CASE REPORT

Chronic Myeloid Leukemia with Rare e1a3 BCR-ABL Transcript

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ABSTRACT

Chronic myelogenous leukemia (CML) results from neoplastic transformation of a hematopoietic stem cell. It is cytogenetically characterized by the presence of Philadelphia chromosome which results from reciprocal translocation t(9;22) that juxtaposes the ABL gene on chromosome 9 with breakpoint cluster region (BCR) on chromosome 22 generating BCR-ABL oncogene. All BCR-ABL fusion proteins display activated tyrosine kinase activity. Their different types are associated with different clinical course and prognosis. We report a rare case of e1a3 BCR-ABL transcript. So far only 4 cases in patients with CML have been reported.

KEY WORDS: *BCR-ABL, e1a3, CML.*

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INTRODUCTION

The BCR-ABL fusion protein is a product of the reciprocal t(9;22) translocation found in chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL). Breaks in the BCR gene occur in one of the following three regions i.e. Major breakpoint cluster region (M-BCR), minor BCR (m-BCR) and micro-BCR (µ-BCR).[1] In majority, M-BCR is involved whereas rearrangements with m-BCR and µ-BCR are rare. Breakpoints in ABL gene are commonly between exon 1a or 1b and a2.[2] Sometimes it occurs within the third intron of the ABL gene.[3]

Several molecular techniques like Southern blot, FISH and Conventional RT-PCR are currently used for the detection of BCR-ABL gene. However, Conventional RT-PCR without cytogenetics can miss the detection of rare cases if proper primers are not used. Multiplex RT-PCR is similar to conventional RT-PCR but includes more than one pair of primers. Presently it is considered a reliable technique to identify typical and atypical BCR-ABL transcripts in a single reaction.[4]

CASE

A 62 years old female came with complains of abdominal pain and fatigue. Physical examination revealed splenomegaly. Her CBC was RBC 3.74x10^9/L, Hb 10.9g/dl, WBC 292.2 x10^9/L , Neutrophils 48%, Promyelocytes 3%, Myelocytes 20%, Metamyelocytes 11%, Bands 7% ,Blasts 4%, Eosinophils 2%, Basophils 1% ,Lymphocyte 1% and PLT count 365x10^9/L . Peripheral and bone marrow findings were consistent with the diagnosis of CML in chronic phase. Hydroxyurea was given to lower the counts.

Multiplex RT-PCR was set up to determine BCR-ABL with break points using Seeplex kit. Whole blood sample was taken and plasma was separated. RNA extraction from plasma was done using Epicenter kit from Quaigen, Germany. Cycling conditions for RT-PCR are shown in table 1.Primers combination in the multiplex RT-PCR allowed simultaneous detection of eight types of BCR-ABL and BCR transcript in one reaction. The expected bands were as follows: 600bp internal control or normal BCR, 1012bp c3a2, 754bp b1a1, 476bp b3a2, 401bp b2a2, 348bp e1a2, 299bp, b3a3, 224bp b2a3 and 174bp e1a3. Quality of the RNA and efficiency of cDNA synthesis was analyzed by amplification of BCR gene as an internal control. The amplified product (600bp) from the BCR gene was the only band detected in BCR-ABL negative patients. Absence of this band indicated procedural failure. Gel was interpretated according to the BCR-ABL marker (M) using Seeplex leukemia detection user manual as a reference. M was used to amplify the approximate size of target product run on a gel electrophoresis. BCR-ABL positive control (PC) was a mixture of BCR-ABL (b2a2, e1a2). Both M and PC were present in the kit.

An e1a3 transcript was identified (Figure 2). To confirm it, RT-PCR of bone marrow aspirate was done . Result was same.

Table 1. Cycling Conditions of Multiplex RT-PCR

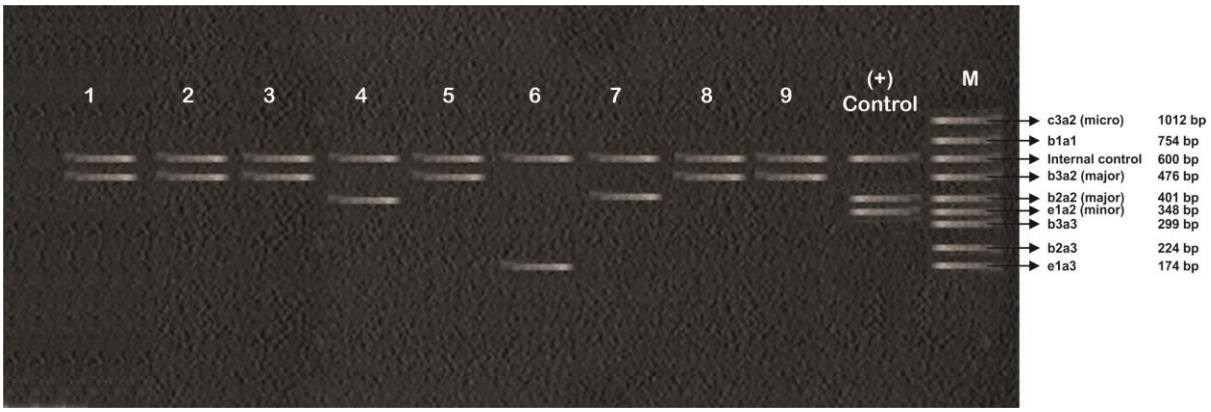
Segment	Number of Cycles	Temperature	Duration
Initial duration	01	94^0C	15 mins
Denaturation	37	94^0C	0.5 min
Annealing	37	60^0C	1.5 mins
Extension	37	72^0C	1.5 mins
Final extension	01	72^0C	10 mins

In Figure 2, Lane M is the marker while lane 10 is the positive control which is a mixture of BCR-ABL (b2a2, e1a2) incorporated in the kit. Lanes 1, 2, 3, 5, 8 and 9 show b3a2 (476 bp) while lanes 4 and 7 show b2a2 (401 bp). In lane 6 minor mutation e1a3 (348 bp) is identified.

