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The Use of DHPLC for Mutation Screening of CEBPA Gene in Acute Myeloid Leukemia

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ABSTRACT

The mutations of CCAAT/enhancer binding protein-alpha (CEBPA) gene are evaluated as a favorable prognostic tool for AML patients. The gold standard method for detection of CEBPA gene mutations is direct sequencing. However, this method has some disadvantages and CEBPA mutations can occur across the whole gene. Thus, it should have a screening test before designating the type of mutation by direct sequencing. This screening method requires an accurate, fast and easy method. The purpose of this study was to evaluate the ability of denaturing high-performance liquid chromatography (DHPLC) method for screening CEBPA mutations. The coding region of CEBPA gene in 40 AML patients was screened by DHPLC and confirmed by direct sequencing. Our results suggested that DHPLC was a useful screening test to detect CEBPA gene mutations in AML patients.

Keywords: CEBPA, mutation, acute myeloid leukemia, DHPLC, screening
INTRODUCTION

CEBPA or CCAAT / enhancer binding protein alpha gene locates on chromosome 19q13.1. It is an intronless gene which encodes a transcription factor protein (C/EBP\(\alpha\)). C/EBP\(\alpha\) is an importance transcription factor in myeloid differentiation and specifically expressed in myeloid lineage. The CEBPA knockout mice present lacking mature granulocytes, whereas, the development of other hematopoietic lineages shows normal proportion. Therefore, it has been assumed that CEBPA inactivation might contribute to the differentiation block specific to AML (Koschmieder et al., 2009).

The mutation of CEBPA can occur throughout the entire gene and various patterns such as deletion, insertion, duplication and point mutation but most mutation are found in two hotspots, N- and C-terminal domains. Patients can have two mutations (involves N- plus C-terminal variant) are called double mutations that are assumed to be biallelic pattern (Green et al., 2010). Mutations of CEBPA gene were suggested to be a good prognostic factor in AML patients, especially in cytogenetically normal AML (Marcucci et al., 2008). Several lines of evidence suggested that overall survival (OS) and disease-free survival (DFS) was significantly longer in AML patients with CEBPA mutations. Some studies show that this advantage is only present in patients with double mutations. This advantage may be results of double mutations which have a specific gene expression pattern, while single mutations and wild type do not. Moreover, patients with double mutations usually show lower mutation in FLT3-ITD that is associated poor prognosis (Paz-Priel and Friedman, 2011). Previous reports performed CEBPA mutation detection as a real-time monitoring marker in AML patients at the relapse period (Smith et al., 2006; Tiesmeier et al., 2003).

Though, the direct sequencing is a gold standard method for detection of CEBPA gene mutations but it is time-consuming, labor-intensive, expensive procedure and requires expertise to interpret results (Ahn et al., 2009). Previously mentioned, CEBPA mutations can occur across the entire coding region of the gene and have various patterns of mutation. Hence, efficient screening test before identifying types of mutations by direct sequencing is necessary. Denaturing high-performance liquid chromatography (DHPLC) is an interesting method to detect any possible genetic alteration (insertions, deletions, duplications and point mutations/polymorphism) (Green et al., 2010). Moreover, DHPLC has been described as a highly sensitive, simple, rapid, inexpensive and facile assay to interpret results (Xiao and Oefner, 2001). In this study, the coding region of CEBPA gene was studied in 40 AML patients by DHPLC to screen CEBPA mutations. Finally direct sequencing method was done to confirm the results. Our data demonstrated that DHPLC method successfully distinguished wild-type and polymorphism/mutation in the patients. Therefore, we suggested that DHPLC was a useful screening test to detect CEBPA gene mutations in AML patients.

MATERIALS AND METHODS

The total of 40 samples of bone marrow or peripheral blood from AML patients was obtained. All samples were leftover specimen of routine assay in Human Genetics Unit, Department of Pathology, Faculty of Medicine Ramathibodi Hospital. The genomic DNA was extracted by using High Pure PCR Template Preparation kits (Roche Diagnostics, USA). The quantity and quality of DNA were evaluated with spectrophotometer. The entire coding region of CEBPA gene was amplified by PCR using 3 pairs of overlapping primers. The PCR products were analyzed by DHPLC using mutation detection program (WAVE® system 4500, Transgenomic® USA) and confirmed by direct sequencing in both directions. The number of nucleotides was according to Genbank Accession No. NM_004364.3.
RESULTS AND DISCUSSION

In this study, 40 samples from AML patients were screened for CEBPA mutations using DHPLC. The results demonstrated that 30 samples presented wild-type chromatogram with one single peak (Figure 1A). Ten samples showed more than one single peaks in one or more fragments, corresponding to genetic alteration (Figure 1B). All samples were confirmed by direct sequencing assay and revealed that 30 samples were wild-type, 7 samples were polymorphism and 3 samples were mutation. These 3 samples presented 5 distinct types of nucleotide variants (c.185-189 del TCGAC, c.904-921 dup AAGGCAAGCAGCGCAAC, c.247del C, c.923-925 dup TGG and c.268 A>T). Two of three samples presented double mutations. All types of CEBPA gene mutations (insertion, duplication, deletion and substitution) were also detected by DHPLC. The sensitivity of DHPLC method to detect the numbers of CEBPA gene mutations is 100% (5/5). Therefore, this result suggested that DHPLC was an effective method for separating between variant positive and normal samples. However, it still requires direct sequencing method for designating mutation types and polymorphism (Figure 2).

Figure 1 DHPLC chromatogram of CEBPA gene: wild-type (A) and polymorphism/mutation (B).

Figure 2 Examples of DHPLC chromatograms and partial sequences of CEBPA gene mutation in AML patients.
CEBPA mutations were detected in 3 samples (7.5%). The frequency was comparable to that of The United States of America (7.3%) (Pabst et al., 2001), United Kingdom (7%) (Green et al., 2010) and Germany (7.7%) (Tiesmeier et al., 2003). The mutations were detected in 2 hotspots. N-terminal mutations were frameshift insertion/deletion mutations while in-frame duplication/deletion mutations were detected in C-terminal (Leroy et al., 2005). One type of polymorphisms detected in TAD2 region was shown in 7 of all patients (17.5%). The c.584-589 dup ACCGC was a 6 nucleotide in-frame duplication polymorphism (P194_H195dup). It was previously described to be a polymorphism (Wouters et al., 2007).

CONCLUSION
Our results demonstrated that DHPLC is a useful screening test to detect CEBPA gene mutations in AML patients. Combination of DHPLC and direct sequencing is an appropriate approach for detecting CEBPA mutations.

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REFERENCES


