	Serpin peptidase inhibitor, clade B	0.0004	3.07	2.86	0.76	−0.21	0.47
CFLAR	CASP8 and FADD-like apoptosis regulator	0.0021	2.95	2.19	0.92	−0.86	0.94
MALAT1	Metastasis associated lung adenocarcinoma	0.0061	2.79	1.85	2.01	−0.86	1.80
PRR20A	Proline rich 20A	0.0019	2.75	2.81	0.84	0.07	0.55
CDKN2B	Cyclin-dependent kinase inhibitor 2B	0.0022	2.72	1.04	0.63	−1.66	0.96
UGT2B15	UDP glucuronosyltransferase 2 family B15	0.0003	2.71	1.88	0.64	−0.83	0.54
CCDC144B	Coiled-coil domain containing 144B	0.0087	2.69	3.19	0.41	0.55	0.72
RFX4	Regulatory factor X, 4	2.5E−05	2.60	1.48	0.49	−1.12	0.52
ID2	Inhibitor of DNA binding 2	0.0006	2.60	1.69	0.39	−0.78	0.66
GZMM	Granzyme M (lymphocyte met-ase 1)	0.0018	2.55	0.25	0.43	−2.31	0.44
ZNF215	Zinc finger protein 215	0.0001	2.55	1.33	0.47	−1.22	0.65
SLC19A3	Solute carrier family 19, member 3	0.0003	2.51	2.26	0.66	−0.25	0.52


