Oncogene (2006) 25, 1118–1124

www.nature.com/onc

SHORT COMMUNICATION

BCR–ABL1 induces aberrant splicing of *IKAROS* and lineage infidelity in pre-B lymphoblastic leukemia cells

F Klein[1], N Feldhahn[1], S Herzog[2], M Sprangers[1], JL Mooster[1], H Jumaa[2] and M Müschen[1]

[1]*Laboratory for Molecular Stem Cell Biology, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany and* [2]*Max-Planck-Institute for Immunobiology, Freiburg, Germany*

Pre-B lymphoblastic leukemia cells carrying a *BCR–ABL1* gene rearrangement exhibit an undifferentiated phenotype. Comparing the genome-wide gene expression profiles of normal B-cell subsets and *BCR–ABL1*$^+$ pre-B lymphoblastic leukemia cells by SAGE, the leukemia cells show loss of B lymphoid identity and aberrant expression of myeloid lineage-specific molecules. Consistent with this, *BCR–ABL1*$^+$ pre-B lymphoblastic leukemia cells exhibit defective expression of *IKAROS*, a transcription factor needed for early lymphoid lineage commitment. As shown by inducible expression of BCR–ABL1 in human and murine B-cell precursor cell lines, BCR–ABL1 induces the expression of a dominant-negative *IKAROS* splice variant, termed IK6. Comparing matched leukemia sample pairs from patients before and during therapy with the BCR–ABL1 kinase inhibitor STI571 (Imatinib), inhibition of BCR–ABL1 partially corrected aberrant expression of IK6 and lineage infidelity of the leukemia cells. To elucidate the contribution of IK6 to lineage infidelity in *BCR–ABL1*$^+$ cell lines, IK6 expression was silenced by RNA interference. Upon inhibition of IK6, *BCR–ABL1*$^+$ leukemia cells partially restored B lymphoid lineage commitment. Therefore, we propose that BCR–ABL1 induces aberrant splicing of *IKAROS*, which interferes with lineage identity and differentiation of pre-B lymphoblastic leukemia cells.
Oncogene (2006) **25,** 1118–1124. doi:10.1038/sj.onc.1209133; published online 3 October 2005

Keywords: lineage commitment; lymphopoiesis; RNA interference; alternative splicing

The *BCR–ABL1* gene rearrangement resulting from the t(9;22)(q34;q11) translocation represents the most frequent recurrent genetic aberration in B lymphoid leukemia in adults (Look, 1997). *BCR–ABL1* fusion genes encode constitutively active tyrosine kinase molecules mostly of a molecular weight of either 190 or 210 kDa (p190 or p210; Laurent *et al*., 2001) and BCR–ABL1 kinase activity is required and sufficient to drive malignant transformation of B-cell precursors in mice (Huettner *et al*., 2000). Pre-B lymphoblastic leukemia cells carrying a *BCR–ABL1* gene rearrangement typically exhibit a differentiation block at the pre-B-cell stage of development (Klein *et al*., 2004, 2005).

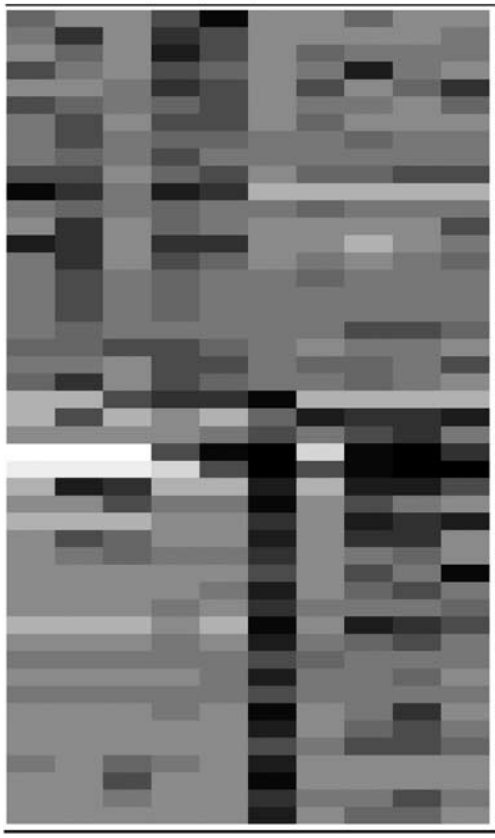
Early B-cell development is guided by a number of transcription factors, including PAX5, E2A, EBF and IKAROS (Busslinger, 2004). In order to search for downstream targets of BCR–ABL1 that contribute to a differentiation block in *BCR–ABL1*$^+$ pre-B lymphoblastic leukemia cells, genome-wide gene expression profiles of this leukemia type were compared with normal hematopoietic progenitor populations by serial analysis of gene expression (SAGE; Velculescu *et al*., 1995).

