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Cytogenetic characterization of a BCR-ABL transduced mouse cell line

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Abstract Most patients with Philadelphia (Ph)-positive acute lymphoblastic leukemia (ALL) show evidence of secondary chromosome aberrations that may influence the course of disease and response to treatment. To better understand how these secondary chromosomal aberrations occur and to investigate whether the p185/p190 BCR-ABL fusion protein may directly induce an increased chromosomal instability and subsequently the appearance of clonal chromosome aberrations, three BRC-ABL (p185/p190)-transduced mouse pre-B cell lines were analyzed by spectral karyotyping and fluorescence in situ hybridization. The human wild-type *BCR-ABL* gene was expressed at a level comparable with that in human Ph-positive leukemias at diagnosis. All *BCR-ABL*–transduced cell lines acquired similar clonal chromosomal aberrations. Trisomy 5 was always present, followed by loss of the Y chromosome, trisomy of chromosomes 12 and 18, and an unbalanced translocation between chromosomes X and 12. Thus, ectopic p185/p190 BCR-ABL expression, such as p210 *BCR-ABL, PML-RARA*, or *C-MYC* transduction, may induce an increased chromosomal instability leading to clonal karyotypic evolution, which may mimic secondary chromosome aberrations in human Ph-positive ALL. © 2005 Elsevier Inc. All rights reserved.

1. Introduction

Philadelphia (Ph)-positive acute lymphoblastic leukemia (ALL) is characterized by the presence of the typical t(9;22)(q34;q11), which leads to a fusion of the *BCR* gene on chromosome 22 and the *ABL* gene on chromosome 9. The p185/p190-kDa BCR-ABL fusion protein is subsequently expressed, with an increased tyrosine kinase activity and impairment of several physiological functions such as control of proliferation or apoptosis [1]. Ph-positive ALL accounts for 2–5% of ALL in children and 25–50% of ALL in adults. The prognosis is generally poor. However, allogenic bone marrow transplantation and treatment with the tyrosine kinase inhibitor imatinib may be effective therapeutic options [2,3]. In contrast to chronic myelogenous leukemia (CML), where the majority of patients have a t(9;22)(q34;q11) as the sole chromosomal aberration at diagnosis, most Ph-positive ALL patients show secondary chromosomal aberrations that may influence the course of disease and response to treatment. To better understand how secondary chromosomal aberrations occur in Ph-positive clones and whether p185/p190 itself may directly induce an increased chromosomal instability and the outgrowth of clones with secondary cytogenetic changes, retroviral vector–mediated expression of fusion proteins in human and mouse cells seems to be an ideal tool. It has been shown recently that retroviral transduction of murine interleukin-3–dependent myeloblastic 32D cl3(G) cells expressing p210 BCR-ABL typical for CML may inhibit the apoptotic signal cascade and can lead to chromosomal instability [4,5]. To investigate whether chromosomal alterations also appear over time in a mouse cell line generated by retroviral expression of the human p185/p190 BCR-ABL fusion protein in murine bone marrow cells [6], spectral karyotyping (SKY) and fluorescence in situ hybridization (FISH) analyses were performed. Here we show that *BCR-ABL*–transduced cell lines acquire clonal chromosomal aberrations (i.e., loss of the Y chromosome; trisomy of chromosomes 5, 12, and 18; and an

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unbalanced translocation between chromosomes X and 12), thus demonstrating an increased chromosomal instability.

2. Materials and methods

2.1. Retroviral transduction

Cloning of the cDNA for wild-type human p185/p190 *BCR-ABL* into pSRα MSTtkneo vectors and retroviral gene transfer into normal murine bone marrow cells were performed [7,8]. As described previously, the BM185 mouse cell line, generated by retroviral expression of the human p185/p190 BCR-ABL fusion protein, developed a pre–B-phenotype, thus exhibiting a typical feature of Ph-positive ALL. In an attempt to study in vivo T-cell responses against surrogate tumor antigens in BM185 cells (data not shown), we have produced derivative cell lines by transducing BM185wt cells with retroviral vectors encoding chicken ovalbumin and green fluorescent protein, yielding the cell lines BM185-ova-gfp and BM185-gfp, respectively.