However recent data suggest that the SH3 domain does not influence the intracellular signalling that regulate proliferation and survival of BCR/ABLp210 transfected cells but is required for full leukaemogeneic potential in vivo. The molecular mechanism underlying the impaired leukaemogenic potential of b3a3-transfected cells appears to involve changes in the adhesion and motility of these cells. Moreover it has been demonstrated that intact ABL SH3 domain is required for induction of STAT5 activity and that an STAT5 dependent pathway plays a crucial role in BCR-ABL leukemogenesis because it is involved in anti apoptotic activity and cell cycle progression

induced by the oncogeneic protein. Studies in murine models of CML have shown that the b3a3 version of BCR-ABL/p210 induces a myeloproliferative disease characterized by a small delay in the onset of disease and by an increased survival compared with the b3a2 version.

Figure 2: Results of RT-PCR



DISCUSSION

Small amounts of p190 transcripts are found in CML patients expressing p210 and are believed to arise from alternative splicing of the longer b2a2/b3a2 component [5]. The rare e1a3 BCR-ABL transcript identified in our study has been previously reported in four[6-8] patients with CML in chronic phase. The first case reported by Roman at al had a benign clinical course with neutrophilic predominance resembling Chronic neutrophilic leukemia (CNL) .The patient was not on any treatment. The 2 patients of CML reported by Al-Ali et al also had a benign clinical course, and achieved complete cytogenetic remission after imatinib therapy. However monocytosis was noted in both cases. Other case of CML in chronic phase with e1a3 transcript reported by Jordi-Martinez-Serra et al also responded well to imatinib therapy, but after 5 months transformed into lymphoid blast crises. Our patient had a benign clinical course with neutrophil predominance resembling CNL

Since e1a3 and e1a2 transcripts have a similar molecular weight one could expect patients with e1a3 transcript to disclose a similar clinical profile[6]. e1a2 a p190 fusion is associated with monocytosis, extramedullary infiltration and disease acceleration resembling chronic myelomonocytic leukemia[5] (CMML) . 2 cases reported by Ali et al showed monocytosis.

The varied clinical course and presentation in all e1a3 patients shows the importance of identification of rare fusion transcripts in CML. Although reports suggest $P190^{BCR-ABL}$CML have an aggressive course with an inferior outcome to Tyrosine kinase inhibitor therapy[9],more cases are required to establish the relationship between e1a3 and their clinical course to p210 CML.

REFERENCES

[1] Deninger MWN, Goldman JM and Melo JV.The molecular biology of chronic myeloid leukemia. Blood.2000; 96:3343-3353.

[2] Yaghmaie M, Ghaffari SH, Ghavamzadeh A, Alimoghaddam K, Jahani M, Mousavi SA, Irvani M, Bahar B, Birbordi I. Frequency of BCR-ABL fusion transcripts in Iranian patients with chronic myeloid leukemia . Arch Iranian Med. 2008; 11(3): 247-251.

[3] Melo J.V. BCR-ABL variants. Bailliere's Clinical Haematology. 1997; 10, 203±222.

[4] Goh HG, Hwang JY, Kim SH, Lee YH, Kim YL and Kim DW. Comprehensive analysis of BCR-ABL transcript types in Korean CML patients using a newly developed Multiplex RT-PCR Translational Research 2006;148:249-256.

[5] Ravandi F, Cortes J, Albitar M, et al. Chronic myelogenous leukemia with p185 (BCR-ABL) expression: characteristics and clinical significance. Br J Haematol.1999; 107:581-586.

[6] Roman J, Jimenez A, Barrios M, Castillejo JA, Maldonado J, Torres A. E1a3 as a unique ,naturally occurring BCR-ABL transcript in an indolent case of chronic myeloid leukemia .Br J Haemtol.2001;114:635-637.

[7] Al-Ali HK, Leibein S, Kovacs I, Hennings F, Niederwieser D, Deininger M. CML with an e1a3 BCR-ABL fusion :rare, benign and a potential diagnostic pitfall. Blood. 2002; 100(3):1092-1093.

[8] Martinez-Serra J, Campo R, Gutierrez A, Antich JL et al. Chronic myeloid leukemia with an e1a3 BCR-ABL fusion protein :transformation into lymphoid blast crisis. Biomarker Research.2014; 2(14):1-4.

[9] Verma D, Kantarjian HM, Jones D, Luthra R, Borthakur G, Verstovsek S et al .CML with p190 BCR-ABL: analysis of characteristics, outcomes and prognostic significance. Blood.2009; 114(11):2232-2235.