BCR–ABL1 interferes with B lymphoid differentiation in BCR–ABL1$^+$ pre-B lymphoblastic leukemia cells

Comparing genome-wide gene expression profiles of leukemia cells from two leukemia cases of *BCR–ABL1*$^+$ pre-B lymphoblastic leukemia with normal pre-B cells by SAGE, a number of myeloid lineage-specific genes, including transcription factors (*MLF2*, *MZF1*, *AML1*, *GATA1*), surface receptors (*CSF3R*, *CSF1R*, *CD14*, *CD11A*) and signaling molecules (*IRAK1*, *MYD88*) were upregulated in the pre-B lymphoblastic leukemia cells as in normal myeloid progenitor cells (Figure 1). Hence, the pattern of gene expression in *BCR–ABL1*$^+$ pre-B lymphoblastic leukemia cells as compared to their normal pre-B-cell counterpart is skewed to the myeloid lineage. Consistent with this, transcription factors and signaling molecules involved in early lymphoid differentiation (*IKAROS*, *E2A*, *IL7Rα*, *RAG1*, *RAG2*, *TdT*) and B lymphoid lineage commitment (*EBF*, *PAX5*) were silenced in *BCR–ABL1*$^+$ pre-B lymphoblastic leukemia cells (Figure 1). These findings lead to the hypothesis that lymphoid lineage commitment is impaired in *BCR–ABL1*$^+$ pre-B lymphoblastic leukemia cells.

We next studied whether derangement of lymphoid lineage commitment and B-cell differentiation in the *BCR–ABL1*$^+$ pre-B lymphoblastic leukemia cells are indeed owing to BCR–ABL1 kinase activity. To this end, we induced BCR–ABL1 expression in a murine B

Correspondence: Dr M Müschen, Laboratory for Molecular Stem Cell Biology, Heinrich-Heine-Universität Düsseldorf, Moorenstr 5, Building 14.80, Düsseldorf 40225, Germany.
E-mail: markus.mueschen@uni-duesseldorf.de
Received 12 January 2005; revised 8 June 2005; accepted 8 June 2005; published online 3 October 2005



UniGene	Gene name	Proposed function	Reference
81994	*GYPC*	erythroid/ myeloid differentiation	Robinson et al., 1981
2175	*CSF3R*	GCSF-receptor	Dong et al., 1996
181002	*MSF1*	related to myeloid leukemia	Osaka et al., 1999
85289	*CD133*	hematopoietic stem cell antigen	Yin et al., 1997
182018	*IRAK1*	IL1 signaling in myeloid cells	Cao et al., 1996
79026	*MLF2*	myeloid leukemia factor 2	Kuefer et al., 1996
169832	*MZF1*	myeloid zinc finger gene 1	Hromas et al., 1991
112255	*NUP98*	related to myeloid leukemia	Ahuja et al., 1999
174142	*CSF1R*	colony-stimulating factor 1 receptor	Gisselbrecht et al., 1987
460463	*IL3Ra*	Shared subunit with GM-CSFR	Kitamura et al., 1991
1817	*MPO*	myeloperoxidase	Weil et al., 1988
89633	*PML*	related to myeloid leukemia	Cleary, 1996
1239	*CD13*	myeloid differentiation antigen	Look et al., 1986
129914	*AML1*	implicated in myelopoiesis	Tanaka et al., 1995
153837	*MNDA*	myeloid nuclear differentiation antigen	Briggs et al., 1994
83731	*CD33*	myeloid differentiation	Peiper et al., 1988
31551	*CBFA2T2*	related to myeloid leukemia	Kitabayashi et al., 1998
765	*GATA1*	myeloid differentiation	Qian et al., 2002
174103	*CD11A*	macrophage differentiation	Springer et al., 1985
196352	*NCF4*	oxidative burst in myeloid cells	Zhan et al., 1996
279751	*SIGLEC8*	myeloid inhibitory receptor	Kikly et al., 2000
82116	*MYD88*	IL1 signaling in myeloid differentiation	Adachi et al., 1998
272537	*TdT*	generation of junctional diversity	Kung et al., 1975
89414	*CXCR4*	SDF1 receptor, pre-B cells	Nagasawa et al., 1996
25648	*CD40*	Proliferation of B cell precursors	Hasbold et al., 1994
96023	*CD19*	B cell co-receptor	Carter & Fearon, 1992
285823	*IGHCM*	IGH m chain	Raff et al., 1976
170121	*CD45*	Antagonizes SHP1, CSK	Pani et al., 1997
101047	*E2A*	Initiation of *IGH* gene rearrangement	Bain et al., 1994
79630	*Iga*	ITAM signaling chain	Flaswinkel et al., 1995
74101	*SYK*	B cell receptor signaling	Kurosaki et al., 1994
237868	*IL7Ra*	Lymphoid differentiation	Uckun et al., 1991
66052	*CD38*	Ligation causes tyrosine phosphorylation	Kitanaka et al., 1999
167746	*BLNK*	Linker in B cell receptor signaling	Fu et al., 1998
82132	*IRF4*	*IGK* and *IGL* gene rearrangement	Lu et al., 2003
2407	*OBF1*	*IGK* gene transcription	Casellas et al., 2002
22030	*PAX5*	Required for pro- to pre-B cell transition	Nutt et al., 1999
158341	*TACI*	Growth control of early B cells	Yan et al., 2001
54452	*IKAROS*	Critical for early B cell development	Kirstetter et al., 2002
1521	*IgMBP2*	Signal transduction through Iga	Grupp et al., 1995
3631	*IgaBP1*	Involvement in B cell receptor signaling	Fukita et al., 1993
159494	*BTK*	Critical for (pre-) B cell receptor signaling	Cheng et al., 1994
192861	*SPIB*	*IGK* and *IGL* gene rearrangement	Su et al., 1996
73958	*RAG1*	V(D)J recombination	Menetski & Gellert, 1990
159376	*RAG2*	V(D)J recombination	Schatz et al., 1989
1101	*OCT2*	Regulates *IGH* gene transcription	Staudt et al., 1988
192824	*EBF*	Required for B lymphopoiesis	Lin & Grosschedl, 1995

