International Journal for Biotechnology and Molecular Biology Research Vol. 2(7), pp. 128-130, July 2011
Available online http://www.academicjournals.org/IJBMBR
ISSN 2141-2154 ©2011 Academic Journals

Short Communication

Authentication of fusion genes in chronic myeloid leukemia

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Accepted 30 May, 2011

Chronic myeloid leukemia is a multifactorial molecular anomaly that confounds the standardization of therapy to date. It is triggered by a broad spectrum of "fused oncoproteins" which are entailed in the disease refractoriness. In Pakistan the molecular diagnosis for leukemia is still in its infancy, as the diagnosis does not efficiently encompass a wide range of the fusion transcripts which are generated as a result of exclusive genomic rearrangements. Two point mutations C944T and T932C of ABL gene were detected which cause complete/partial imatinib resistance with limelight NUP98-LEDGF fusion transcript. It will be helpful in understanding primary resistance of molecularly targeted cancer therapies.

Key words: Allele-specific oligonucleotide polymerase chain reaction (ASO-PCR), leukemia, myeloid, chronic, imatinib.

INTRODUCTION

Hematological malignancies are the most prevalent molecular anomalies around the world. Fusion transcripts have been widely characterized in various diseases. These fusion transcripts are generated by unfaithful repair of DNA that results in illegitimate genomic rearrangements (Ammad et al., 2011). Imatinib mesylate (Gleevec) is the frontline drug for the clinical management of chronic myeloid leukemia (CML) (O'Dwyer et al., 2003). It is a matter of great concern that BCR-ABL kinase domain (KD) is the hotspot for point mutations. These mutations induce steric hindrance and drastically abrogate Imatinib mesylate binding by impairing critical interactions between ABL and Imatinib mesylate (Azam et al., 2003). Lens epithelium derived growth factor (LEDGF) is a transcriptional co-activator that contains coiled-coil domains that mediate self-association of the NUP-X chimaeric proteins and are documented in leukemic patients (Hussey et al., 2001; Hussey and Dobrovic, 2002; Grand et al., 2005; Morerio et al., 2005). These fusion transcript profiles are still unexplored in the leukemic patients of Pakistan. We tried to check out the prevalence of fusion transcripts with focus on NUP98-LEDGF, this is first study conducted and documented.

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Abbreviations: ASO-PCR, allele-specific oligonucleotide-PCR; **CML,** chronic myeloid leukemia; **LEDGF,** lens epithelium derived growth factor; **NUP-98,** Nucleoporin-98.

MATERIALS AND METHODS

Forty blood samples of patients with CML, which were receiving imatinib treatment from January 2009, to November 2010 were obtained from different hospitals of Lahore. DNA/RNA extraction and PCR amplification were done by using materials and method previously used by Catherine et al. (2002). Blood samples were subjected to genomic DNA extraction method.

