

Differential expression and alternative splicing of cell cycle genes in imatinib-treated K562 cells

Jing Liu¹ · Jin Lin¹ · Lin-Feng Huang¹ · Bo Huang¹ · Yan-Mei Xu¹ · Jing Li² · Yan Wang¹ · Jing Zhang¹ · Wei-Ming Yang¹ · Qing-Hua Min¹ · Xiao-Zhong Wang¹

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Abstract Cancer progression often involves the disorder of the cell cycle, and a number of effective chemotherapeutic drugs have been shown to induce cell cycle arrest. The purpose of this study was to comprehensively investigate the effects of imatinib on the expression profile of cell cycle genes in the chronic myeloid leukemia (CML) K562 cell line. In addition, we also investigated alternative splicing of the cell cycle genes affected by imatinib, since an important relationship has been shown to exist between RNA splicing and cell cycle progression. Exon array analysis was performed using total RNA purified from normal and imatinib-treated K562 cells. We identified 185 differentially expressed genes and 277 alternative splicing events between the two cell groups. A detailed analysis by reverse transcription-PCR (RT-PCR) of key genes confirmed the experimental results of the exon array. These results suggested that treatment of K562 cells with imatinib shifts the expression and alternative splicing profiles of several cell cycle-related genes. Importantly, these findings may help improve imatinib treatment strategies in patients with CML and may be useful for imatinib resistance research and CML drug development.

Keywords Imatinib · K562 · Chronic myeloid leukemia · Cell cycle · Gene expression profile · Alternative splicing

Introduction

The traditional subdivisions of the standard cell cycle are divided into four main phases designated as G₁, S, G₂, and M [1]. In addition, a fifth state, called G₀, is a quiescent state that accounts for the majority of non-proliferating cells in the human body. The cell cycle is controlled by numerous mechanisms that ensure correct cell division [2]. Previous studies have demonstrated that cell cycle abnormalities are closely related to many cancers [3]. Consequently, targeting components of the cell cycle, such as cyclin-dependent kinases (CDKs), presents unique opportunities for drug discovery, and a number of potential molecular targets for novel anticancer agents have been identified in cell cycle control mechanisms [4].

Imatinib is a tyrosine kinase inhibitor that specifically targets the breakpoint cluster region-Abelson murine leukemia (BCR-ABL) fusion protein and was originally developed in the 1990s for treating chronic myeloid leukemia (CML) and some rare forms of stomach cancer. Imatinib has been shown to potentially inhibit proliferation and induce apoptosis in BCR-ABL⁺ cells. In addition, a number of studies have reported the effects of imatinib on cell cycle-related genes in other types of cancers [5–8]. However, to date, no studies have comprehensively investigated the effect of imatinib on cell cycle-related genes.

Most human genes produce multiple mRNA isoforms through alternative splicing, which produces multiple different transcripts from one gene. Recent studies suggest that up to 94 % of all human genes undergo alternative splicing, and aberrant pre-mRNA splicing is a critical

Jing Liu and Jin Lin contributed equally to this paper.

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✉ Xiao-Zhong Wang
wangxiaozhong@ncu.edu.cn

¹ Department of Clinical Laboratory, The Second Affiliated Hospital of Nanchang University, No. 1 Min De Road, Nanchang 330006, China

² Department of Clinical Laboratory, The First Affiliated Hospital of Nanchang University, Nanchang 330006, China

Table 1 The primers and amplification conditions used in this study

Gene name	Sequence (5'→3') forward reverse	Reaction condition	Splicing isoform	Product size (bp)
CENPE	AAAACCTGCTCGTATGCTAT GTGGTTCTGTCGGTCTCTA	35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min	–	441
SLC4A1	CCGCCCCGACCTCTCTCAC GCCTCACGAAGCCCAGCAC	35 cycles at 94 °C for 30 s, 61.8 °C for 30 s, and 72 °C for 1 min	–	455
TBX3	CCCGAAGAAGAGGTGGAGGACGAC TCTTCGGCCATTTCAGTGTCCCG	35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min	Variant L Variant S	651 591
PLCB1	AGCAGATCCTGGATGAAAAGCCCA TCCGATGACGCTGGAGTGGC	35 cycles at 94 °C for 30 s, 64 °C for 30 s, and 72 °C for 1 min	Variant L Variant S	423 305
β-actin	CGGGAAATCGTGCGTGAC TGAAGGTGGACAGCGAGG	35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min	–	443

component of the pathogenesis of neoplasias [9]. Not surprisingly, genes involved in the cell cycle also undergo alternative splicing and may play important roles in tumor development; however, little is known about the role of precise splicing in cell cycle progression [10, 11].

In our previous study, we have used the Affymetrix Human Exon 1.0 ST array which contains almost 5.4 million probes groups into 1.4 million probe sets for interrogating over 1 million exon clusters to investigate the different splices of apoptotic genes between K562 cells and imatinib-treated K562 cells. As the same, this powerful tool has used to identify that genes and exons are differentially expressed in normal and imatinib-treated K562 cells in this study. Through combining exon array and several special commercial softwares for gene level expression analysis, we complete this study conveniently and persuasively.

Materials and methods

We have done similar work in our previous study, so a brief introduction of methods of this study was given in this paper. First, human myeloid leukemia cell line K562 were cultured in Roswell Park Memorial Institute 1640 (RPMI-1640; Hyclone, USA) media supplemented with 10 % fetal bovine serum (FBS; Hyclone) in a 5 % CO₂ humidified atmosphere at 37 °C, and divided into two groups which include imatinib-treated cells and untreated controls. Second, total RNA was extracted from 10⁷ K562 cells of treatment and control groups using the TRIzol reagent (Tiangen, China) following the manufacturer's instructions. RNA samples were labeled according to the GeneChip Whole Transcript (WT) Sense Target Labeling Assay as provided by the manufacturer (<http://www.affymetrix.com>) and hybridized to the Human Exon