Figure 1 SAGE analysis of differentially expressed genes in BCR–ABL1$^+$ pre-B lymphoblastic leukemia cells and normal pre-B cells. cDNA synthesis, SAGE analysis, cloning and sequencing of SAGE concatemers was carried out as described previously (Feldhahn *et al*., 2002; Müschen *et al*., 2002; Klein *et al*., 2003). A total of 592 000 SAGE tags were collected for 10 SAGE profiles. All SAGE libraries were normalized to 100 000 tags. Leukemia cells from two cases of *BCR–ABL1*$^+$ pre-B lymphoblastic leukemia (case II, p190 and case IX, p210; Klein *et al*., 2004) and normal bone marrow populations and mature B-cell subsets were compared by SAGE. Upregulated genes are depicted in black, downregulated genes in white and intermediate-expressed genes in gray. For each gene, a proposed function with a reference is given. SAGE extracts a 14-bp fragment of any transcript in the analysed cell population as a unique identifier (SAGE tag) of an expressed gene using the reference database UniGene. By counting the number of SAGE tags per 100 000, the representation of a transcript within the transcriptome can be quantified. Normal bone marrow populations analysed include CD34$^+$ HSC, CD15$^+$ myeloid progenitor cells (CMP), CD7$^+$ CD10$^+$ T lymphoid progenitor cells (TLP) and CD10$^+$ CD19$^+$ pre-B cells (pre-B). In addition, CD19$^+$ CD27$^-$ naïve B cells (NBC), CD20$^+$ CD77$^+$ germinal center B cells (GCB), CD19$^+$ CD27$^+$ memory B cells (MBC) and CD19$^+$ CD138$^+$ plasma cells (PC) were analysed. In both leukemia cases (p190 and p210), the cells harbor a V_HDJ_H gene rearrangement on one allele and a DJ_H gene rearrangement on the second allele (Klein *et al*., 2004). However, *IGK* and *IGL* loci were in germline configuration in both cases (not shown). An *IGH VDJ* gene rearrangement in the absence of *IGK* or *IGL* gene rearrangement defines a pre-B-cell stage of development. We therefore sorted SAGE data based on the ratio of SAGE-tag frequencies in *BCR–ABL1*$^+$ pre-B lymphoblastic leukemia cells to normal pre-B cells. SAGE data were sorted according to the ratio between SAGE-tag counts in pre-B cells and in *BCR–ABL1*$^+$ ALL cases.

lymphoid cell line carrying an inducible *BCR–ABL1* transgene (Klucher *et al*., 1998; Figure 2a and b). BCR–ABL1 expression was induced by addition of doxycycline as monitored by measurement of BCR–ABL1 mRNA expression and Western blot analysis (Figure 2b). Induced expression of BCR–ABL1 resulted in upregulation of mRNA levels for the myeloid lineage-specific genes *Csfr1* and *Gata1* and downregulation of mRNA levels for *Il7rα*, which is critical for early lymphoid development. Of note, inducible BCR–ABL1 expression also resulted in aberrant splicing of *IKAROS*, leading to the expression of a dominant-negative form of Ikaros (IK6; Figure 2a). These findings indicate that BCR–ABL1 can promote lineage infidelity in the murine B-cell precursor line. However, mRNA levels for *Oct2*, *Obf1* and *Pax5* remained unchanged.

Attenuation of lymphoid lineage commitment was also linked to BCR–ABL1 kinase activity in *BCR–ABL1*$^+$ pre-B lymphoblastic leukemia cells directly isolated from leukemia patients (Figure 2c and d). From three leukemia patients, matched sample pairs of leukemia cells were available before and during treatment with the BCR–ABL1 kinase inhibitor STI571 (Imatinib). Comparing leukemia cell samples before and during STI571 therapy by flow cytometry, inhibition of BCR–ABL1 kinase activity resulted in downregulation of the myeloid antigens GM-CSFRα, IL3Rα and CD13 (Figure 2c). Conversely, STI571-treated leukemia cells