2.2. Reverse-transcriptase and real-time polymerase chain reaction for the detection of BCR-ABL expression

Total RNA was isolated from the cell lines with Trizol (Invitrogen, Karlsruhe, Germany) and the RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA quality was measured using the Agilent device. Reverse transcriptase polymerase chain reaction (RT-PCR) for the detection of the *BCR-ABL* e1a2 fusion transcript was performed as proposed by the BIOMED-1 concerted action [9]. The amplification products were analyzed on a 2% agarose gel. The expression level of BCR-ABL was determined using a light-cycler PCR (Roche, Basel, Switzerland) according to the manufacturer's instructions. The human leukemia cell lines SUP-B15 and K 562 (DSMZ Braunschweig, Germany) were used as controls for the detection of the *BCR-ABL* fusion transcript. Primers were designed to use mouse *β-actin* as the housekeeping gene. As second control, RNA extracted from mouse bone marrow cells (C57Bl6) was used to show the origin of the vector construct.

2.3. Sequencing

Since point mutations (e.g., in the ABL kinase domain) may mediate drug resistance to STI571 therapy in patients with CML and Ph-positive ALL by reactivating the oncogenic potential of the *BCR-ABL* fusion gene [10–13], the RT-PCR product was completely sequenced using a sequencing kit and an automated sequencer (both from Beckman-Coulter, Krefeld, Germany) according to the manufacturer's instructions.

2.4. Cell culture and chromosome preparation

The transduced BCR-ABL–positive cells were cultured at 37°C in Dulbecoo's modified Eagle's medium containing 10% heat-inactivated fetal calf serum, 2% glutamine, and 1% penicillin/streptomycin, and were used for chromosome preparation immediately after the second transduction. Therefore, cells were treated with Colcemid for 1 hour at a concentration of 0.035 μg/mL, incubated in 0.075 mol/L KCl for 20 minutes at 37°C, and fixed in a freshly prepared mixture of methanol/acetic acid (3:1) at room temperature. Cell suspension was dropped onto glass slides in a climate chamber (Polymer, Kassel, Germany) at 22°C and 48% humidity.

2.5. SKY

The three cell lines were cytogenetically characterized by using SKY, as described by Schröck et al. [14]. For SKY analysis, metaphase chromosomes were hybridized for 3 days with a SKY kit for mouse chromosomes (Applied Spectral Imaging, Migdal HaEmek, Israel). Detection of signals was carried out according to the manufacturer's instructions. We used the SpectraCube system (Applied Spectral Imaging) for image acquisition and the SKYView imaging software (Applied Spectral Imaging) for chromosomal analysis. For molecular cytogenetic investigation, 15 metaphases from each cell line were analyzed.

2.6. FISH

To better characterize the unbalanced translocation t(X;12), a probe for the mouse chromosome X (BAC clone RP23-382J5) hybridizing to region F2 was generated. BAC DNA was labeled by random priming with SpectrumGreen-dUTP (Vysis, Downers Grove, IL) as described [15] and then hybridized to metaphase chromosomes. After detection, image acquisition was performed using EasyFISH software (Applied Spectral Imaging).

3. Results

3.1. RT-PCR, real-time PCR, and sequencing proved the expression of the wild-type human BCR-ABL transgene in the BCR-ABL–transduced mouse cell lines

RT-PCR analysis of BM185 wild-type cells showed that the e1a2 fusion transcript, typical for Ph-positive ALL, was present. Sequencing revealed no mutations in the *BCR-ABL* transgene. Thus, the human wild-type *BCR-ABL* e1a2 fusion transcript is expressed in BM185wt cells. Using light-cycler real-time PCR, the BCR-ABL expression level was determined. In the BM185wt cell line and the human *BCR-ABL*–positive cell line SUP-B15, the relation of the cycle threshold (Ct) values after amplification of the housekeeping gene *β-actin* and *BCR-ABL* was 0.8. An amplification of *BCR-ABL* was not detectable in mouse bone marrow cells (Fig. 1).

