Int J Hematol (2008) 87:184–188
DOI 10.1007/s12185-008-0031-5

CASE REPORT

A variant transcript, e1a3, of the minor BCR–ABL fusion gene in acute lymphoblastic leukemia: case report and review of the literature

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Received: 11 June 2007 / Revised: 4 October 2007 / Accepted: 13 November 2007 / Published online: 6 February 2008
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Abstract We report a rare case of adult Philadelphia chromosome (Ph)-positive acute lymphoblastic leukemia (ALL) with an e1a3 fusion transcript. A 25-year-old female consulted our hospital for leukocytosis and thrombocytopenia. She was diagnosed with Ph-positive precursor B cell ALL. The patient's BCR–ABL fusion gene showed the e1a3 transcript. She received bone marrow transplantation (BMT) in the first complete remission (CR). However, the disease relapsed 4 months later, and she received a second BMT in the second CR, which caused lethal venoocculusive disease. The duration of the total clinical course was 18 months. We established a new cell line from the patient's leukemic cells at the time of relapse, which is very rare and useful for study as an atypical Ph-positive ALL model. The literature on Ph-positive leukemia lacking ABL exon 2 was also reviewed.

Keywords Philadelphia chromosome · Acute lymphoblastic leukemia · e1a3 · New cell line

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1 Introduction

The Philadelphia chromosome (Ph) is a chromosomal abnormality associated with some specific malignant diseases. It is found in more than 95% of patients with chronic myelogenous leukemia (CML), in 30% of adults and in 2–5% of children with acute lymphoblastic leukemia (ALL), and in occasional cases of acute myelogenous leukemia (AML). The translocation between chromosomes 9 and 22 in Ph, referred to as t(9;22)(q34;q11), results in the formation of a BCR–ABL fusion gene, and is considered as the cause of leukemogensis. The BCR–ABL gene has recently been reported to show variation according to the position of the breakpoint in BCR and/or ABL. Cases with the BCR–ABL transcript lacking ABL exon 2 (a2) have anecdotally been reported, among which one or two of the b2a3, b3a3, and e1a3 fusion transcripts have been detected. In this paper, we describe a rare case of adult Ph-positive precursor B cell ALL with the e1a3 fusion transcript, the clinical course, and a new cell line established from the leukemic cells. The literature on Ph-positive leukemia lacking ABL a2 was also reviewed.

2 Patient and methods

2.1 Patient

A 25-year-old female was admitted to our hospital for leukocytosis and thrombocytopenia in May 2004. The white blood cell (WBC) count was 38.8×10^9/L with 74% lymphoblasts, the hemoglobin level was 12.7 g/dl, and the platelet count was 23×10^9/L. The bone marrow (BM) was hypercellular and karyotype analysis showed 46XX, t(9;22)(q34;q11). Immunophenotypical analysis showed

reactivity with antibodies for CD10, CD19, and HLA-DR. She was diagnosed with Ph-positive precursor B cell ALL, and leukemic cells were shown to have the e1a3 fusion transcript at 170,000 copies/μg RNA by the real-time quantitative polymerase chain reaction (RQ-PCR). Remission induction therapy consisting of daunorubicin, cyclophosphamide (CPA), predonisolone, vincristine (VCR), and L-asparaginase was carried out according to the Japan Adult Leukemia Study Group ALL202 protocol, with the result of complete remission (CR) with 6.0% via fluorescence in situ hybridization (FISH), although RQ-PCR was not done. After two courses of consolidation therapy, she received bone marrow transplantation (BMT) with a CY/TBI (cyclophosphamide, total body irradiation) regimen from a HLA full-matched unrelated donor. The BCR–ABL fusion transcript was detected at 2.5% (FISH) and 8,200 copies/μg RNA (RQ-PCR) just before BMT and 4.0% (FISH) and 50> copies/μg RNA (RQ-PCR) one month later after BMT. Graft-versus-host disease (GVHD) prophylaxis was carried out using cyclosporine (CYA) and short-term methotrexate (MTX) with no GVHD, and the discontinuation of CYA 4 months later after BMT. However, the disease relapsed 4 months later after BMT (almost at the same time as the point of CYA discontinuation) with a WBC count of 36.6×10^9/L. The BM was hypercellular and karyotype analysis showed complicated additional abnormalities besides t(9;22)(q34;q11). Immunophenotypical analysis showed positivity for CD10, CD19, CD34, and HLA-DR. The BCR–ABL fusion transcript was identified at 71.0% and 270,000 copies/μg RNA by FISH and RQ-PCR, respectively. The second CR was obtained with hyper CVAD (CPA, VCR, doxorubicin, and dexamethasone) and high-dose MTX/Ara-C (MTX, cytarabine) [1] with 4.0% (FISH) and 50> copies/μg RNA (RQ-PCR). Thereafter, imatinib mesylate was given as the maintenance therapy. She again received BMT with BU/L-PAM (busulfun, melphalan) regimen from another HLA full-matched unrelated donor. CR had been maintained with minimal residual disease of 4.0% (FISH) and 50> copies/μg RNA (RQ-PCR) just before the second BMT. However, she died of venoocclusive disease 13 days later after that BMT. The duration of the total clinical course was 18 months.