Table 2 Functional classification of differentially expressed genes

Annotation cluster	Enrichment score	GO ID	GO term	Count	Percent (%)	<i>p</i> _value
1	83.16	0022402	Cell cycle process	109	58.92	6.87E-106
		0022403	Cell cycle phase	92	49.73	1.58E-92
		0000279	M-phase	75	40.54	3.14E-74
		0051301	Cell division	67	36.22	2.16E-65
		0007067	Mitosis	62	33.51	1.96E-66
2	21.49	0000082	G1/S transition of mitotic cell cycle	14	7.57	2.11E-13
3	12.62	0051726	Regulation of cell cycle	52	28.11	4.37E-41
		0000075	Cell cycle checkpoint	24	12.97	8.16E-24
4	10.56	0006974	Response to DNA damage stimulus	28	15.14	3.01E-13
		0006281	DNA repair	18	9.73	1.61E-07
5	10.53	0031570	DNA integrity checkpoint	15	8.11	2.54E-15
		0000077	DNA damage checkpoint	14	7.57	2.35E-14

Please refer to Supplemental Table 1 for the gene probe IDs of genes that were included in each group

1.0 ST arrays for 16 h according to the Affymetrix protocol. Third, data was analyzed by easyExon software (<http://microarray.ym.edu.tw/easyexon>) and the Affymetrix Power Tools (APT) package. Differentially expressed genes ($p < 0.05$) between the two cell groups were functionally annotated and classified by using the functional annotation tool of database for annotation, visualization, and integrated discovery (DAVID) <http://david.abcc.ncifcrf.gov/> [12], which provides integrated solutions for the annotation and analysis of genome-scale datasets derived from high-throughput technologies [13]. Last, reverse transcription-PCR (RT-PCR) was used to confirm the differentially expressed and alternatively spliced genes detected by the Human Exon 1.0 ST arrays. The primers and reaction conditions for each transcript are shown in Tables 1 and 2. Images of the gels were analyzed by Quantity One 4.6.2 software (Bio-Rad, USA). For gene analysis, ratios of gray values between the target gene and β -actin were calculated, and for exon analysis, the ratios of gray values between splice variants were calculated in untreated and imatinib-treated cells, respectively. For the detail of those methods, refer to our previous study [1].

Results

Gene level expression differences induced by imatinib treatment

Using the easyExon software, we conducted a t test filtration at the gene level of the two K562 cell groups. No other information was needed. For selecting differentially expressed genes, the criteria for probe set filtration were set as follows: fold change (FC) ≥ 2 or ≤ 0.5 with a statistical significance of $p < 0.05$ (calculated by a student's t test). Based on these criteria, we identified 2193 transcripts with expression patterns that were significantly different between control and imatinib-treated K562 cells.

The transcript cluster filtration tool of easyExon was used to reduce the number of positive hits based on (i) GO term and (ii) gene name or accession number. Using GO term “cell cycle” for filtration, we obtained a gene list that contained 185 cell cycle-related genes, of which 147 (79.46 %) were down-regulated and 38 (20.54 %) were up-regulated. The number of genes exhibiting decreased expression was far greater than those having increased expression. Based on FC values,

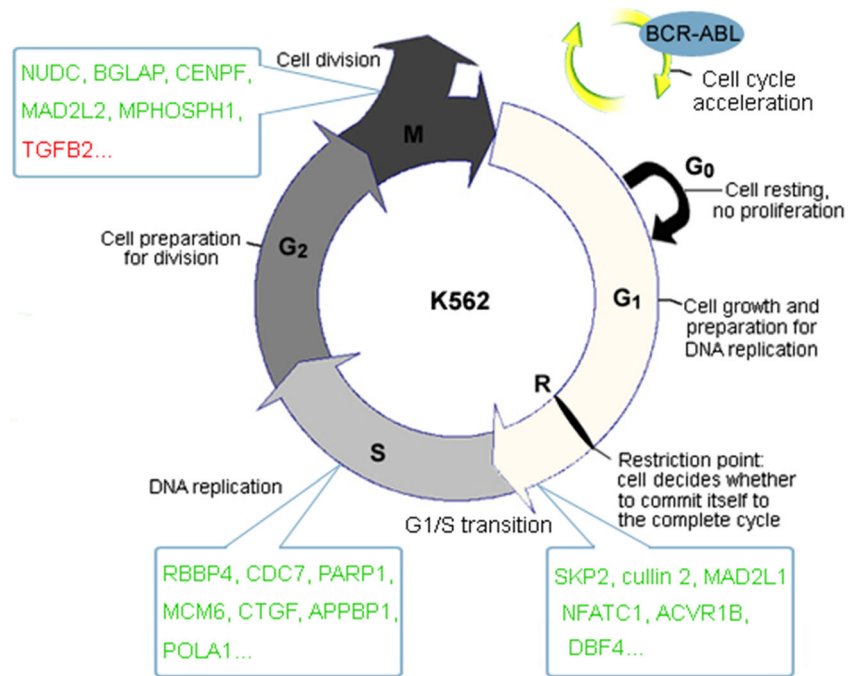
Table 3 Cell cycle-related genes that were significantly changed in K562 cells in response to imatinib treatment (the 10 most up-regulated and the 10 most down-regulated genes)