-2.49
0
2.49
0.178
1
5.62
CML
Control
PRMT2 protein arginine methyltransferase 2 (PRMT2), transcript variant 4, mRNA.
TRIM15 tripartite motif containing 15 (TRIM15), mRNA.
PUS10 Pseudouridylate synthase 10
RTKN rhotekin (RTKN), transcript variant 2, mRNA.
LIN7C lin-7 homolog C (C. elegans) (LIN7C), mRNA.
KRT15 keratin 15 (KRT15), mRNA.
MAPK10 mitogen-activated protein kinase 10 (MAPK10), transcript variant 4, mRNA.
PRR20A proline rich 20A (PRR20A), mRNA.
PRR20A proline rich 20A (PRR20A), mRNA.
SERPINB9 serpin peptidase inhibitor, clade B (ovalbumin), member 9 (SERPINB9), mRNA.
SLC19A3 solute carrier family 19, member 3 (SLC19A3), mRNA.
KCNQ1OT1 KCNQ1 opposite strand/antisense transcript 1 (non-protein coding) (KCNQ1OT1),
KCNQ1OT1 KCNQ1 opposite strand/antisense transcript 1 (non-protein coding) (KCNQ1OT1),
HSP90AB2P heat shock protein 90kDa alpha (cytosolic), class B member 2, pseudogene (HSP
ORC4 origin recognition complex, subunit 4 (ORC4), transcript variant 4, mRNA.
ORC4 origin recognition complex, subunit 4 (ORC4), transcript variant 4, mRNA.
MLLT4 Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); t
PGM5P2 phosphoglucomutase 5 pseudogene 2 (PGM5P2), non-coding RNA.
CCDC144B coiled-coil domain containing 144B (pseudogene) (CCDC144B), non-coding RNA.
XKR9 XK-related protein 9. [Source:Uniprot/SWISSPROT;Acc:Q5GH70]
Prostate-specific P775P mRNA sequence
ASTN2 astrotactin 2 (ASTN2), transcript variant 6, mRNA.
OVARIAN CANCER RELATED PROTEIN OVN9-3
GM2A GM2 ganglioside activator (GM2A), transcript variant 1, mRNA.
PPP1R3F Protein phosphatase 1, regulatory subunit 3F
METTL21A methyltransferase like 21A (METTL21A), transcript variant 2, mRNA.
VPS13B vacuolar protein sorting 13 homolog B (yeast) (VPS13B), transcript variant 3,
NFS1 NFS1 nitrogen fixation 1 homolog (S. cerevisiae)
GATC glutamyl-tRNA(Gln) amidotransferase, subunit C homolog (bacterial) (GATC), tr
GIGYF2 GRB10 interacting GYF protein 2 (GIGYF2), transcript variant 2, mRNA.
PTPLA Protein tyrosine phosphatase-like (proline instead of catalytic arginine), me
UEVLD UEV and lactate/malate dehyrogenase domains
TMEM120B transmembrane protein 120B (TMEM120B), mRNA.
AF012329 Human Endogenous Retrovirus K1,2-10 5′ retroviral regulatory termina
PMAIP1 Phorbol-12-myristate-13-acetate-induced protein 1
LCE2C late cornified envelope 2C (LCE2C), mRNA.
MCL1 myeloid cell leukemia sequence 1 (BCL2-related) (MCL1), nuclear gene encoding
GADD45B Growth arrest and DNA-damage-inducible, beta
SAT1 spermidine/spermine N1-acetyltransferase 1 (SAT1), transcript variant 1, mRNA
ORC4 origin recognition complex, subunit 4 (ORC4), transcript variant 5, mRNA.
Q6PID2_HUMAN MGC4836 protein. [Source:Uniprot/SPTREMBL;Acc:Q6PID2]
MIPOL1 Mirror-image polydactyly 1
FREM2 FRAS1 related extracellular matrix protein 2
BNIP3L BCL2/adenovirus E1B 19kDa interacting protein 3-like
BNIP3L BCL2/adenovirus E1B 19kDa interacting protein 3-like
P40
Q96AS0_HUMAN ZNF471 protein (Fragment). [Source:Uniprot/SPTREMBL;Acc:Q96AS0]
UGT2B15 UDP glucuronosyltransferase 2 family, polypeptide B15 (UGT2B15), mRNA.
RETINOBLASTOMA 1
CFLAR CASP8 and FADD-like apoptosis regulator (CFLAR), transcript variant 5, mRNA.
OR1J1 olfactory receptor, family 1, subfamily J, member 1 (OR1J1), mRNA.
AXDND1 Axonemal dynein light chain domain containing 1
TRNAU1AP TRNA selenocysteine 1 associated protein 1
MHC CLASS I POLYPEPTIDE-RELATED SEQUENCE B; MHC CLASS I-LIKE MOLECULE PERB11.
RAB11FIP1 Rab11 family-interacting protein 1 (Rab11-FIP1) (Rab-coupling protein). [Sour
ATP6V0D2 ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d2 (ATP6V0D2), mRNA.
MCC mutated in colorectal cancers (MCC), transcript variant 1, mRNA.
YME1L1 YME1-like 1 (S. cerevisiae)
ARHGAP35 Rho GTPase activating protein 35 (ARHGAP35), mRNA.
FOS FBJ murine osteosarcoma viral oncogene homolog (FOS), mRNA.
JUNB jun B proto-oncogene (JUNB), mRNA.
ID2 inhibitor of DNA binding 2, dominant negative helix-loop-helix protein (ID2),
GADD45B growth arrest and DNA-damage-inducible, beta (GADD45B), mRNA.
O00627_HUMAN Endogenous retrovirus H D1 leader region/integrase-derived ORF1 ORF2, and pu
IGHA1 Immunoglobulin heavy constant alpha 1
HDLBP High density lipoprotein binding protein
CACUL1 CDK2-associated, cullin domain 1 (CACUL1), mRNA.