2.2 RQ-PCR and sequence analysis

Samples were taken from the patient's bone marrow at the time of diagnosis. The RQ-PCR assay and sequence analysis were based on a previous report [2]. The nucleotide sequence is accessible under the following NCBI accession number: AF113911.

2.3 Cell culture

Cells were obtained from the patient's bone marrow at relapse with informed consent in accordance with the Declaration of Helsinki. Mononuclear cells (MNCs) were purified by Ficoll centrifugation (Ficoll-Paque Plus, Amersham, Uppsala, Sweden), washed in phosphate-buffered saline (PBS), and cultured in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 2 mM L-glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml), and 10% (vol/vol) heat-inactivated fetal calf serum (FCS; Gibco, Grand Island, NY, USA) at 37°C in a humidified atmosphere containing 5% CO_2. Cultures were fed once weekly by replacing one half of the growth medium with fresh medium. MNCs did not require any supplementation since cells immediately showed spontaneous proliferating activity.

3 Results

3.1 RQ-PCR and sequence analysis

An amplified band of 123 base pairs (bp) was detected when analyzed with the forward primer 5′ATCGTGGGCGTCCGCAAGAC3′ and reverse primer 5′GGCTTCACACCATTCCCCAT3′, which were parts of BCR e1 and ABL a3 regions, respectively (Fig. 1). Sequence analysis showed the deletion of ABL a2 in the PCR product, which corresponded to an e1a3 fusion transcript (Fig. 2).

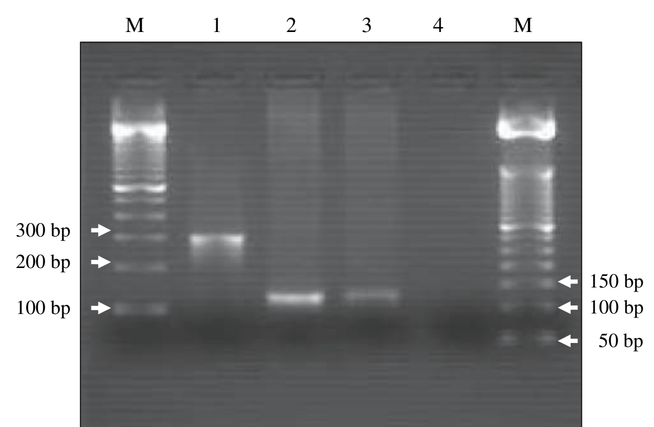

Fig. 1 RQ-PCR analysis. RQ-PCR analysis was performed with the primers described in the text. *Lane 1* e1a2-positive control (297 bp), *Lane 2* the present case at diagnosis (123 bp), *Lane 3* the present case just before the first BMT, *Lane 4* negative control, *M* molecular weight marker

1 gtaccagccc taccagagca tctacgtcgg gggcatgatg gaaggggagg gcaagggccc
61 gctcctgcgc agccagagca cctctgagca ggagaagcgc cttacctggc cccgcaggtc
121 ctactccccc cggagttttg aggattgcgg aggcggctat accccggact gcagctccaa
181 tgagaacctc acctccagcg aggaggactt ctcctctggc cagtccagcc gcgtgtcccc
241 aagccccacc acctaccgca tgttccggga caaaagccgc tctccctcgc agaactcgca
301 acagtccttc gacagcagca gtccccccac gccgcagtgc cataagcggc accggcactg
361 cccggttgtc gtgtccgagg ccacc**atcgt gggcgtccgc aagac**cgggc agatctggcc
421 caacgatggc gagggcgcct tccatggaga cgcagaagcc cttcagcggc cagtagcatc
481 tgactttgag cctcagggtc tgagtgaagc cgctcgttgg aactccaagg aaaaccttct
541 cgctggaccc agtgaaaatg accccaacct tttcgttgca ctgtatgatt ttgtggccag
601 tggagataac actctaagca taactaaagg tgaaaagctc cgggtcttag gctataatca
661 ca**atggggaa tggtgtgaag cc**caaaccaa aaatggccaa ggctgggtcc caagcaacta
721 catcacgcca gtcaacagtc tggagaaaca ctcctggtac catgggcctg tgtcccgcaa
781 tgccgctgag tatctgctga gcagcgggat caatggcagc ttcttggtgc gtgagagtga
841 gagcagtcct ggccagaggt ccatctcgct gagatacgaa gggagggtgt accattacag
901 gatcaacact gcttctgatg gcaagctcta cgtctcctcc gagagccgct tcaacaccct
961 ggccgagttg gttcatcatc attcaacggt ggccgacggg ctcatcacca cgctccatta
1021 tccagcccca aagcgcaaca agcccactgt ctatggtgtg tcccccaact acgacaagt