Gene probe ID	^a Fold change	Gene title	Gene symbol	Accession no.
2780172	0.14	Centromere protein E, 312 kDa	CENPE	NM_001813
3599811	0.16	Kinesin family member 23	KIF23	NM_138555
3105467	0.17	E2F transcription factor 5, p130-binding	E2F5	NM_001951
2783715	0.19	MAD2 mitotic arrest deficient-like 1 (yeast)	MAD2L1	NM_002358
3368304	0.19	Wilms tumor 1	WT1	NM_024424
3565663	0.19	Disks, large homolog 7 (Drosophila)	DLG7	NM_014750
3750785	0.20	Sperm associated antigen 5	SPAG5	NM_006461
2489545	0.20	Hexokinase 2	HK2	NM_000189
2914777	0.20	TTK protein kinase	TTK	NM_003318
2333136	0.20	Cell division cycle 20 homolog (<i>S. cerevisiae</i>)	CDC20	NM_001255
2976768	3.95	CBP/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	CITED2	NM_006079
2902444	3.99	Allograft inflammatory factor 1	AIF1	NM_001623
3963754	5.14	Structural maintenance of chromosomes 1B	SMC1B	NM_148674
2415084	5.21	Jun oncogene	JUN	NM_002228
3359432	5.66	Cyclin-dependent kinase inhibitor 1C (p57, Kip2)	CDKN1C	NM_000076
2887309	6.41	Dual specificity phosphatase 1	DUSP1	NM_004417
3387259	9.94	Sestrin 3	SESN3	NM_144665
2372858	13.96	Regulator of G-protein signaling 2, 24 kDa	RGS2	NM_002923
3836266	26.38	FBJ murine osteosarcoma viral oncogene homolog B	FOSB	NM_006732
3759006	59.05	Solute carrier family 4, anion exchanger, member 1 (erythrocyte membrane protein band 3, Diego blood group)	SLC4A1	NM_000342

Please refer to Supplemental Table 2 for a more comprehensive list of differentially expressed cell cycle-related genes in K562 cells treated with imatinib

^a Gene expression levels were expressed as the mean of fold change, which was calculated as the ratio between the average values of expression in imatinib-treated K562 cells relative to the average values of control K562 cells. Fold change > 2.0 , up-regulated; Fold change < 0.5 , down-regulated

Fig. 1 The cell cycle stages and differentially expressed cell cycle genes of imatinib-treated K562 cells. BCR-ABL can accelerate K562 cell cycle, which can be specifically inhibited by imatinib. Some genes involved in key steps of the K562 cell cycle are listed in the chart in *red* (up-regulation) and *green* (down-regulation)



the 10 most up-regulated and the 10 most down-regulated genes are shown in Table 3.

Previous studies have demonstrated that BCR-ABL can directly promote cell cycle entry in the absence of

growth factors in both primary leukemic cells and BCR-ABL transfected cell lines [14]. In addition, BCR-ABL expression is sufficient to induce G₁-to-S phase transition, DNA synthesis, and activation of cyclin-dependent

Table 4 Alternative splicing probe sets of cell cycle-related genes that were significantly changed in K562 cells in response to imatinib treatment (the 10 most up-regulated and the 10 most down-regulated probe sets)

Gene probe ID	Exon probe ID	Splice index	Gene title	Gene symbol	Accession no.
3875195	3875230	0.21	Minichromosome maintenance complex component 8	MCM8	NM_182802
3442641	3442673	0.29	CD163 molecule-like 1	CD163L1	NM_174941
2423017	2423077	0.3	Ecotropic viral integration site 5	EVI5	NM_005665
3124180	3124205	0.3	PIN2-interacting protein 1	PINX1	NM_017884
3817698	3817712	0.3	Ubiquitin-like, containing PHD and RING finger domains, 1	UHRF1	NM_013282
3661065	3661098	0.31	Retinoblastoma-like 2 (p130)	RBL2	NM_005611
2813524	2813541	0.32	RAD17 homolog (<i>S. pombe</i>)	RAD17	NM_002873
3575567	3575640	0.34	Forkhead box N3	FOXN3	NM_005197
3257338	3257378	0.34	M-phase phosphoprotein 1	MPHOSPH1	NM_016195
3533435	3533458	0.34	Pinin, desmosome associated protein	PNN	NM_002687
3463112	3463149	4.43	E2F transcription factor 7	E2F7	NM_203394
3690550	3690563	4.49	Seven in absentia homolog 1 (<i>Drosophila</i>)	SIAH1	NM_001006610
3956433	3956483	4.58	CHK2 checkpoint homolog (<i>S. pombe</i>)	CHEK2	NM_001005735
3750767	3750770	4.64	Sperm associated antigen 5	SPAG5	NM_006461
3816509	3816515	5.3	Growth arrest and DNA damage-inducible, beta	GADD45B	NM_015675
3307680	3307701	5.5	DNA cross-link repair 1A (PSO2 homolog, <i>S. cerevisiae</i>)	DCLRE1A	NM_014881
3736290	3736292	6	Baculoviral IAP repeat-containing 5 (survivin)	BIRC5	NM_001168
3258168	3258174	6	M-phase phosphoprotein 1	MPHOSPH1	NM_016195
3368304	3368362	6.57	Wilms tumor 1	WT1	NM_024424
3145107	3145124	8.14	Cyclin E2	CCNE2	NM_057749

Please refer to Supplemental Table 3 for a more comprehensive list of alternatively spliced cell cycle-related genes in K562 cells treated with imatinib