CDKN2B cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4) (CDKN2B), transcrip
TBKBP1 TBK1 binding protein 1 (TBKBP1), mRNA.
MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 7 INTERACTING PROTEIN 2 ISOFOR
RABL2B RAB, member of RAS oncogene family-like 2B (RABL2B), transcript variant 5, mR
MICAL3 microtubule associated monoxygenase, calponin and LIM domain containing 3 (MI
PTRF polymerase I and transcript release factor (PTRF), mRNA.
NLRC3 NLR family, CARD domain containing 3
TUBGCP5 tubulin, gamma complex associated protein 5 (TUBGCP5), transcript variant 1,
RASA4 RAS p21 protein activator 4 (RASA4), transcript variant 1, mRNA.
SCAF11 SR-related CTD-associated factor 11
RFX4 Regulatory factor X, 4 (influences HLA class II expression)
ST6GAL2 ST6 beta-galactosamide alpha-2,6-sialyltranferase 2 (ST6GAL2), transcript var
PCDH11Y Protocadherin 11 Y-linked
Heterochromatic block map Yq12 transcribed DYZ1 sequence mRNA, partial sequen
CDRT1 CMT1A duplicated region transcript 1 (CDRT1), mRNA.
LRP6 low density lipoprotein receptor-related protein 6 (LRP6), mRNA.
ELOVL5 ELOVL fatty acid elongase 5
MALAT1 metastasis associated lung adenocarcinoma transcript 1 (non-protein coding) (
FKBP14 FK506 binding protein 14, 22 kDa (FKBP14), transcript variant 1, mRNA.
SLC2A10 solute carrier family 2 (facilitated glucose transporter), member 10 (SLC2A10
LOC280665 Anti-CNG alpha 1 cation channel translation product-like
GZMM granzyme M (lymphocyte met-ase 1) (GZMM), transcript variant 2, mRNA.
PVRIG poliovirus receptor related immunoglobulin domain containing (PVRIG), mRNA.
WFDC3 WAP four-disulfide core domain 3 (WFDC3), mRNA.
CRLF1 cytokine receptor-like factor 1 (CRLF1), mRNA.
LRRC14B leucine rich repeat containing 14B (LRRC14B), mRNA.
NCOR2 Nuclear receptor corepressor 2
LFNG LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase (LFNG), transcri
SLC38A4 solute carrier family 38, member 4 (SLC38A4), transcript variant 1, mRNA.
CCDC136 coiled-coil domain containing 136 (CCDC136), transcript variant 2, mRNA.
CRHR2 corticotropin releasing hormone receptor 2 (CRHR2), transcript variant 5, mRN
ESAM endothelial cell adhesion molecule (ESAM), mRNA.
ARPP21 cAMP-regulated phosphoprotein, 21kDa (ARPP21), transcript variant 2, mRNA.
KRIT1 KRIT1, ankyrin repeat containing (KRIT1), transcript variant 2, mRNA.
HMBOX1 Homeobox containing 1
DNAI2 dynein, axonemal, intermediate chain 2 (DNAI2), transcript variant 2, mRNA.
ATN1 atrophin 1 (ATN1), transcript variant 2, mRNA.
TEF thyrotrophic embryonic factor (TEF), transcript variant 2, mRNA.
APOA2 apolipoprotein A-II (APOA2), mRNA.
H2AFZ H2A histone family, member Z (H2AFZ), mRNA.
C1QBP complement component 1, q subcomponent binding protein (C1QBP), nuclear gene
HSPA8 heat shock 70kDa protein 8 (HSPA8), transcript variant 1, mRNA.
GCSH glycine cleavage system protein H (aminomethyl carrier) (GCSH), nuclear gene
HMGN2 high mobility group nucleosomal binding domain 2 (HMGN2), mRNA.
TXN thioredoxin (TXN), transcript variant 1, mRNA.
METTL5 methyltransferase like 5 (METTL5), mRNA.
NUCB2 nucleobindin 2 (NUCB2), mRNA.
TMEM14B transmembrane protein 14B (TMEM14B), transcript variant 1, mRNA.
SNRPE small nuclear ribonucleoprotein polypeptide E (SNRPE), mRNA.
PPA1 pyrophosphatase (inorganic) 1 (PPA1), mRNA.
DEK DEK oncogene (DEK), transcript variant 2, mRNA.
FABP5 fatty acid binding protein 5 (psoriasis-associated) (FABP5), mRNA.
NDUFA8 NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 8, 19kDa (NDUFA8), nuclea
SERBP1 SERPINE1 mRNA binding protein 1 (SERBP1), transcript variant 4, mRNA.
TFDP1 transcription factor Dp-1 (TFDP1), transcript variant 1, mRNA.
CCT2 chaperonin containing TCP1, subunit 2 (beta) (CCT2), transcript variant 2, mR
EEF1B2 eukaryotic translation elongation factor 1 beta 2 (EEF1B2), transcript varian
SOCS2 suppressor of cytokine signaling 2 (SOCS2), mRNA.
H2AFV H2A histone family, member V (H2AFV), transcript variant 4, mRNA.
BRP44 brain protein 44 (BRP44), transcript variant 1, mRNA.
TTC27 tetratricopeptide repeat domain 27 (TTC27), transcript variant 1, mRNA.
HINT1 histidine triad nucleotide binding protein 1 (HINT1), transcript variant 1, m
HNRNPA1 heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1), transcript variant 1, m
HIST1H4C histone cluster 1, H4c (HIST1H4C), mRNA.