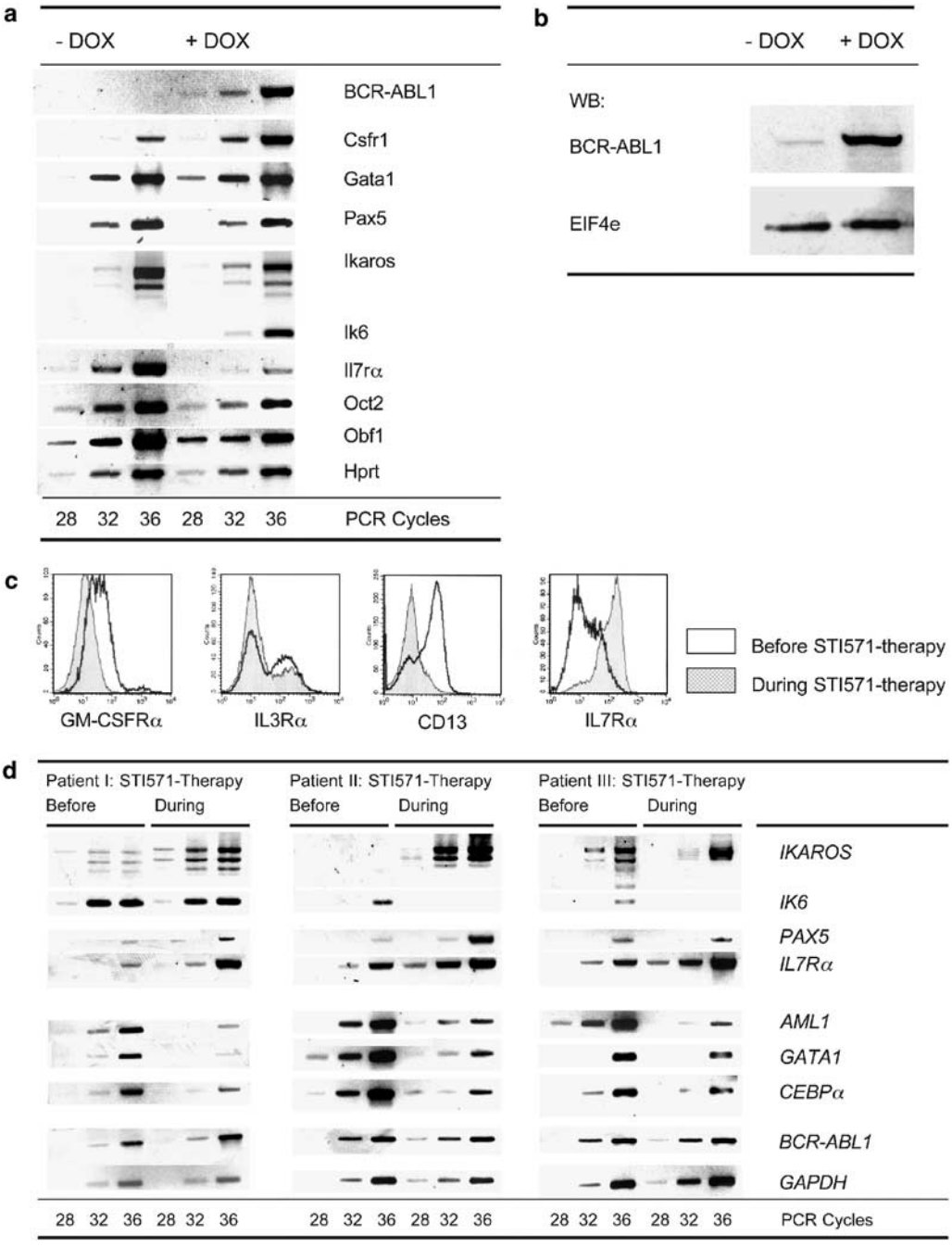


Figure 2 BCR–ABL1 kinase activity results in lineage infidelity and differentially regulates the expression of lymphoid versus myeloid-specific genes. (**a**, **b**) Lymphoid derivative of the murine cell line TONB210 (Klucher *et al*., 1998) carrying an inducible *BCR–ABL1* transgene under the control of a doxycycline-dependent promoter was used for inducible expression of a *BCR–ABL1* transgene. The cells were cultured in the presence or absence of 1 μg/ml doxycycline (DOX). In the absence of BCR–ABL1 expression, the cells remain viable in the presence of 2 ng/ml IL-3. BCR–ABL1 expression was induced by addition of 1 μg/ml doxycycline and verified by Western blot analysis (**b**). EIF4e was used as a loading control. mRNA levels for human BCR–ABL1 and murine Csfr1, Gata1, Pax5, Ikaros splice variants, Il7rα, Oct2, Obf1 and Hprt were monitored (**a**). From three leukemia patients, matched leukemia sample pairs (patients I–III correspond to cases XIII, XIV and XVIII in Klein *et al*., 2004) before (white histograms) and during (gray histograms) therapy with the BCR–ABL1 kinase inhibitor STI571 were analysed by flow cytometry for surface expression of GM-CSFRα, IL3Rα, CD13 and IL7Rα (**c**). One representative case out of three is shown. These patient samples were also subjected to RT–PCR analysis for mRNA expression of *IKAROS* splice variants, *PAX5*, *IL7Ra* and the myeloid transcription factors *AML1*, *GATA1*, *CEBPα*, and normalized for cDNA amounts and leukemia cell content by amplification of *BCR–ABL1* and *GAPDH* cDNA fragments (**d**). Specificity of the inhibitory effect of STI571 on BCR–ABL1 with respect to B lymphoid versus myeloid lineage markers was tested using three *MLL-AF4*$^+$ leukemia cell lines (BEL1, RS4;11 and SEM) that did not carry a *BCR–ABL1* gene rearrangement, yet exhibited a mixed lineage (lymphoid/myeloid) phenotype. These three cell lines were cultured in the presence or absence of 10 μmol/l STI571 for 48 h. STI571 had no effect on expression levels (carried out by FACS analysis) of myeloid-lineage related surface molecules GM-CSFRα, IL3Rα and CD13 and the lymphoid lineage marker IL7Rα (not shown). Primers used for semiquantitative RT–PCR analysis are listed in Supplementary Table 1.

