



## Analysis of *BCR/ABL* transcripts in healthy individuals

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Genet. Mol. Res. 12 (4): 4967-4971 (2013)

Received February 5, 2013

Accepted July 18, 2013

Published October 24, 2013

DOI <http://dx.doi.org/10.4238/2013.October.24.8>

**ABSTRACT.** The chimeric oncogene *BCR/ABL*, which is the product of reciprocal translocation between chromosomes 9 and 22, is a known molecular marker of chronic myeloid leukemia (CML), and is related to the major factors involved in leukemogenesis. Some previous studies have also reported the presence of this oncogene in peripheral blood cells of healthy individuals. In this study, we investigated the presence of *BCR/ABL* transcripts in peripheral blood of individuals aged 40 years or more without symptoms of CML. The presence of *BCR/ABL* transcripts was observed in 2 of the 30 individuals analyzed. The genesis of *BCR/ABL* transcripts and its presence in healthy individuals are topics of ongoing debate. The risks and biological implications of the presence of *BCR/ABL* transcripts in healthy individuals are challenging issues that remain to be elucidated.

**Key words:** Chronic myeloid leukemia; Chromosome rearrangements; *BCR/ABL*

## INTRODUCTION

Chromosome rearrangements in somatic cells are one of the major factors involved in cancer development. One of the most studied chromosomal rearrangements is a reciprocal t(9;22) chromosomal translocation that leads to the Philadelphia chromosome (Nowell and Hungerford, 1960), which is present in 95% of chronic myeloid leukemia (CML) cases (Rowley, 1973). This rearrangement results in the production of the BCR/ABL fusion protein, which is responsible for the oncogenic status of cells, promoting imbalance in the cell cycle, the acquisition of growth factors, and resistance to apoptosis (Di Bacco et al., 2000; Burke and Carroll, 2010). In CML, the vast majority of breakpoints in the *BCR* gene occur within the major breakpoint cluster region (M-bcr), and the resultant BCR-ABL mRNA molecules encode a p210<sup>BCR/ABL</sup> fusion protein with a b2a2 or a b3a2 junction (Melo, 1996).

Some studies have shown the presence of b3a2 and b2b2 transcripts at low levels in peripheral blood cells of healthy individuals without any clinical symptoms or hematological leukemia (Biernaux et al., 1995; Bose et al., 1998). According to Biernaux et al. (1995), age influences *BCR/ABL* positivity in healthy individuals, and is rarely detected in healthy children. This is most likely due to the accumulation of molecular and cellular damage with increasing age.

Bose et al. (1998) detected transcripts of p210<sup>BCR/ABL</sup> proteins in leukocytes of 4 of 15 (27%) non-affected subjects. These data revealed that the gene expression of *BCR/ABL* in healthy subjects should be further examined as such knowledge might enable early diagnosis of genetic transformants. Thus, the purpose of this study was to investigate the presence of *BCR/ABL* transcripts in a group of asymptomatic individuals with respect to CML.

## MATERIAL AND METHODS

### Subjects

With the approval of the Human Ethics Committee of the Assis Gurgacz Faculty, samples were collected from peripheral blood of 30 non-related asymptomatic volunteers aged above 40 years, who did not have any previous hematological or genetic disorder. Informed consent was obtained from all individuals participating in the study. Our sample consisted of 20 female subjects and 10 male subjects.

### Molecular analysis of BCR/ABL

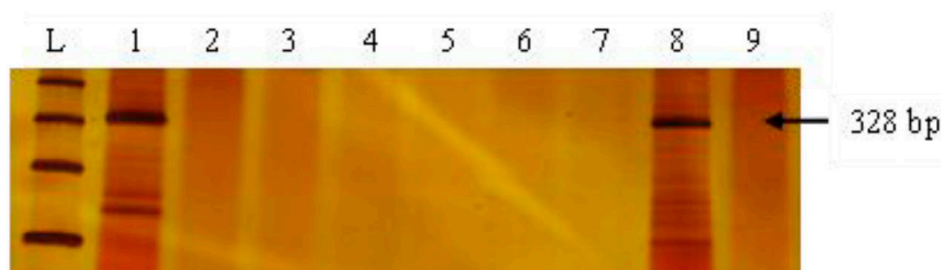
Total cellular RNA was extracted from peripheral blood leukocytes using the Trizol reagent (Invitrogen, USA) according to manufacturer instructions. The material was then re-suspended in 18  $\mu$ L sterile diethylpyrocarbonate-treated water. The cDNA was generated from 6  $\mu$ L total RNA using the outer antisense primer and the cDNA GeneAmp RNA PCR synthesis kit (Perkin Elmer, USA).

The primers used for amplification of *BCR/ABL* mRNA were obtained from the sequence deposited in GenBank (accession No. AJ 131466): LM1 outer sense: 5'-TTCAGAAGCTTCTCCCTG-3'; LM2 outer antisense: 5'-CTCCACTGGCCACAAAAT-3'; LM3 inner sense: 5'-TTCAGAAGCTTCTCCCTGACATCCG-3'; LM4 inner antisense:

5'-CTCCACTGGCCACAAAATCATAACAG-3'. The sense primers LM1 and LM3 were derived from the b2 exon of M-bcr, and the antisense primers LM2 and LM4 were derived from exon a2 of *ABL*. After nested-polymerase chain reaction (PCR), a fragment of 328 or 253 bp was generated in the presence or absence of exon b3, respectively. The conditions for both PCR cycles were as follows: 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200 μM dNTP, and 1.25 U Taq polymerase, and consisted of an initial denaturation step at 93°C for 2 min followed by 35 cycles of 93°C for 40 s, 60°C for 30 s, 72°C for 40 s, and a final extension at 72°C for 10 min in a thermocycler (ThermalCycler Gradient, Eppendorf, USA). The amplified product was analyzed by electrophoresis on 10% polyacrylamide gel for 1 h, and visualized by silver staining. The samples were analyzed in triplicate to avoid observing false-positive results.