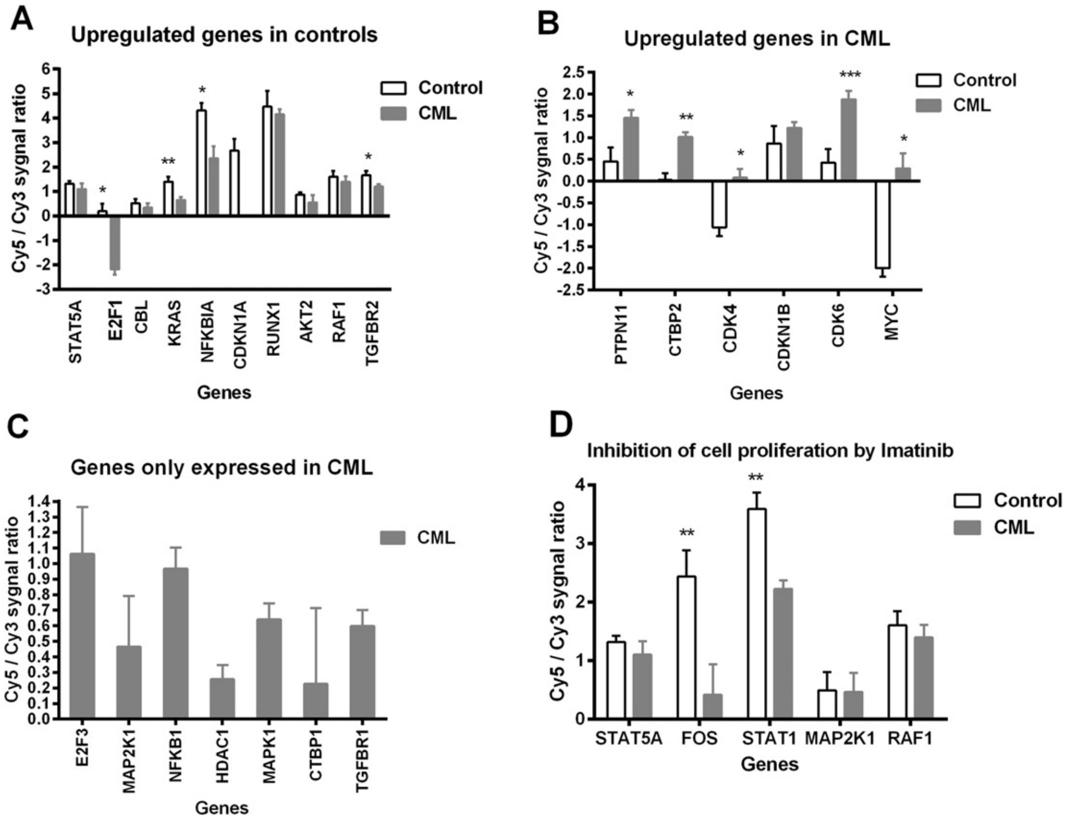


Fig. 3. *BCR–ABL* activated gene expression profile in CD34$^+$ cells of CML. (A) Upregulated genes in controls. (B) Upregulated genes in CML. (C) Genes expressed only in CML. (D) Inhibition of cellular proliferation after Imatinib therapy.

peripheral blood of chronic phase CML subjects. Comparing the controls and CML CD34$^+$ cells we found that 64 genes were overexpressed exclusively in CML CD34$^+$ cells more than 2 fold. Their products are involved in the regulation of different cellular functions including cell cycle (*WDR5*, *WDR18*), apoptosis (*CASP3*, *CASP6*), tumor suppression (*ARMCX1*), and regulation of transcription (*ZNF180*). Expanded microarray analysis of granulocytes revealed a significant difference in expression pattern of 39 genes between CML and healthy donors. In contrast to presented *DEFA1* and *DEFA3* upregulated gene expression in CD34$^+$ cells of chronic phase CML subjects, genes responsible for anti-pathogen response (*DEFA1*, *DEFA3*, *DEFA4*) were downregulated in blast phase CML cells [13].