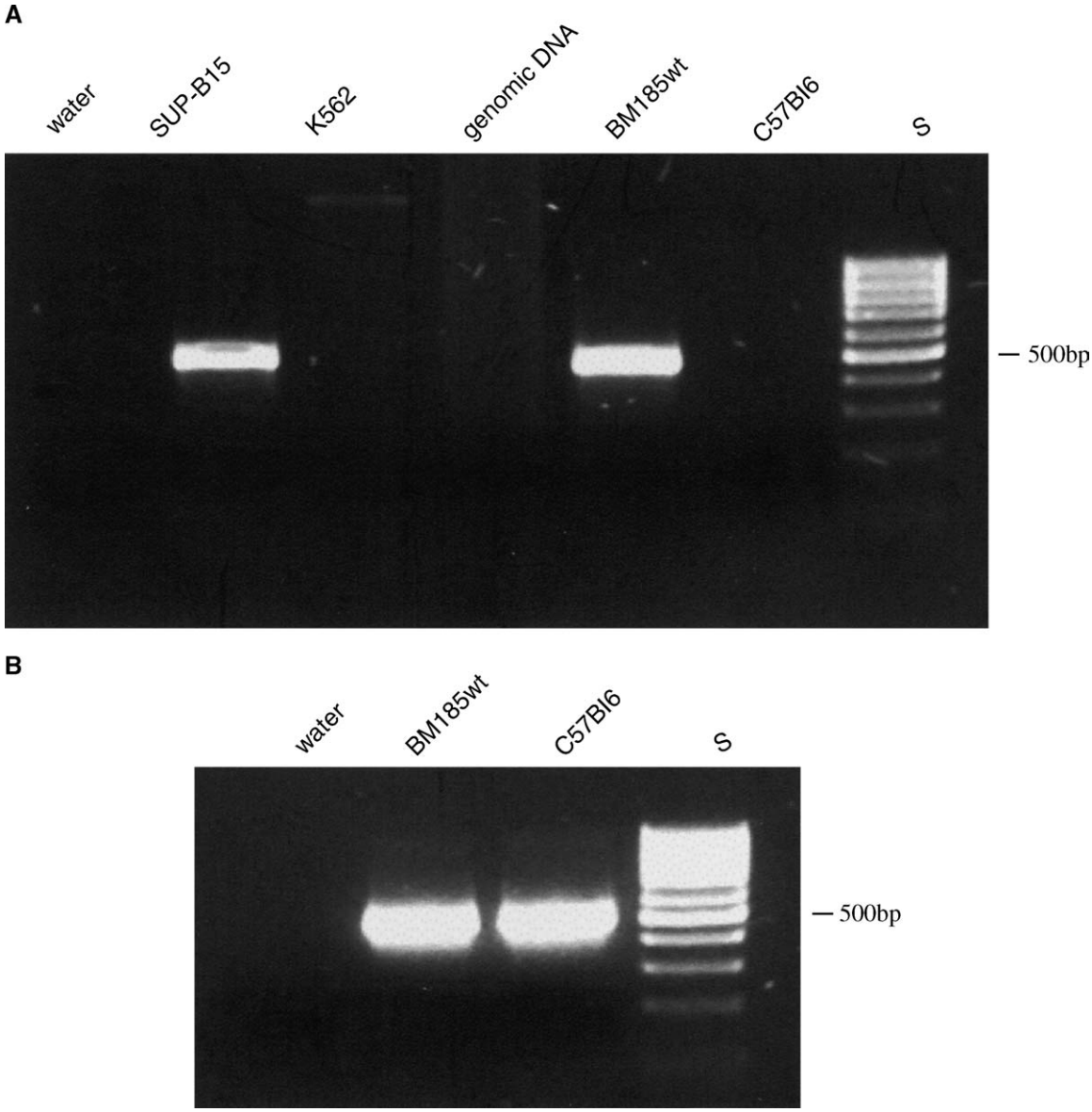

Fig. 1. (A) Detection of the e1-a2 *BCR-ABL* fusion transcript with RT-PCR. Water (contamination control); human leukemia cell line SUP-B15 (positive control); K562 cells (negative control); human genomic DNA; BM 185wt; C57Bl6 (bone marrow cells); S, 100–base pair (bp) length standard. (B) Amplification of the housekeeping gene *β-actin*. Water (contamination control); BM 185wt; C57Bl6 (bone marrow cells); S, 100-bp length standard.

3.2. SKY and FISH reveal clonal aberrations in BCR-ABL–transduced cell lines

Cytogenetic findings are summarized in Table 1. Trisomy 5 was present in all cells. In addition, loss of the Y chromosome, trisomy 18, trisomy 12, or a derivative chromosome 12 resulting from an unbalanced T(XE?;12D) were found in BM185wt, BM185-gfp, and BM185-ova-gfp cells. A representative metaphase analyzed by SKY is shown in Fig. 2.

To quantify the cells carrying +Der(12)T(XE?;12D), we performed FISH analysis on metaphase chromosomes. Fig. 3 shows metaphase chromosomes of BM185wt with two signals for chromosome region XF2 instead of one signal, as expected in a male cell. Fifteen percent of the BM185wt cells and 12% of ovalbumin-green fluorescent protein (GFP)–expressing BM185 cells showed the signal constellation indicative of +Der(12)T(XE?;12D). It can thus be concluded that +Der(12)T(XE?;12D) is a clonal alteration. FISH analysis confirmed that there were no GFP-expressing cells with +Der(12)T(X?E;12D).