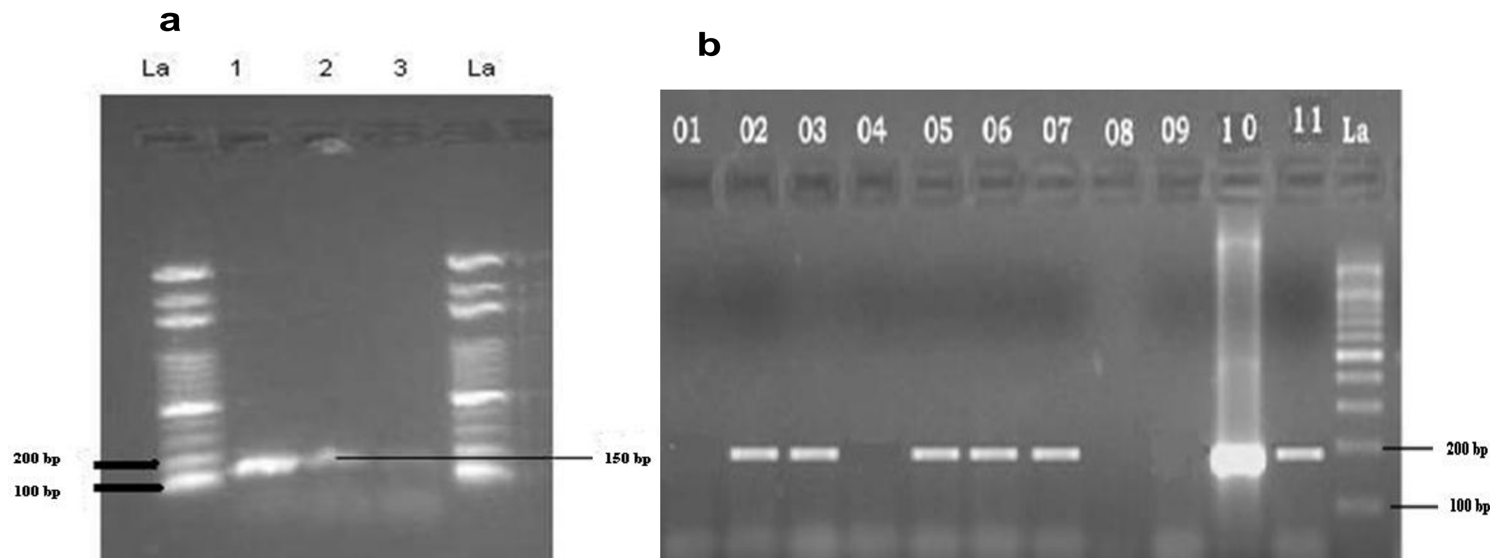

Figure 1. Analysis of C944T and T932C mutations by ASO-PCR. (a) Lane 1 and 5: DNA ladder of 1 kb, Lane 2, 3 and 4: patients positive with C944T mutation. (b) Lane 1: Negative control, Lane 2, 3, 5, 6, 7, 10 and 11: Patients positive with T932C mutation. The rest of the patients were negative.

PCR amplification

PCR mix preparation: A 20 µl PCR reaction was performed containing 1 µl of DNA (50 ng/µl), 2 µl 10X PCR buffer (Fermentas, USA), 25 pM of both forward and reverse primers, 300 M each of dATP, dGTP, dCTP and dTTP, 1.5 mM $MgCl_2$, 1.5 U Taq polymerase and 12 µl PCR water. To characterize fusion genes in the given samples, the extracted DNA from the blood was subjected to ASO-PCR. For the detection of two point mutations, cytosine to thymine at the position 944 of ABL gene and thymine to cytosine at the position 932 of ABL gene, mutation specific primers were used, and it was previously reported by Catherine et al. (2002). For the amplification of NUP98-LEDGF, forward primer to NUP98 exon 5 (1F: 5′-AGTACTAGCAGTGGAGGACTCTT-3′) and a reverse primer to LEDGF exon 9 (1R: 5′-CTCTTCATCCTTCTTAGGCTGCT-3′) were used and it was previously reported by Grand et al. (2005).

RESULTS AND DISCUSSION

ABL is a protein kinase that is involved in the phosphorylation of various downstream molecules but myristoylation in the hydrophobic region of the protein makes it silent by auto inhibition. Contrarily, if there is a fusion of BCR and ABL, it de-represses the activity of ABL. The activation of ABL is restored as soon as there is a fusion of BCR with ABL and this fusion protein is hyper-activated to disturb the spatial-temporal behavior of the signaling (Mian et al., 2009). To treat this pathology, imatinib is used to extinguish and dampen the effect of BCR-ABL. According to contemporary findings there is a paradigm shift from sensitivity towards resistance. In the patients with CML, C944T mutation was detected (in 3 patients). Cytosine to thymine mutation (mutation 1) at the position 944 of ABL gene was confirmed by appearance of the amplicons at 150 bp (Figure 1a). Thymine to cytosine mutation (mutation 2) at position 932 of the ABL gene was detected in (seven) imatinib resistant CML patients by using mutation specific primers. On 2% agarose gel, 174 bp specific bands were observed in patients positive for thymine to cytosine mutation at 932 of ABL gene (Figure 1b).