upregulated IL7Rα surface expression. In agreement with this, we found that inhibition of BCR–ABL1 kinase activity during STI571 therapy results in increased mRNA levels of *IL7Rα*, while mRNA levels of myeloid transcription factors *AML1*, *GATA1* and *CEBPα* were downregulated (Figure 2d).

BCR–ABL1 induces defective expression of IKAROS

Previous studies showed that loss of functional *IKAROS* prevents normal B-cell development (Georgopoulos *et al*., 1994; Kirstetter *et al*., 2002). Since the expression of a dominant-negative splice variant IK6 can inhibit B-cell differentiation (Tonnelle *et al*., 2001), lineage infidelity in human pre-B lymphoblastic leukemia cells may result from BCR–ABL1-induced derangement of *IKAROS* pre-mRNA splicing, leading to aberrant expression of IK6. Furthermore, the expression of dominant-negative *IKAROS* splice variants lacking the DNA-binding domain was recently reported in both childhood and adult B-cell lineage acute lymphoblastic leukemia (Sun *et al*., 1999; Nakase *et al*., 2000). Studying *IKAROS* isoform expression in *BCR–ABL1*$^+$ pre-B lymphoblastic leukemia, expression of dominant-negative IK6 was detected in six of seven primary cases (cases XIII–XIX in Klein *et al*., 2004) and two of three cell lines (in BV173 and SUP-B15, but not in Nalm1 cells).

As shown by us and others, BCR–ABL1 can induce aberrant splicing of various genes (Perrotti and Calabretta, 2002), including SLP65 (Jumaa *et al*., 2003; Klein *et al*., 2004), PYK2 (Salesse *et al*., 2004) and BTK (Feldhahn *et al*., 2005). Consistent with BCR–ABL1-induced derangement of *IKAROS* pre-mRNA splicing, patient-derived leukemia cells express dominant-negative IKAROS (IK6) before, but not during extended therapy with the BCR–ABL1 kinase inhibitor STI571 (Figure 2d).

To directly analyse the effect of BCR–ABL1 expression on *IKAROS* pre-mRNA splicing in human pre-B lymphoblastic leukemia cells, 697 cells that carry an *E2A-PBX1*, but not a *BCR–ABL1* gene rearrangement, and only exhibit expression of full-length *IKAROS* were transiently transfected with expression vectors encoding either GFP only or BCR–ABL1 and GFP (Figure 3a). For both transfections, GFP$^+$ and GFP$^-$ cells were sorted and separately analysed for the expression of *IKAROS* splice variants. Expression of the BCR–ABL1 kinase in transfected cells was verified by Western blot analysis (Figure 3b). As shown in Figure 3a, induced expression of BCR–ABL1 results in aberrant splicing of *IKAROS*, leading to the expression of IK6.

Inhibition of BCR–ABL1 kinase corrects splicing and nuclear localization of IKAROS in pre-B lymphoblastic leukemia cells

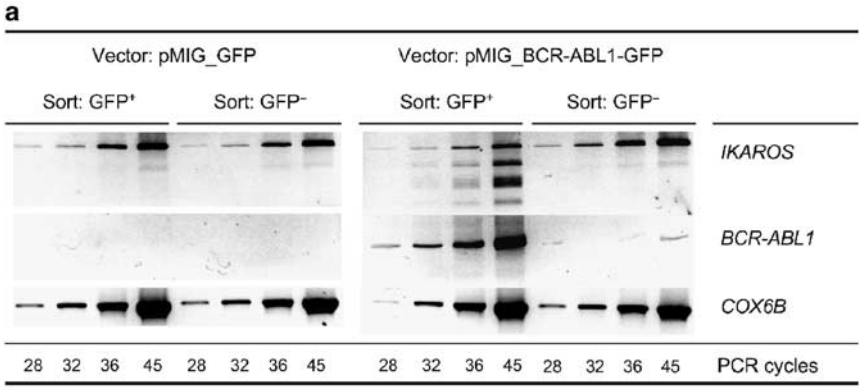
Treatment of the leukemia cells with STI571 for 4 days induced selective outgrowth of differentiating subclones that downregulate IL3Rα expression and exhibit surface IgM expression (not shown), which indicates the pre-B to immature B-cell transition (Klein *et al*., 2005). We studied mRNA expression of *IKAROS* in undifferentiated leukemia cells and in IgM$^+$ MACS-enriched differentiating subclones. Whereas undifferentiated leukemia cells predominantly express dominant-negative IK6, expression of functional IKAROS transcripts was largely restored in IgM$^+$ differentiating subclones (Figure 3c).