The largest difference between chronic phase CML subjects and normal donors was obvious in CD34$^+$ cells, including the downregulation of genes encoding inhibitors of cell proliferation in chronic phase CML [15]. Among genes linked to the inhibition of cellular proliferation by Gleevec: *STAT1* and *FOS* had significantly decreased expression in CML, whereas *MAP2K1* and RAF1 had very similar level of expression in CML and controls. The following genes *CDK4*, *CDK6*, *MYC*, *CTBP2*, and *PTPN11* had increased expression and *NFKBIA*, *E2F1*, *KRAS* and *TGFBR2* genes had reduced expression in CML-associated genes of CD34$^+$ cells. Extracellular-regulated kinase (ERK) was significantly upregulated in primary *BCR–ABL*-positive cells (MAPK1). Regarding genes involved in the PI3K pathway, its substrate AKT and the downstream molecules NFκB and Bcl-xl were significantly upregulated in CML CD34$^+$ cells [4]. According to our data, *AKT* and *Bcl-xl* had decreased expression while *NFKB*1 had increased expression in CML CD34$^+$ cells, but no significant difference was observed. Moreover, the upregulation has been reported in CD34$^+$ compartment of proteins within the STAT pathway and MYC, and downregulation of MDM2, MEK, AKT and NFκB proteins [16]. It has been reported that the *BCR–ABL* adapter protein CRKL has also been upregulated in CML CD34$^+$ cells, but not in our results at mRNA level. Within the TGF-β signaling pathway, other report has shown that *TGF-β1* itself as well as *SMAD2* and *SMAD4* were significantly upregulated in CML CD34$^+$ cells, in contrast to our results [4].

The bone marrow CD34$^+$ cells expressed 9 cell cycle driving genes at particularly higher levels than circulating CD34$^+$ cells [17]. According to those results cycling activity of bone marrow CD34$^+$ cells was higher than in peripheral blood CD34$^+$ cells. We found a significant downregulation of *FOS* in CML CD34$^+$ cells. FOS had a role in growth suppression and apoptosis of many cell types. Therefore, the downregulation of FOS might be liable for increased proliferation of CML CD34$^+$ cells [5]. In accordance to our results, the upregulation of *CDK4* gene expression has been reported in CML, as a gene involved in cell cycle [13]. The upregulation of the E2F1 transcription factor lead to a molecular mechanism that initiated the proliferation of hematopoietic stem and progenitor cells [17]. The cell cycle-initiating transcription factor E2F1, that

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Fig. 2. Hierarchical clustering of genes expressed in CML and control CD34$^+$ cells. Hierarchical clustering of statistically significant different ($p < 0.05$) gene expression, between CML and control CD34$^+$ cells, determined by Student's t-test. The color indicates the relative fold expression of each gene: red indicates increased expression, green negative expression, black not changed expression, while gray stands for absent expression per each examined sample. The total gene expression of CML and control CD34$^+$ cells is also clustered (upper image), representing similarities among examined cells. The genes and arrays correlations are uncentered. The gene description is provided in Table 3

Table 4
BCR–ABL activated signaling pathway related genes in CD34$^+$ cells of CML and healthy control origin.

Symbol	Gene description	p value	Mean differ	Mean contr	SD	Mean CML	SD
AKT2	v-akt murine thymoma viral oncogene homolog 2			0.87	0.18	0.55	0.68
CBL	Cbl proto-oncogene, E3 ubiquitin protein ligase			0.52	0.43	0.34	0.42
CDK4	Cyclin-dependent kinase 4	0.02	−1.58	−1.06	0.29	0.08	0.50
CDK6	Cyclin-dependent kinase 6	0.003	−1.42	0.42	0.63	1.88	0.47
CDKN1A	Cyclin-dependent kinase inhibitor 1A			2.66	0.69		
CDKN1B	Cyclin-dependent kinase inhibitor 1B			0.87	0.9	1.23	0.31
CRK	v-crk sarcoma virus CT10 oncogene homolog			1.5	0	−0.08	0.42
CTBP1	C-terminal binding protein 1			−0.42	0	0.23	1.09
CTBP2	C-terminal binding protein 2	0.007	−1.08	0.03	0.31	1.01	0.27
E2F1	E2F transcription factor 1	0.03	2.00	0.19	0.69	−2.18	0.44
E2F3	E2F transcription factor 3			0.65	0	1.06	0.74
FOS	FBJ murine osteosarcoma viral oncogene homolog	0.007	1.79	2.46	0.87	0.42	1.16
GRB2	Growth factor receptor-bound protein 2					1.8	0.19
HDAC1	Histone deacetylase 1					0.26	0.21
HDAC2	Histone deacetylase 2					−0.5	0.14
KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	0.003	1.06	1.4	0.51	0.34	0.31
MAP2K1	Mitogen-activated protein kinase kinase 1			0.8	0	0.46	0.57
MAPK1	Mitogen-activated protein kinase 1					0.64	0.18
MYC	v-myc myelocytomatosis viral oncogene homolog	0.018	−2.93	−2.0	0.27	0.29	0.85
NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1			0.57	0	0.97	0.30
NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, α	0.02	1.95	4.31	0.72	2.36	1.21
PIK3CB	Phosphoinositide-3-kinase, catalytic, β polypeptide					1.18	0.13
PIK3R1	Phosphoinositide-3-kinase, regulatory subunit 1			0.72	0	1.34	0.02
PTPN11	Protein tyrosine phosphatase, non-receptor type 11	0.04	−1.14	0.45	0.46	1.46	0.43
RAF1	v-raf-1 murine leukemia viral oncogene homolog 1			2.06	0	1.66	0.32
RUNX1	runt-related transcription factor 1			4.47	1.1	4.15	0.56
STAT1	Signal transducer and activator of transcription 1	0.003	1.30	3.6	0.55	2.22	0.36
STAT5A	Signal transducer and activator of transcription 5A			1.32	0.24	1.10	0.57
TGFBR1	Transforming growth factor, β receptor 1					0.6	0.23
TGFBR2	Transforming growth factor, β receptor II	0.023	0.48	1.67	0.18	1.20	0.14