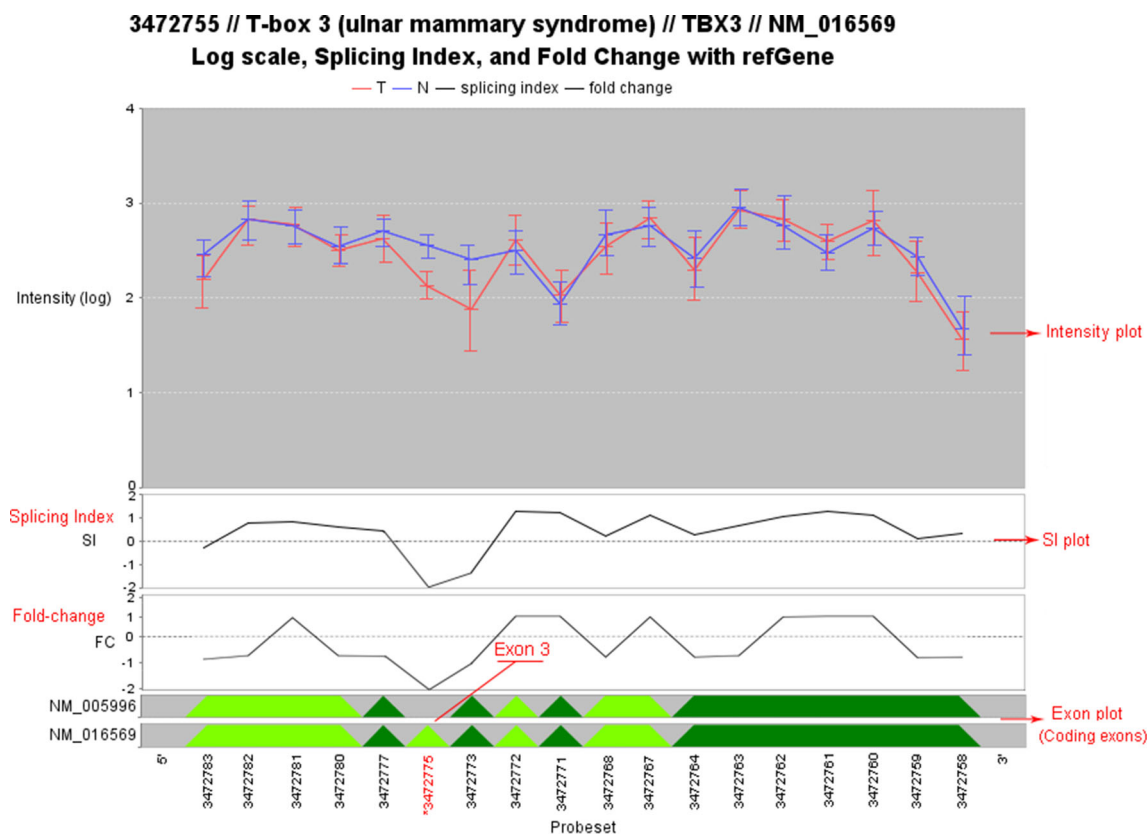


Fig. 2 The transcript cluster of TBX3. The transcript with exon 3 was down-regulated in the imatinib-treated K562 cell line (red line). This exon was targeted by probe set 3472775 and found to be differentially

expressed using the MIDAS test (labeled in red). The fold change of this probe set between untreated and imatinib-treated cells was significant and greater than 2

kinases (Fig. 1). Therefore, in our study, we classified the 185 genes according to their specific functions by using the DAVID functional annotation tool (Table 2). The group enrichment score, which is the geometric mean in log scale of p values from the corresponding annotation cluster, was used to rank the biological significance of the genes. The p values associated with each annotation terms inside each clusters are exactly the same meaning/values as p values (Fisher exact/EASE score) shown in the regular chart report for the same terms (http://david.abcc.ncifcrf.gov/helps/functional_annotation.html#E4).

Exon-level expression differences induced by imatinib treatment

Through using the easyExon analysis which with a limitation of probe sets in a transcript cluster from 4 to 40, a total of 331 genes that represented by one or several of the probe sets is obtained. Those alternatively spliced genes are different expressions in the imatinib-treated K562 cells contrast with the K562 cells, and most of these probe sets had a change in expression of less than threefold. We set the easyExon “GO

Biological Process” parameter to cell cycle, which was related to the transcript-affiliated gene name or accession number, and identified that 190 genes from the original set of 331 are cell cycle-related genes. Each of these genes contained at least one probe set meeting the above criteria, which represented a total of 227 probe sets. Table 4 lists the 10 most up-regulated and the 10 most down-regulated probe sets.

In this circumstance, there are three statistical methods available for exon-level filtration in the easyExon software, including Affymetrix Microarray Detection of Alternative Splicing (MIDAS), Alternative Splice Analysis of Variance (Partek AS ANOVA) and pattern-based correlation (PAC), we used the default Affymetrix MIDAS. The criteria for the probe set filtration for selecting alternatively spliced genes were set as follows: fold change (FC) ≥ 2 or ≤ 0.5 with a statistical significance of $p < 0.05$.

Detection of alternative splicing events in response to imatinib treatment

From the graphic presentation of analysis results, we were able to identify whether a specific isoform was up- or down-regulated in the imatinib-treated cells [15]

(Fig. 2). After identifying probe sets that were differentially spliced in response to imatinib treatment (Supplemental Table 3), we detected changes of two randomly chosen internal exons (T-box transcription factor (TBX3) and phospholipase C, beta 1 (PLCB1)) between untreated and imatinib-treated K562 cells with using the easyExon program for experimental validation. For TBX3 (NM_016569), the probe set (ID3472775, indicated in red) that targeted exon 3 was down-regulated in imatinib-treated K562 cells. The expression fold change of this probe set was larger than 2 (labeled in red) and was statistically significant (marked by an asterisk). These results indicated the presence of two TBX3 isoforms in K562 cells: one with exon 3 and another without exon 3. In addition, the isoform lacking exon 3 was up-regulated in imatinib-treated K562 cells (Fig. 2). For PLCB1 (NM_015192), the probe set (ID 3875823, indicated in red) targeting exon 30 was up-regulated in imatinib-treated K562 cells. In addition, two spliceosomes were detected in K562 cells, which suggested that alternative splicing was affected by imatinib treatment (Fig. 3). Using this same process, we

identified other transcripts that undergo alternative splicing as well.

RT-PCR validation of differentially expressed and alternatively spliced genes

We confirmed the transcript level of CENPE and SLC4A1 on the basis of the largest and smallest fold changes in imatinib-treated K562 cells, respectively. Three independent samples were treated with 1 μ M imatinib or dimethyl sulfoxide (DMSO) to assess differential expression by RT-PCR. As shown in Fig. 4, the mRNA levels of CENPE and SLC4A1 had the decreased and increased tendency in the imatinib-treated K562 cells compared to the controls respectively, which basically confirmed and validated the results obtained from the exon array.

Using primers targeting the exons that flank the exon of interest, we also carried out RT-PCR validation of differential splicing in TBX3 and PLCB1 mRNA transcripts from imatinib-treated K562 cells. The results indicated a difference in the ratio of isoforms in response to imatinib treatment for probe sets 3472775 (TBX3) and 3875823 (PLCB1) (Fig. 5).