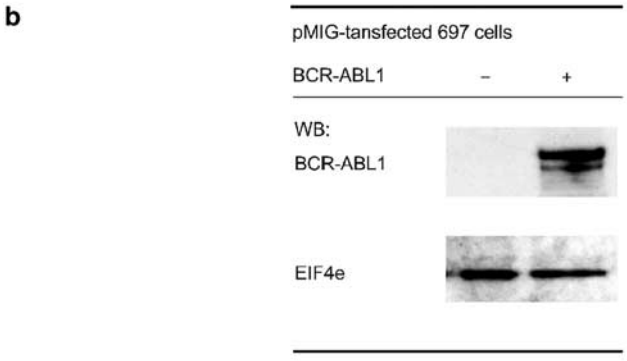
While nuclear localization of IKAROS is critical for its function as a transcription factor, in two untreated leukemia cell lines expressing either p190 or p210 BCR–ABL1 proteins, IKAROS protein is localized in the cytoplasm, which is consistent with the expression of non-DNA-binding IK6 (Figure 3d). However, differentiating subclones that were MACS-enriched for IgM expression exhibit a nuclear pattern of staining for IKAROS, which is consistent with expression of DNA-binding forms of IKAROS (Figure 3d).

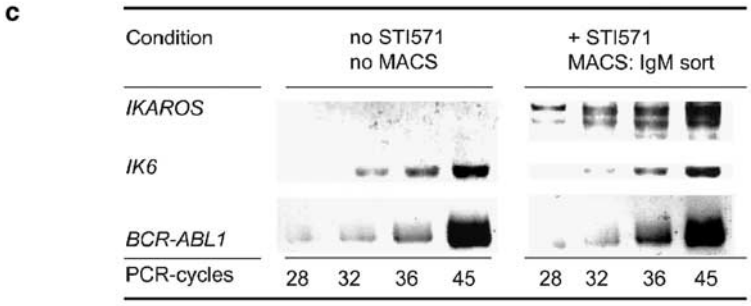
Silencing of dominant-negative IK6 partially restores B lymphoid lineage commitment

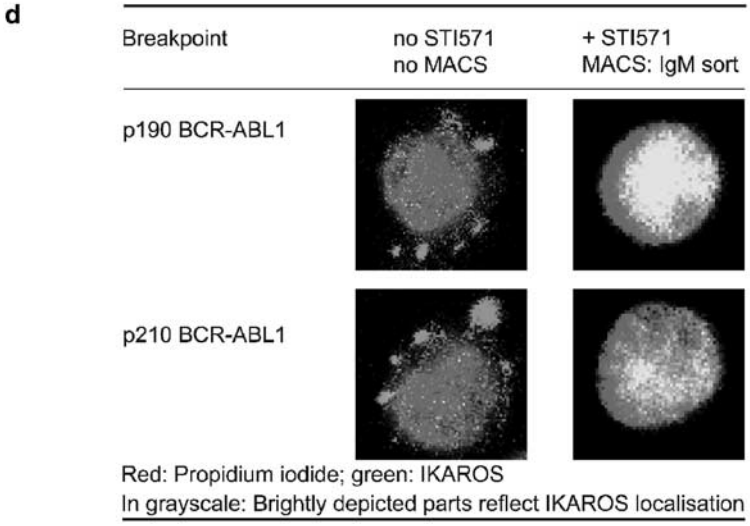
Expression of dominant-negative IK6 can effectively block B-cell differentiation of hematopoietic progenitor cells (HSC) from umbilical cord blood (Tonnelle *et al*., 2001). In the absence of functional IKAROS, hematopoietic stem cells cannot give rise to lymphoid cells and are exclusively diverted into the myeloid lineage (Georgopoulos *et al*., 1994).

In order to provide evidence for a possible link between the expression of IK6 and lineage infidelity in pre-B lymphoblastic leukemia cells, we silenced IK6 expression in two BCR–ABL1$^+$ pre-B lymphoblastic leukemia cell lines (SUP-B15 and BV173). Three different siRNA duplices against IK6 and one nontargeting siRNA duplex were used in transfection experiments. All siRNA duplices were labeled with a Cy2 fluorochrome to identify transfected cells. Cy2$^+$ cells carrying siRNAs were sorted by FACS and subjected to RT–PCR analysis. In both cell lines, IK6 expression was significantly diminished (Figure 4). Using cells transfected with a nontargeting siRNA duplex as a reference, mRNA levels for the V(D)J-recombinase molecule *RAG1* and the B-cell-specific component of the surrogate light chain λ5 were increased, while mRNA levels of the myeloid transcription factor *GATA1* were reduced in parallel with IK6. However, no differences were detected for the mRNA expression levels of *PAX5* (Figure 4). Specificity of the IK6-targeting siRNA duplices was controlled by co-amplification of GAPDH mRNA levels, which remained stable (Figure 4). We conclude that BCR–ABL1-induced expression of IK6 contributes to lineage infidelity observed in *BCR–ABL1*$^+$ pre-B lymphoblastic leukemia cells. However, we cannot exclude that molecules other than IK6 also contribute to BCR–ABL1-induced loss of lineage determination.