promoted cell cycle progression, showed higher expression in bone marrow CD34$^+$ cells than in peripheral blood CD34$^+$ cells [18]. In our study, *E2F1* gene expression was downregulated in CML CD34$^+$ cells compared to control CD34$^+$ cells.

Results of this study highlighted the difference in gene expression between primitive and mature cells of CML and control subjects, with the accent on the CD34$^+$ cells that direct the pathogenic course of malignancy. The presence of *BCR–ABL* fusion gene significantly modified the observed genes in PI3K/AKT, MAPK and TGF-β signaling pathways, enhancing its influence on CD34$^+$ cell proliferation, apoptosis and cell growth.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bcmd.2015.08.002.

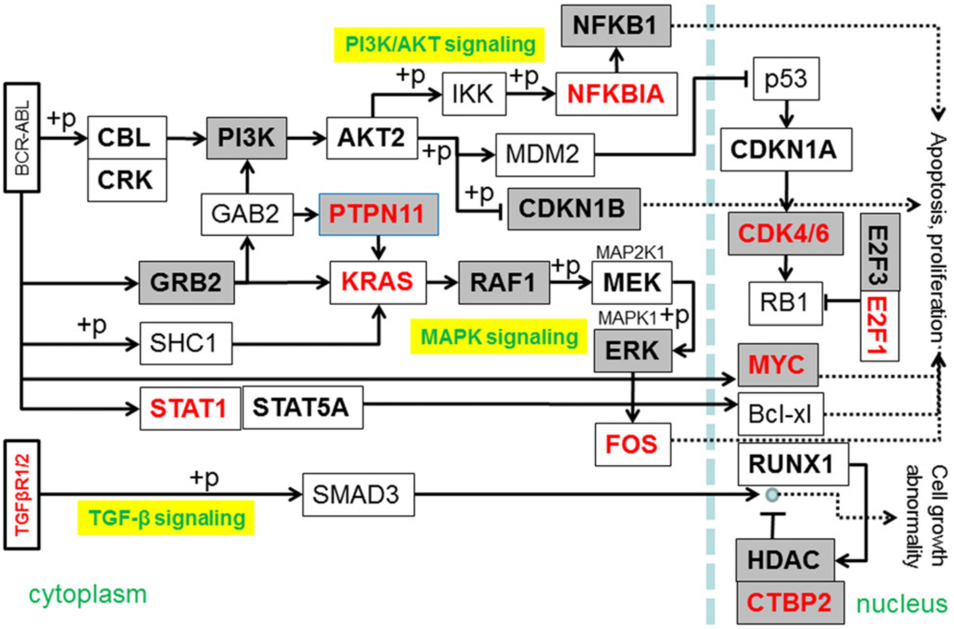

Fig. 4. *BCR–ABL* activated signaling pathways in CD34$^+$ cells of CML origin. (+p) phosphorylation; → stimulation, ⊥ inhibition; dotted lines define gene function; bolded gene symbols in empty boxes represent downregulated genes, while in gray boxes represent upregulated genes in CML vs. controls (corresponding to Table 4). Non-bolded gene symbols in empty boxes represent unexpressed or sporadically expressed genes. Red bolded gene symbols represent significantly different genes in CML vs. controls.

Acknowledgments

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