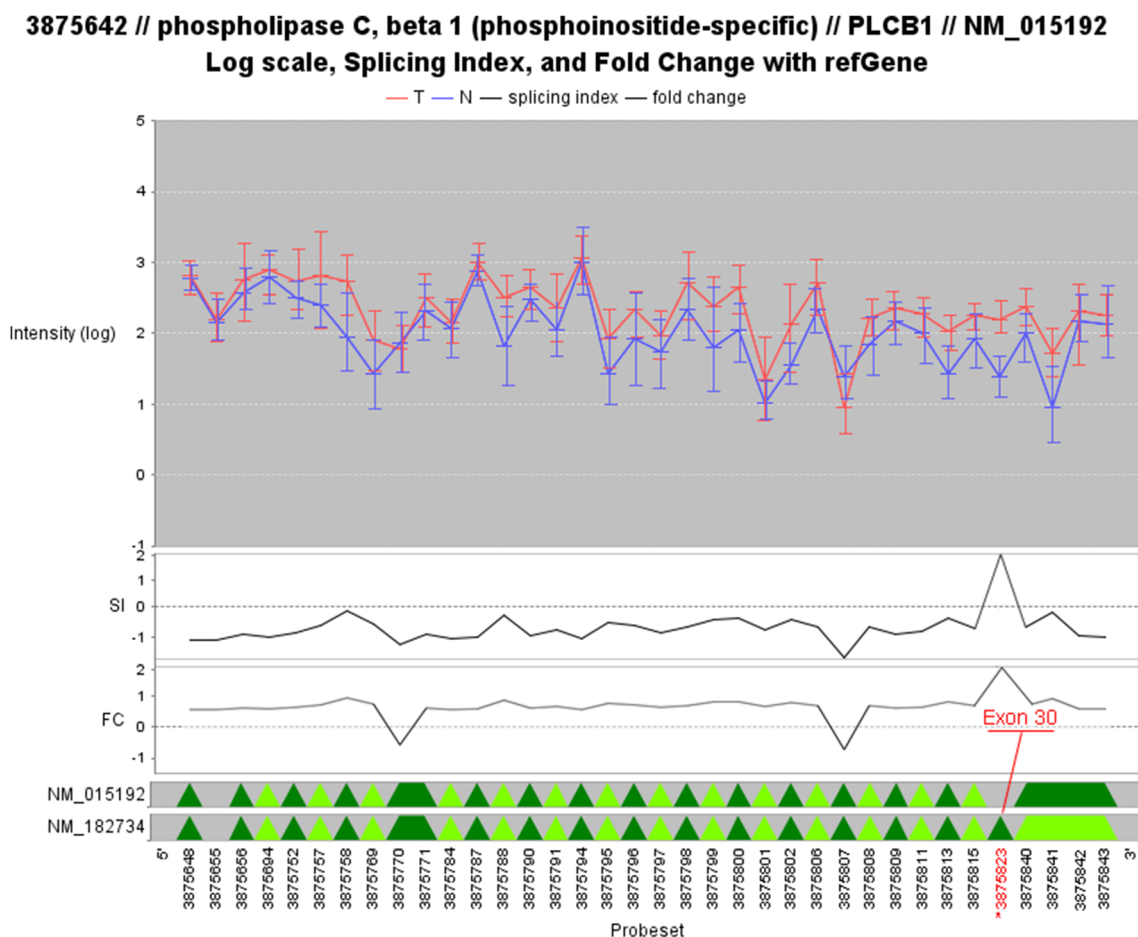


Fig. 3 The transcript cluster of PLCB1. The 30th exon is up-regulated in the imatinib-treated K562 cell line (red line). This exon was targeted by probe set 3875823 and was identified by MIDAS (labeled in red)

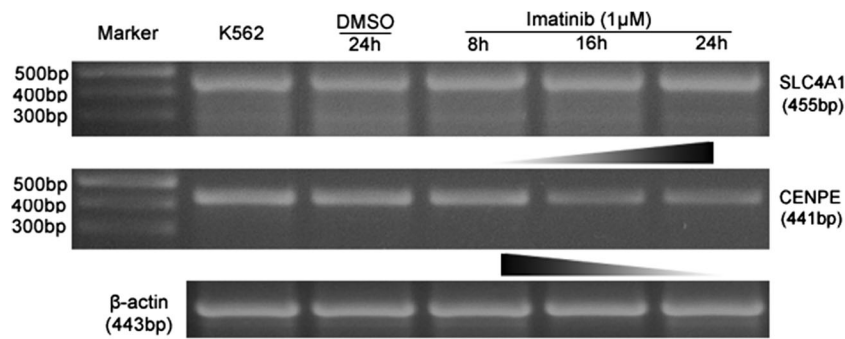


Fig. 4 RT-PCR validation of differentially expressed genes in imatinib-treated K562 cells. Representative bands of SLC4A1 and CENPE mRNA expression in K562 cells. β -actin was used as an internal control. Three independent samples were treated with 1 μ M imatinib or DMSO at

various time points. An increase and decrease in the relative expression of SLC4A1 and CENPE was observed in a time-dependent manner, respectively

Importantly, the relative abundance of TBX3 and PLCB1 transcripts was consistent with the trend of SI values calculated from the exon array data (Fig. 5).

Discussion

Imatinib is considered to be the most effective drug for the treatment of chronic phase CML with a relatively good safety profile. In this study, we employed the Human Exon 1.0 ST array together with easyExon software to assess the comprehensive profile of differentially expressed genes and alternative splicing events in imatinib-treated K562 cells, with a focus on cell cycle-related genes. A total of 185 differentially expressed genes and 227 alternative splicing events were identified from all analyzed cell cycle genes, several of which were

successfully validated by RT-PCR. Our results revealed that imatinib treatment induces aberrant gene expression and alternative splicing events during various points in the cell cycle of K562 cells (Fig. 1). Our results also demonstrated that the exon array technology is reliable and sensitive enough to detect differential expression at the gene and exon level in this cell line.