## RESULTS

Positive amplifications of mRNA *BCR/ABL* were obtained in two samples (6.67%), which were both female subjects over 61 years of age. There were no positive samples observed among male subjects. These results were consistent with b3a2 chromosomal fusion, as shown in Figure 1.



**Figure 1.** Expression of *BCR-ABL* transcripts in peripheral blood cells from asymptomatic subjects. PCR products were submitted to electrophoresis on 10% silver-stained acrylamide gels. Two samples (*lanes 1 and 8*) showed 328-bp amplicons, corresponding to b3a2 transcript. *Lanes 2, 3, 4, 5, 6, 7, 9* = absence of *BCR-ABL* transcript. *Lane L* = a 100-bp ladder.

## DISCUSSION

The detection of Philadelphia chromosomes and the evaluation of the expression of *BCR/ABL* transcripts are considered as the initial events of CML, and are recommended in therapeutic evaluations in diagnosed individuals. The expression of *BCR/ABL* results in the formation of chimeric proteins with abnormal tyrosine kinase activity, p210<sup>BCR/ABL</sup>, which exert oncogenic effects throughout the course of the disease (Saglio et al., 1996; Kurzrock et al., 2003). *BCR/ABL* proteins lead to an unbalanced cell cycle, increasing the activity of tyrosine kinase, and continuing the cycle even in the absence of growth factors (Di Bacco et al., 2000; Fernandes-Luma, 2000; Goldman et al., 2009).

A limited number of studies have examined *BCR/ABL* rearrangements in healthy individuals, although the biological effect of the expression of this oncogene in asymptomatic individuals has been discussed previously. Biernaux et al. (1995) found a 30.14% (N = 73)

positivity rate of *BCR/ABL* in healthy subjects between 20 and 80 years of age, while Bose et al. (1998) found a rate of 27% in a group (N = 15) aged between 23 and 46 years. In the present study, the two positive samples were found in subjects over 61 years of age.

Aging is known to be related to the increased incidence of cancer due to increased genetic instability associated with the accumulation of irreparable DNA lesions, and the relatively longer period of exposure to carcinogens (Jackson and Bartek, 2009). Thus, it is possible that the relative risk is proportional to age, increasing in accordance with the accumulation of mutations in precursor cells that are responsible for the pathogenesis of leukemia. Moreover, the genesis of *BCR/ABL* and its mechanisms of interaction with cellular components have also been discussed. The exposure to environmental factors, such as radiation, has been associated with the origin of mutations that are precursors to hematological diseases, although such diagnoses are dependent on other manifestations.

Chromosomal translocations and gene fusions are not sufficient to generate malignancy (Bose et al., 1998). Rather, a combination of factors is necessary: the gene fusion is capable of producing proteins with oncogenic activity without degradation, it originated in a primitive hematopoietic progenitor, and it shows proliferative potential. It is likely that the *BCR/ABL* transcripts found in healthy individuals differ from those found in leukemic individuals, which produce truncated proteins that are unable to promote proliferative advantage in these cells.

The development of CML is related to pathogenic co-factors, such as immunological defects or a second genetic aberration (Kurzrock et al., 2003). The stage of differentiation of the positive *BCR/ABL* cell should be critical for disease development. Thus, the expression of this gene (or of the fusion products) should not be the only factor exerting important biological effects on mature cells (Brassescio et al., 2009). Recently, Cosset et al. (2011) reported the upregulation of the oncogene *TWIST-1* at both the RNA and protein levels in tyrosine kinase inhibitor resistant cell lines of CML patients. This overexpression of the *TWIST-1* oncogene might represent a novel key prognostic factor that could be potentially useful for optimizing therapy and diagnostics of CML (Cosset et al., 2011).

Although the detection of low levels of *BCR/ABL* transcripts in CML patients is used in disease control strategies, new observations have demonstrated that malignant cell persistence is not necessarily dependent on higher activity in *BCR/ABL* signaling pathways (Jilani et al., 2008; Corbin et al., 2011; Nguyen et al., 2011). The reverse transcription-PCR technique is extremely sensitive, and can detect primary transcripts even at low levels of expression. Different types of *BCR-ABL* junctions are found in the diagnosis of CML, which fall into two main forms, b3a2 and b2a2, and two sporadic forms, e1a2 and e19a2 (Biernaux et al., 1995; Bose et al., 1998; Barbosa et al., 2000). In the present study, we were able to demonstrate the presence of *BCR/ABL* transcripts in healthy individuals even within a small sample size from south Brazil.

Bayraktar and Goodman (2010) reported the case of a 39-year-old man who was asymptomatic for CML with normal blood cell count, but was positive for *BCR/ABL*, as detected by fluorescence *in situ* hybridization and real-time PCR. This case presented a challenging question related to the appropriate conduct for treatment and monitoring, which leads to an important dichotomy in therapy: either 1) treat the patient whose only characteristic of the disease in question is the persistent positivity for *BCR/ABL*, thus potentially unnecessarily exposing him to the adverse effects of medication without a defined therapeutic goal, or 2) wait for the eventual development of leukemia with close monitoring of the evolution of *BCR/ABL* transcripts (Bayraktar and Goodman, 2010).

The detection of these transcripts in asymptomatic individuals demonstrates an important biological implication: the identification of mRNA *BCR/ABL* does not necessarily directly indicate leukemic disease, but may instead indicate a risk condition for malignant transformation.

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