Fig. 2 Sequence analysis. Amplified bands were formed when analyzed with forward and reverse primers contained in the BCR e1 and ABL a3 regions, respectively (*bold*). A band of 123 bp was formed when the ABL a2 region (174 bp, *underlined*) was deficient in the present case, while a band of 297 bp was formed in the e1a2-positive control

3.2 Establishment of the FUN cell line

The cultured cells began to proliferate actively 15 weeks after the initial culture, and were subcloned. After about 20 weeks, cell growth became continuous and the cell line "FUN" was established. The FUN cells proliferate as free-floating cells and have been maintained continuously for over 33 months with a doubling time of about 30 h. The FUN cells were negative for Epstein Barr virus DNA by reverse transcriptase PCR (RT-PCR) (data not shown). Expression of the e1a3 fusion transcript was detected on FUN cells by RT-PCR (data not shown).

3.3 Surface antigen and karyotype of FUN cell line

CD10 and CD19 were expressed on FUN cells, while CD34 and HLA-DR were not. The karyotype of FUN cells was revealed to be t(9;22)(q34;q11) by G-banding.

4 Discussion

BCR–ABL fusion gene shows variation according to the position of the breakpoint in BCR and/or ABL. In nearly all patients with the BCR–ABL gene, the BCR–a2 junction is detected, such as b2a2 and/or b3a2, e1a2, and e19a2, which are transcribed into major, minor, and micro bcr–abl messenger RNA, respectively. A BCR–ABL gene lacking ABL a2 has; however, been recognized by several groups (Table 1) [3–22].

The incidence of the BCR–ABL gene lacking ABL a2 is theoretically 0.3%, assuming that the breakpoint in ABL is equally distributed [4]. The number of reported BCR–a3 cases is, however, small compared to the calculated number based on the theoretical frequency of BCR–a3 cases.

A possible reason for this mismatch may be due to the methodology of RT-PCR, as pointed out previously [4]. Actually, we missed the e1a3 fusion transcript at first in the present case by RQ-PCR using the primer corresponding to ABL a2 sequences in spite of the existence of t(9;22)(q34;q11) by G-banding. We performed another RQ-PCR with the primer corresponding to ABL a3 sequences, resulting in the appearance of an e1a3 fusion transcript band. There might be more cases that present BCR–a3 fusion transcripts if an adequate primer becomes routinely used in RT-PCR. Although limited to Korean CML patients, the frequency of BCR–a3 cases was 0.36% (2 of 548) when appropriately diagnosed, which was in accordance with the theoretical frequency [21]. Alternatively, it is possible that the breakpoint in ABL does not occur randomly for unknown reason(s), resulting in a lower frequency of BCR–a3 fusion transcripts than theoretically expected.

The low WBC count was suggested to be characteristic of leukemia with a deletion of ABL a2 [6, 7, 16, 23]. Table 1 shows the reported cases of leukemia with BCR–a3 fusion transcripts so far. This seems to suggest nothing in particular in terms of the WBC count in these rare cases, ranging from 8.4 to 209.0 $\times 10^9$/L in ALL and from 3.7 to 416.0 $\times 10^9$/L in CML. From a genetic point of view, the WBC count ranges from 3.7 to 189.5 $\times 10^9$/L in e1a3 cases, from 32.9 to 416.0 $\times 10^9$/L in b2a3 cases, and from 9.0 to 95.8 $\times 10^9$/L in b3a3 cases. The WBC count is not necessarily low, although more cases are needed to draw a definite conclusion.

RQ-PCR analysis with the primer corresponding to ABL a3 sequences showed a 123-bp band in the present case, in comparison with an e1a2-positive control band (297 bp), suggesting that the ABL a2 region (174 bp) was completely deficient, which was confirmed by sequence analysis (Figs. 1, 2).