It is known that the CDK family of serine/threonine kinases is at the core of mammalian cell cycle pathways and controls cell cycle progression by phosphorylating proteins that function at specific cell cycle stages (Fig. 6) [16, 17]. This current understanding has provided a collective rationale for targeting CDKs and associated regulatory molecules as a therapeutic strategy for cancer [18]. The selective interruption of cell cycle regulation in cancer cells by interfering with CDKs provides a novel and potentially effective strategy for killing the

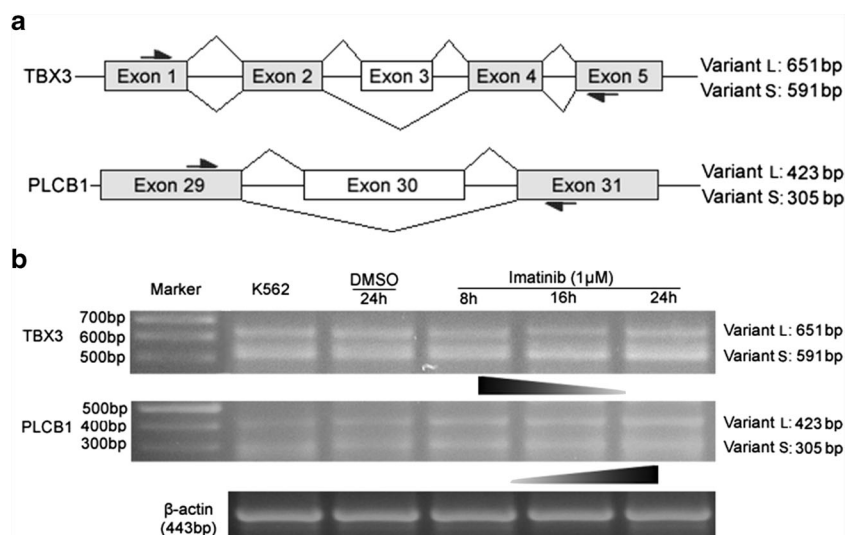
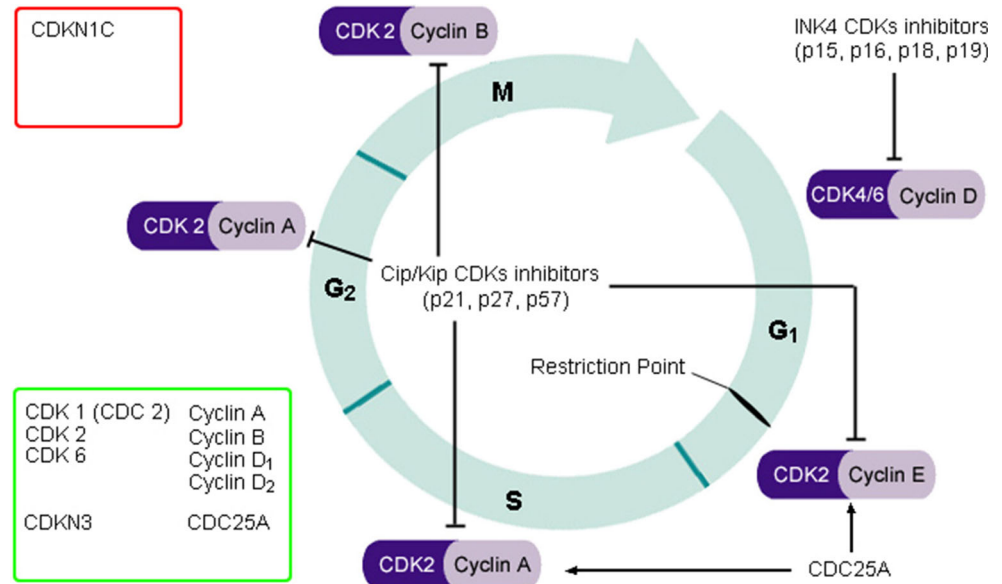


Fig. 5 Imatinib regulates TBX3 and PLCB1 splicing in K562 cells. K562 cells were treated with 1 μ M of imatinib, total RNA was extracted, and RT-PCR was used to validate the alternative splicing of TBX3 and PLCB1. **a** A schematic representation of alternative splicing of TBX3 exon 3 and PLCB1 exon 30 as well as the resulting mRNAs. The RT-PCR primer positions are schematically presented (black arrows). **b**

RT-PCR validation of alternatively spliced TBX3 and PLCB1. Agarose gel electrophoresis showing larger (exon-included) and smaller (exon-skipped) isoforms. An decrease and increase in the variant L/variant S ratio of TBX3 and PLCB1 was observed, respectively, in a time-dependent manner. β -actin was used as an internal control. The lane designated Marker contains a 100-bp ladder

Fig. 6 Regulation of CDK activity in the cell cycle. The four major mechanisms of CDK regulation are cyclin binding, CAK phosphorylation, regulatory inhibitory phosphorylation, and binding of CDK inhibitory subunits (CKIs). Genes involved in these mechanisms that are regulated by imatinib are listed in boxes. Up-regulated genes are indicated in the *red box* and down-regulated genes are indicated in the *green box*



cells. Flavopiridol (alvocidib) was the first CDK inhibitor to be tested in clinical trials after being identified from an anticancer agent screen in 1992 [19]. More specific second-generation CDK inhibitors that target single CDKs have since been developed and are currently being investigated in pre-clinical and clinical studies (reviewed in reference [18]). In the present study, we found that some CDK family members (i.e., CDK1, CDK2, and CDK6) and CDK regulators (i.e., cyclin A, cyclin B, and CDC25A) were markedly down-regulated in

imatinib-treated K562 cells (Fig. 6), including key regulators in the G1/S transition, G2/M transition, and S phase progression. Our observations are consistent with findings by Du et al. [20] showing that the combination of arsenic trioxide (ATO) and imatinib can induce programmed cell death in K562 cells more effectively and efficiently than imatinib alone. The direct down-regulation of CDKs and CDK-related genes may abolish the cell cycle machinery, which may be a prerequisite for apoptosis in imatinib-treated CML