a
Vector: pMIG_GFP
Vector: pMIG_BCR-ABL1-GFP
Sort: GFP+
Sort: GFP−
Sort: GFP+
Sort: GFP−
IKAROS
BCR-ABL1
COX6B
28 32 36 45 28 32 36 45 28 32 36 45 28 32 36 45
PCR cycles


b
pMIG-tansfected 697 cells
BCR-ABL1 – +
WB:
BCR-ABL1
EIF4e


c
Condition
no STI571
no MACS
+ STI571
MACS: IgM sort
IKAROS
IK6
BCR-ABL1
PCR-cycles
28 32 36 45 28 32 36 45


d
Breakpoint
no STI571
no MACS
+ STI571
MACS: IgM sort
p190 BCR-ABL1
p210 BCR-ABL1
Red: Propidium iodide; green: IKAROS
In grayscale: Brightly depicted parts reflect IKAROS localisation

Taken together, these findings show that BCR–ABL1 kinase activity (i) induces lineage infidelity in pre-B lymphoblastic leukemia cells, (ii) interferes with IKAROS pre-mRNA splicing and (iii) that aberrant expression of IK6 contributes to lineage infidelity. Therefore, we propose that defective lineage commitment and B-cell differentiation in *BCR–ABL1*$^+$ pre-B lymphoblastic leukemia cells can occur as a consequence of BCR–ABL1-induced derangement of *IKAROS* expression.

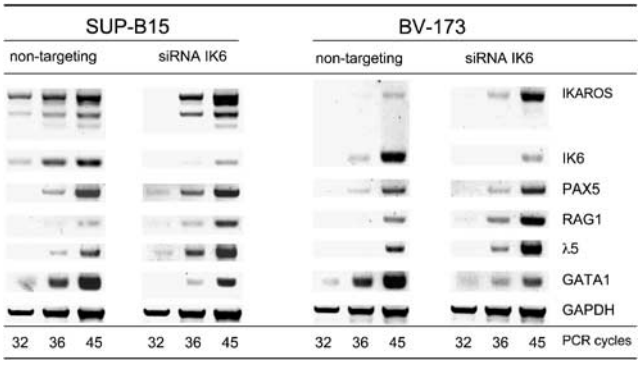


Figure 4 Specific silencing of IK6 partially restores lineage determination in *BCR–ABL1*$^+$ pre-B leukemia cells. *BCR–ABL1*$^+$ pre-B lymphoblastic leukemia cell lines (SUP-B15 and BV173) were transfected either with a pool of siRNA duplices targeting IK6 or a nontargeting siRNA duplex serving as a negative control. Three different IK6 siRNAs were designed and synthesized (MWG Biotech, Ebersberg, Germany) to target the junction of exons 2 and 7, which defines the *IKAROS* splice variant IK6 (sequence data available from EMBL/GenBank under accession number AM085310). The control nontargeting siRNA duplex did not match a known mRNA sequence. The IK6-specific siRNA duplexes were designed according to the guidelines described by Dr Thomas Tuschl at http://www.rockefeller.edu/labheads/tuschl/sirna.html. All siRNA duplices were labeled with Cy2, using an siRNA labeling kit (Ambion, Austin, TX, USA) according to the manufacturer's protocol. siRNAs were applied to the cells with a final concentration of 100 nmol/l for each siRNA, using Oligofectamine (Invitrogen) in Opti-MEM1 medium (Invitrogen) according to the manufacturer's instructions. After 24 h, leukemia cells were re-transfected with labeled siRNAs and subsequently incubated for further 24 h. The silencing effect of siRNAs for IKAROS isoform IK6 was controlled by RT–PCR analysis of IK6 in Cy2$^+$ sorted cells. siRNA-transfected cells were subjected to RT–PCR analysis as previously described and investigated for the expression of *IKAROS*, *IK6*, *PAX5*, *RAG1*, *λ5*, *GATA1* and *GAPDH*. Oligonucleotides used for semiquantitative RT–PCR analysis are listed in Supplementary Table 1.

Abbreviations

IGH, immunoglobulin heavy chain; SAGE, serial analysis of gene expression; siRNA, short-interfering RNA.

Acknowledgements

We would like to thank Martin Krönke (Köln), Janet D Rowley (Chicago) and Klaus Rajewsky (Boston) for continuous support, Wolf-Karsten Hofmann (Berlin) for provision of leukemia samples, George Q Daley (Cambridge) for provision of TONB210, and Stefanie Jauch and Peter Wurst for excellent technical assistance. FK is supported by the Studienstiftung des deutschen Volkes and the Köln Fortune program of the Faculty of Medicine of the University of Cologne. NF is supported by a fellowship of the José-Carreras-Leukemia Foundation. MM is supported by the Deutsche Forschungsgemeinschaft through the *Emmy-Noether*-Programm, the German José-Carreras-Leukemia-Foundation (grant to MM), the Deutsche Krebshilfe through joint project grant 'Mechanisms of Malignant Lymphoma' (MM) and the Ministry of Science and Research for North Rhine-Westphalia through the Stem Cell Network NRW (to MM).