The ABL a2 region encodes a part of the *Src* homology (SH) 3 domain. The SH3 domain is considered to play a negative regulatory role in the kinase domain (SH1) [24]. Therefore, the lack of a SH3 domain might result in a more aggressive form of Ph-positive leukemia. On the other hand, the SH3 domain is required for STAT5 activation by BCR–ABL protein, leading to full leukemogenesis [25]. So, deletion of the SH3 domain might induce a less progressive clinical course. Although the role of the SH3 domain in leukemogenesis is still controversial, lack of the SH3 domain did not have an effect on the more favorable

Table 1 Summary of leukemia patients with the BCR–ABL transcript lacking ABL exon 2

Case No.	Age/sex	BCR–ABL transcript	Type of leukemia	WBC count (× 10^9/L)	Clinical outcome	Duration of follow up (months)	Authors	Published year
1	61/F	b2a3	ALL	209.0	Dead	14	Soekarman	1990
2	39/F	e1a3	ALL	8.4	Dead	9	Soekarman	1990
3	59/M	b2a3	CML	254.0	Alive	30	van der Plas	1991
4	Unknown	b2a3	Unknown	Unknown	Unknown	–	Tuszynski	1993
5	3/F	b3a3	ALL	11.4	Dead	41	Inukai	1993
6	39/F	b3a3	CML	9.0	Alive	Unknown	Iwata	1994
7	1/F	e1a3	ALL	95.0	Dead	54	Iwata	1994
8	27/M	Unknown	CML	Unknown	Dead	Unknown	Paldi-Haris	1994
9	19/M	b3a3 + b2a3	CML	42.0	Dead	34	Polak	1998
10	86/F	b2a3 + e1a2	AML	Unknown	Unknown	–	Paietta	1998
11	23/M	b3a3	CML	95.8	Alive	60	Amabile	1999
12	68/F	e1a3 + b2a3	CML	38.4	Alive	20	Martnelli	1999
13	69/M	b3a3	CML	18.0	Alive	36	Tiribelli	2000
14	51/M	b3a3	CML	19.9	Alive	126	Tiribelli	2000
15	Unknown	b2a3	CML	Unknown	Unknown	–	Wilson	2000
16	Unknown	e1a3	ALL	Unknown	Unknown	–	Wilson	2000
17	62/F	b2a3	CML	78.0	Alive	Unknown	Guillaume	2000
18	75/F	e1a3	CML	18.5	Alive	64	Roman	2001
19	33/M	b2a3	CML	32.9	Alive	19	Otazu	2002
20	64/F	e1a3	CML	53.2	Alive	62	Al-Ali	2002
21	41/M	e1a3 + e1a2	CML	189.5	Alive	56	Al-Ali	2002
22	49/M	b2a3	CML	87.0	Alive	24	Liu	2003
23	31/M	b2a3	CML	416.0	Alive	35	Snyder	2004
24	Unknown	b2a3	CML	Unknown	Unknown	–	Goh	2006
25	42/M	e1a3	CML	3.7	Alive	Unknown	Goh	2006
26	39/M	b2a3	CML	163.0	Alive	22	Pienkowska-Grela	2007
27	25/F	e1a3	ALL	38.8	Dead	18	Present case	2008

clinical course, at least, in the present case, considering early relapse after the first BMT.

The reported BCR–a3 cases showed different clinical outcomes (Table 1). A poor prognosis was recognized in all ALL cases, as in typical ALL cases that have a BCR–a2 transcript. The outcomes in CML cases in blastic crisis were also poor [8, 9]. In some CML cases, especially in recent, newly diagnosed CML cases, the chronic phase has been continuing for a long time mainly with imatinib mesylate [17–22]. This prognostic difference between ALL and CML might be due to the essential variation in leukemic cells themselves in terms of aggressiveness.

A cell line derived from an ALL case with the b3a3 fusion transcript was previously established [6]. We established another new cell line called "FUN" from the leukemic cells of the present case at the time of relapse. The surface antigens and karyotype of FUN cells are partly different from those of the original leukemic cells in the patient. The disappearance of CD34 and HLA-DR in the antigen might be due to transformation during cell culture, and the disappearance of complicated additional abnormalities in the karyotype might be due to the selection of simple Ph-positive leukemic cells during cell culture. Regardless, this is, to our knowledge, the first Ph-positive cell line with the e1a3 fusion transcript. Although the number of patients with this atypical fusion transcript might be limited, this may offer a useful model system for investigating Ph-positive leukemia.

In conclusion, the Ph-positive leukemia with the BCR–a3 fusion transcript is a rare disease, but more cases could be identified when properly diagnosed with an adequate method. The further accumulation of cases would help elucidate the pathology and characteristics of the disease.

Acknowledgments We thank BML Inc. for helping us with the experiment.

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