Syntenic regions of the mouse chromosome 5 are distributed on human chromosomes 2, 4, 6, 7, 12, 13, and 22. Syntenic regions of mouse chromosome 18 are found on human chromosomes 2, 5, 10, and 18, and syntenic regions of mouse chromosome 12 on human chromosomes 2, 7, and 14. Genes located on mouse chromosome X are found on human chromosomes X, Y, and 3 [16].

Table 1
Cytogenetic results of BM185 wild-type and derivative cells using spectral karyotyping

Investigated cell lines	Karyotype	Percentage of cells with +Der(12)T(XE?;12D)
BM185wt	41,XY,+5[8]/40~44[a],idem, +18,+Der(12)T(XE?;12D), +mar[cp7]	15%
BM185-gfp	41,XY,+5[8]/37~43[a],X, −Y,+5,+12,+18[cp7]	—
BM185-ova-gfp	41, XY,+5[6], 39~40,X,-Y, +5[8]/36[a],XY,+5, +Der(12)T(XE?;12D),+18[1]	12%

Abbreviations: wt, wild-type; gfp, green fluorescent protein; ova-gfp, ovalbumin-green fluorescent protein.

For quantification of cells carrying the translocation Der(12)T (XE?;12D), FISH analysis was carried out using a probe hybridizing to region F2 of the X chromosome.

[a] Some of these metaphases showed losses of different chromosomes, most likely due to technical artefacts.

4. Discussion

In this study, we used a p185/p190 *BCR-ABL*–transduced mouse pre-B cell line as a model for Ph-positive ALL to investigate whether *BCR-ABL* induces an increased chromosomal instability and leads to the appearance of secondary chromosomal aberrations. p185/p190 *BCR-ABL* cDNA had been transduced into normal murine bone marrow cells that can be assumed to have a normal karyotype. Thus, any karyotypic evolution should be the effect of p185/p190 BCR-ABL. However, effects of retroviral transduction itself cannot be ruled out. Unfortunately, cells from mice transplanted with a noncoding vector (i.e., "mock-transduced" cell lines) are not available for cytogenetic characterization.