References

Busslinger M. (2004). *Annu Rev Immunol* **22**: 55–79.
Feldhahn N, Klein F, Mooster JL, Hadweh P, Sprangers M, Wartenberg M *et al*. (2005). *J Exp Med* **201**: 1837–1852.
Feldhahn N, Schwering I, Lee S, Wartenberg M, Klein F, Wang H *et al*. (2002). *J Exp Med* **196**: 1291–1305.
Georgopoulos K, Bigby M, Wang JH, Molnar A, Wu P, Winandy S *et al*. (1994). *Cell* **79**: 143–156.
Huettner CS, Zhang P, Van Etten RA, Tenen DG. (2000). *Nat Genet* **24**: 57–60.
Jumaa H, Bossaller L, Portugal K, Storch B, Lotz M, Flemming A *et al*. (2003). *Nature* **423**: 452–456.
Klein F, Feldhahn N, Harder L, Wang H, Wartenberg M, Hofmann WK *et al*. (2004). *J Exp Med* **199**: 673–685.

Figure 3 BCR–ABL1 induces aberrant splicing of IKAROS in human pre-B lymphoblastic leukemia cells. Pre-B lymphoblastic leukemia cells carrying an *E2A-PBX1* gene rearrangement were transiently transfected through electroporation with an expression vector encoding only GFP (pMIG_GFP) or BCR–ABL1 and GFP (pMIG_BCR–ABL1/GFP). Expression of the BCR–ABL1 kinase was identified by Western blot analysis (**b**). EIF4e was used as loading control. At 24 h after electroporation, for each transfection, GFP$^+$ and GFP$^-$ cells were sorted, subjected to RNA isolation, cDNA synthesis and analysed for the expression of *IKAROS* splice variants (**a**). cDNA amounts were normalized for *COX6B* mRNA levels. BCR–ABL1$^+$ pre-B lymphoblastic leukemia cells (BV173) were cultured in the presence or absence of 10 μmol/l STI571 for 4 days. Differentiating IgM$^+$ subclones were enriched by MACS as described previously (Klein *et al*., 2004). Undifferentiated *BCR–ABL1*$^+$ pre-B lymphoblastic leukemia cells (IgM$^-$) and differentiating subclones (IgM$^+$) were subjected to RT–PCR analysis for *IKAROS* isoform expression (**c**). IK6 expression (IKAROS splice variant lacking exons 3–6) was identified by sequence analysis. (Sequence data are available from EMBL/GenBank under accession number AM085310) cDNA amounts were normalized by amplification of BCR–ABL1 fusion transcripts. Localization of IKAROS (**d**) was studied by confocal laser microscopy in *BCR–ABL1*$^+$ pre-B lymphoblastic leukemia cells (BV173 and SUP-B15) expressing either a p210 or a p190 BCR–ABL1 fusion molecule, respectively. For localization of IKAROS, undifferentiated and MACS-enriched IgM$^+$ differentiating subclones were stained with propidium iodide (nucleus) and with anti-IKAROS antibodies (colored image only in HTML version). Nuclear or cytoplasmic localization of IKAROS was analysed using primary antibodies from Santa Cruz Biotechnology (Santa Cruz, CA, USA) together with anti-rabbit IgG-Cy2 (Jackson Immunoresearch). Cells were fixed with 0.4% paraformaldehyde and incubated for 10 min in 90% methanol on ice and subjected to confocal laser-scanning microscopy as described previously (Klein *et al*., 2003).

Klein F, Feldhahn N, Lee S, Wang H, Ciuffi F, von Elstermann M *et al*. (2003). *Proc Natl Acad Sci USA* **100**: 6747–6752.

Klein F, Feldhahn N, Mooster JL, Sprangers M, Hofmann WK, Wernet P *et al*. (2005). *J Immunol* **174**: 367–375.

Klucher KM, Lopez DV, Daley GQ. (1998). *Blood* **91**: 3927–3934.

Kirstetter P, Thomas M, Dierich A, Kastner P, Chan S. (2002). *Eur J Immunol* **32**: 720–730.

Laurent E, Talpaz M, Kantarjian H, Kurzrock R. (2001). *Cancer Res* **61**: 2343–2355.

Look AT. (1997). *Science* **278**: 1059–1064.

Müschen M, Lee S, Zhou G, Feldhahn N, Barath VS, Chen J *et al*. (2002). *Proc Natl Acad Sci USA* **99**: 10014–10019.

Nakase K, Ishimaru F, Avitahl N, Dansako H, Matsuo K, Fujii K *et al*. (2000). *Cancer Res* **60**: 4062–4065.

Perrotti D, Calabretta B. (2002). *Oncogene* **21**: 8577–8583.

Salesse S, Dylla SJ, Verfaillie CM. (2004). *Leukemia* **18**: 727–733.

Sun L, Heerema N, Crotty L, Wu X, Navara C, Vassilev A *et al*. (1999). *Proc Natl Acad Sci USA* **96**: 680–685.

Tonnelle C, Bardin F, Maroc C, Imbert AM, Campa F, Dalloul A *et al*. (2001). *Blood* **98**: 2673–2680.

Velculescu VE, Zhang L, Vogelstein B, Kinzler KW. (1995). *Science* **270**: 484–487.

Supplementary Information accompanies the paper on the Oncogene website (http://www.nature.com/onc).