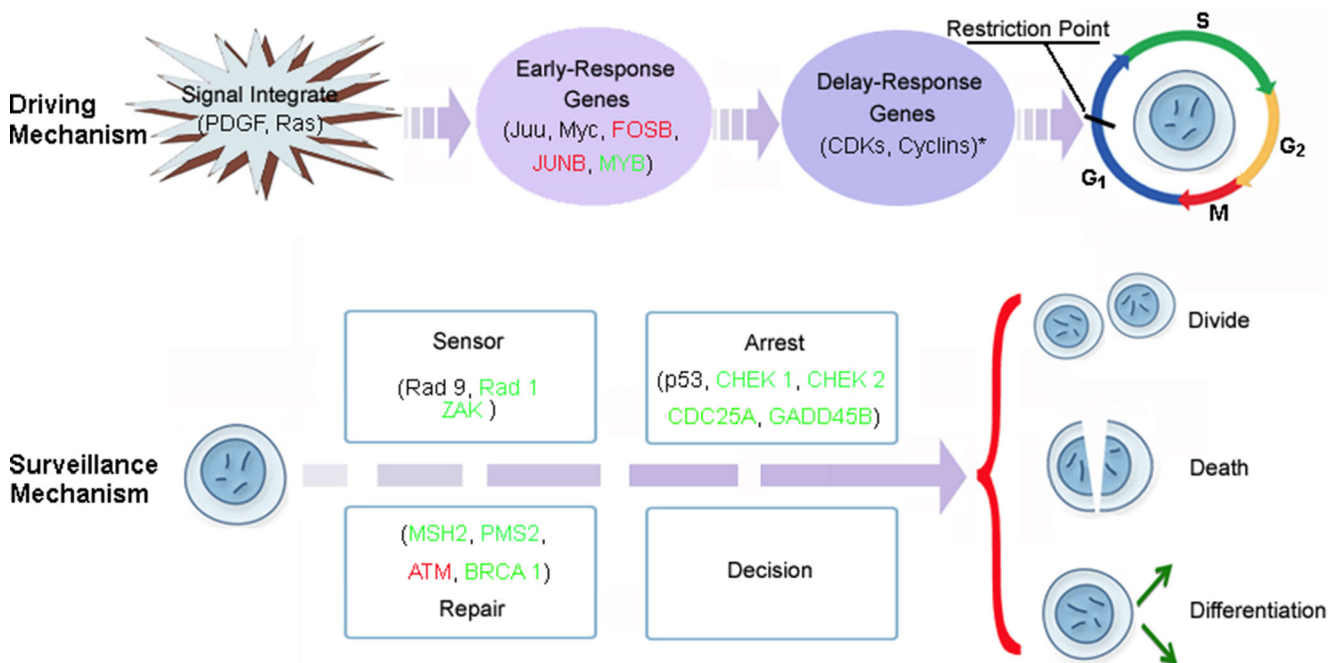


Fig. 7 Ideogram illustration of dynamic changes underlying imatinib-mediated cell cycle regulation in K562 cells. Cell cycle driving and surveillance are the two key biological mechanisms of cell cycle control. Some genes involved in these mechanisms and modulated by

imatinib are marked in *red* (up-regulation) and *green* (down-regulation). Genes that have no change in expression are indicated in *black*. Note: the expression changes of CDK and cyclin families are shown in Fig. 6

cells. Based on these findings, we hypothesize that imatinib also inhibits CDK family members that directly regulate the cell cycle.

Cell cycle driving and surveillance are the two key biological mechanisms of cell cycle control. External stimuli from growth factors or mitotic signals (i.e., platelet-derived growth factor (PDGF) and Ras) initiates the transcription of early response genes (i.e., *Juu*, *Fos*, and *Myc*) followed by delayed-response genes (i.e., cyclins and CDKs), which allow cells to pass through the cell cycle checkpoints and proliferate. In our study, we found that the expression of several genes related to these two mechanisms underwent a significant change in imatinib-treated K562 cells (Fig. 7). From the gene expression profile, we concluded that imatinib can prevent cells from entering the cell cycle and can inhibit DNA repair in cells (Fig. 7), which is consistent with the findings by Majsterek et al. [21]. It has been suggested that BCR-ABL may contribute to the resistance of CML cells to imatinib by stimulating DNA repair [22]. Therefore, additional studies exploring the influence of imatinib on DNA repair will be very useful for understanding drug resistance in human leukemia.

Another major finding of our study was that treatment of K562 cells with imatinib markedly shifted the number of alternative splicing events of cell cycle-related genes. Alternative splicing involves differential exon selection of a gene transcript to generate mRNA and protein isoforms with structural and functional diversity [23]. In addition, it has been demonstrated that alternative splicing is closely related to cell cycle progression [24, 25]. However, little is known about the detailed functional role of precise splicing control in cell cycle progression. Based on our study, aberrant splicing of such a large number of cell cycle-related genes suggests that alternative splicing of cell cycle genes is a prerequisite for imatinib-induced apoptosis. This conclusion is in agreement with the findings by Ahn et al., which suggested that cell cycle defects may be manifested by specific splicing defects [11]. Previous studies have also suggested that abnormal alternative splicing is associated with malignant phenotypes, such as chemoresistance and invasive properties [23]. As mentioned previously, BCR-ABL may contribute to the resistance of CML cells to chemotherapy drugs, such as imatinib, through the stimulation of DNA repair, which is part of the cell cycle surveillance mechanism. Based on these analyses, we speculate that the abnormal splicing of cell cycle-related genes may play a significant role in the resistance of CML cells to imatinib, which could serve as a target for developing novel therapeutic strategies for treating CML.

In conclusion, using exon arrays, we have discovered that imatinib induces wide-ranging effects on the expression level and alternative splicing of cell cycle-related genes in K562 leukemic cells. This information may help improve the

imatinib treatment strategies in patients with CML and may be useful for imatinib resistance research. Although several of the imatinib-induced alternative splicing events were successfully validated by RT-PCR in our study, a more comprehensive validation is necessary to provide a complete analysis of the current findings.