In a double-transgenic mouse model for acute promyelocytic leukemia (APL), it has been shown that expression of both *PML-RARA* and *BCL2* leads to a rapid development of leukemia and that these leukemias are cytogenetically characterized by predominant numerical alterations (i.e., trisomies syntenic to secondary changes of human AML M3) [17]. In all *BCR-ABL* (p185/p190)-transduced mouse pre-B cell lines analyzed in this study, most of the chromosomal aberrations were also trisomies. The most frequent change was trisomy 5, followed by trisomy 18, trisomy 12, and loss of the Y chromosome. Numerical chromosome aberrations, particularly whole chromosome gains, occur frequently in

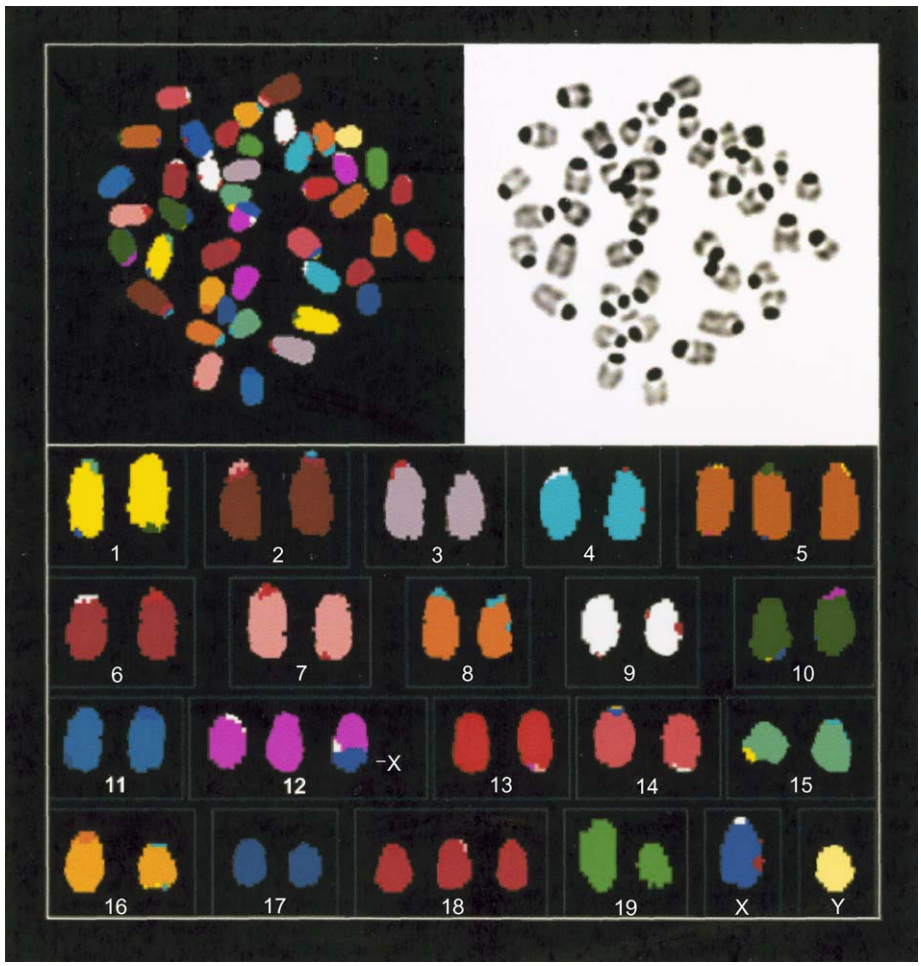

Fig. 2. Molecular cytogenetic analysis of a metaphase spread of a BM185wt cell using SKY. The following were evident: a trisomy 5, a trisomy 18, and in addition to two normal chromosomes 12, a Der(12)T(XE?;12D).