In present study we screened two mutations in CML patients which are in concordance with the

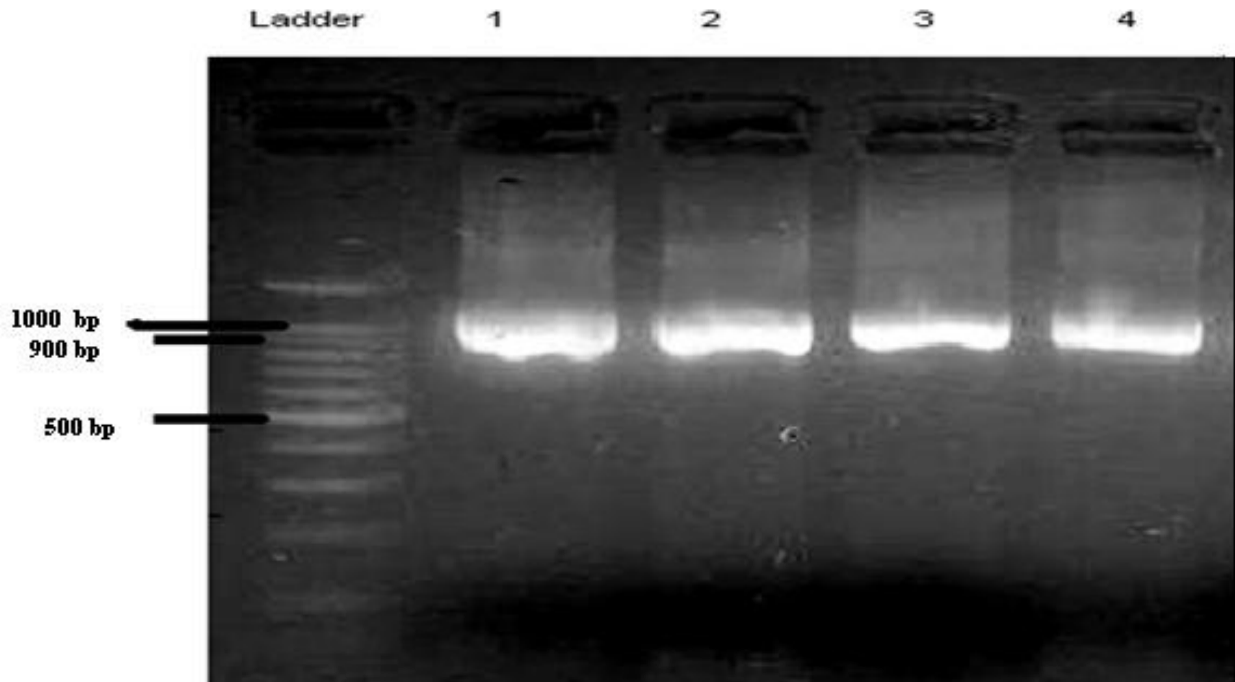

Figure 2. Amplification of NUP98-LEDGF. The bands show amplification of NUP98-LEDGF at 971 bp in 1, 2, 3 and 4.1 kb Ladder is used.

results of Catherine et al. (2002). We did not find a double mutation in a single patient which is in discordance with the results reported by Zafar et al. (2004). Samples were either positive for C944T or T932C. We have characterized and documented yet another fusion transcript NUP98-LEDGF in CML patients from Lahore (Figure 2). In the previous studies only case study was conducted to elucidate the molecular aspects of the fused genes. However, we have identified this chimeric transcript in (four) CML patients. It will be a great utility in clinical management of drug treatment and holds a potential for providing a reliable tool in diagnosis.

ACKNOWLEDGEMENTS

The corresponding author sincerely express thanks the family and research supervisor Ammad Ahmad Farooqi for participating in the study. The entire work was supported by National Institute for Genomics and Advanced Biotechnology, NARC Islamabad, Pakistan.

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