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Conflicts of interest None

References

- Weinberg R, Lundberg A. Control of the cell cycle and apoptosis. *Eur J Cancer*. 1999;35(4):531–9.
- Vermeulen K, Van Bockstaele DR, Berneman ZN. The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. *Cell Prolif*. 2003;36(3):131–49.
- Senese S, Lo Y, Huang D, Zangle T, Gholkar A, Robert L, et al. Chemical dissection of the cell cycle: probes for cell biology and anti-cancer drug development. *Cell Death Dis*. 2014;5(10):e1462.
- Alexander A, Keyomarsi K. Exploiting cell cycle pathways in cancer therapy: new (and old) targets and potential strategies. *Nuclear Signaling Pathways and Targeting Transcription in Cancer*. Springer; 2014; 337–72.
- Nishimura N, Furukawa Y, Sutheesophon K, Nakamura M, Kishi K, Okuda K, et al. Suppression of ARG kinase activity by Sti571 induces cell cycle arrest through up-regulation of CDK inhibitor p18/Ink4c. *Oncogene*. 2003;22(26):4074–82.
- Roussidis A, Mitropoulou T, Theocharis A, Kiamouris C, Papadopoulos S, Kletsas D, et al. Sti571 as a potent inhibitor of growth and invasiveness of human epithelial breast cancer cells. *Anticancer Res*. 2004;24(3A):1445–8.
- Uziel O, Fenig E, Nordenberg J, Beery E, Reshef H, Sandbank J, et al. Imatinib mesylate (Gleevec) downregulates telomerase activity and inhibits proliferation in telomerase-expressing cell lines. *Br J Cancer*. 2005;92(10):1881–91.
- Yin T, Wu Y-L, Sun H-P, Sun G-L, Du Y-Z, Wang K-K, et al. Combined effects of As4s4 and Imatinib on chronic myeloid leukemia cells and BCR-ABL oncoprotein. *Blood*. 2004;104(13):4219–25.
- Liu J, Huang B, Xiao Y, Xiong H-M, Li J, Feng D-Q, et al. Aberrant expression of splicing factors in newly diagnosed acute myeloid leukemia. *Onkologie*. 2012;35(6):335–40.
- Prinos P, Gameau D, Lucier J-F, Gendron D, Couture S, Boivin M, et al. Alternative splicing of SYK regulates mitosis and cell survival. *Nat Struct Mol Biol*. 2011;18(6):673–9.
- Ahn E-Y, DeKolver RC, Lo M-C, Nguyen TA, Matsuura S, Boyapati A, et al. Son controls cell-cycle progression by coordinated regulation of RNA splicing. *Mol Cell*. 2011;42(2):185–98.
- Dennis Jr G, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, et al. David: database for annotation, visualization, and integrated discovery. *Genome Biol*. 2003;4(5):3.
- Yin L-M, Jiang G-H, Wang Y, Wang Y, Liu Y-Y, Jin W-R, et al. Use of serial analysis of gene expression to reveal the specific regulation of gene expression profile in asthmatic rats treated by acupuncture. *J Biomed Sci*. 2009;16(1):46.
- Jonuleit T, van der Kuip H, Miething C, Michels H, Hallek M, Duyster J, et al. BCR-ABL kinase down-regulates cyclin-

- dependent kinase inhibitor P27 in human and murine cell lines. *Blood*. 2000;96(5):1933–9.
15. Chang T-Y, Li Y-Y, Jen C-H, Yang T-P, Lin C-H, Hsu M-T, et al. Easyexon—a java-based Gui tool for processing and visualization of affymetrix exon array data. *BMC Bioinformatics*. 2008;9(1):432.
 16. Morgan DO. Cyclin-dependent kinases: engines, clocks, and microprocessors. *Annu Rev Cell Dev Biol*. 1997;13(1):261–91.
 17. Hunter T, Pines J. Cyclins and cancer II: cyclin D and CDK inhibitors come of age. *Cell*. 1994;79(4):573–82.
 18. Sausville EA. Complexities in the development of cyclin-dependent kinase inhibitor drugs. *Trends Mol Med*. 2002;8(4):S32–S7.
 19. Kelland LR. Flavopiridol, the first cyclin-dependent kinase inhibitor to enter the clinic: current status. *Expert Opin Investig Drugs*. 2000;9(12):2903–11.
 20. Du Y, Wang K, Fang H, Li J, Xiao D, Zheng P, et al. Coordination of intrinsic, extrinsic, and endoplasmic reticulum-mediated apoptosis by Imatinib mesylate combined with arsenic trioxide in chronic myeloid leukemia. *Blood*. 2006;107(4):1582–90.
 21. Majsterek I, Arabski M, Czechowska A, Pytel D, Morawiec Z, Morawiec-Bajda A, et al. Imatinib (Sti571) inhibits DNA repair in human leukemia oncogenic tyrosine kinase-expressing cells. *Zeitschrift für Naturforschung C*. 2006;61(11/12):896.
 22. Blasiak J, Gloc E, Pertynski T, Drzewoski J. DNA damage and repair in BCR/ABL-expressing cells after combined action of idarubicin, Sti571 and amifostine. *Anti-Cancer Drugs*. 2002;13(10):1055–60.
 23. Chang J-G, Yang D-M, Chang W-H, Chow L-P, Chan W-L, Lin H-H, et al. Small molecule amiloride modulates oncogenic Rna alternative splicing to devitalize human cancer cells. *PLoS One*. 2011;6(6):e18643.
 24. Lundgren K, Allan S, Urushiyama S, Tani T, Ohshima Y, Friendewey D, et al. A connection between pre-mRNA splicing and the cell cycle in fission yeast: *cdc28+* is allelic with *prp8+* and encodes an RNA-dependent ATPase/helicase. *Mol Biol Cell*. 1996;7(7):1083–94.
 25. Burns CG, Ohi R, Mehta S, O'Toole ET, Winey M, Clark TA, et al. Removal of a single A-tubulin gene intron suppresses cell cycle arrest phenotypes of splicing factor mutations in *saccharomyces cerevisiae*. *Mol Cell Biol*. 2002;22(3):801–15.