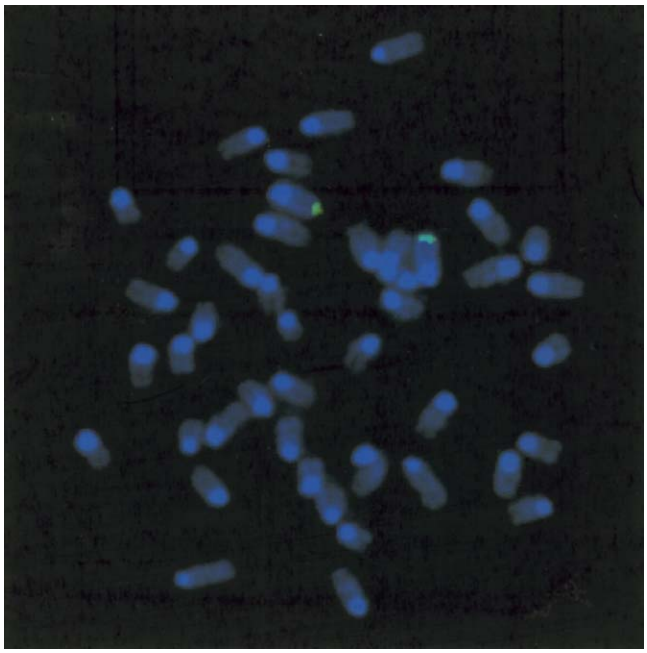

Fig. 3. FISH analysis on metaphase chromosomes of the BM185wt cell line detecting the chromosome X and the Der(12)T(XE?;12D). The FISH probe was generated using the BAC RP23-382J5 clone.

human Ph-positive ALL as well. Moreover, additional Philadelphia chromosomes, losses of 9p, complete or partial monosomy of chromosome 7 (including 7p), and hyperdiploidy are typical secondary changes [18]. Whereas additional Philadelphia chromosomes may lead to an overexpression of *BCR-ABL*, the genes deregulated by other additional chromosomal abnormalities have not yet been identified. Heerema et al. [19] showed that secondary chromosomal alterations occurred in 61% of investigated cases with childhood Ph-positive ALL, most frequently affecting chromosomes 9, 22, 7, 14, and 8.

Since the syntenic regions of mouse chromosomes 5, 18, 12, X, and Y are distributed among many human chromosomes, the relevant chromosomal regions remain unclear. Thus, cytogenetic results found in the murine system are not easily translatable to humans and point out the difficulty in narrowing down critical chromosome regions and identifying the genes that cooperate in leukemogenesis or disease progression.

The *BCR* gene in mice is located on chromosome 10B5.3, whereas the *ABL* gene is located on chromosome 2B [16]. As expected, no alterations in these genes were observed in any of the p185/p190 *BCR-ABL*–transduced mouse cell lines analyzed.

Since all cytogenetically studied p185/p190-transduced cell lines showed very similar chromosome aberrations, we believe that the karyotypic evolution observed in these cell lines has not been induced by the retroviral vector–mediated transduction but, rather, is an effect of p185/p190 *BCR-ABL* function. Measurement of genomic instability in preleukemic p185/p190 *BCR-ABL* transgenic mice revealed an increased frequency of insertions and deletions in kidney and spleen tissues [20]. Furthermore, murine IL-3–dependent myeloblastic 32D cl3(G) cells retrovirally transduced to express p210 *BCR-ABL* developed numerical and structural chromosomal alterations even 2 months after infection. Interestingly, clonal structural alterations affected mainly chromosomes 4 and 12, resulting in a Der(12)T(4E1;12A1) [5]. In this study, chromosome 12 also was involved in both numerical and structural aberrations. The breakpoints in chromosome 12, however, seem to be different. Moreover, an increased frequency of deletions and insertions was found in p190 *BCR-ABL* transgenic mice that may be explained by the downregulation of BRCA1, a key molecule in DNA repair, or by the inhibition of nucleotide excision repair activity by BCR-ABL [21,22]. In conclusion, this study supplies further evidence that chromosomal instability leading to karyotypic evolution may be a result of *BCR-ABL* expression.

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