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Research paper

New rapid method to detect BCR-ABL fusion genes with multiplex RT-qPCR in one-tube at a time

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ABSTRACT

Fast identification of BCR-ABL fusion genes is critical for precise diagnosis, risk stratification and therapy scheme selection in leukemia. More convenient methods are needed for quickly detection of the BCR-ABL fusion genes. Multiplex fluorescent reverse transcription quantitative real-time PCR (Multiplex RT-qPCR) methods are developed for detection of the at least 14 subtypes of BCR-ABL fusion genes in one tube at a time by using patients' bone marrow samples. The new Multiplex RT-qPCR method could quickly detect BCR-ABL fusion genes with sensitivity up to 10–10^6 copies. It can detect the fusion genes in patients' bone marrow samples containing any subtypes of the major bcr (M-bcr) e13a2, e14a2, e13a3 and e14a3, the minor bcr (m-bcr) e1a2 and e1a3, the micro bcr (μ-bcr) e19a2 and e19a3, and the nano bcr (n-bcr) e6a2 and e6a3. The specificity is comparable to the FISH methods. The cutoff for clinical diagnosis of BCR-ABL(+) is also determined by testing in clinical chronic myeloid leukemia samples. This is a new fast method with high sensitivity and specificity for clinical detection of BCR-ABL fusion genes. It will benefit the precise diagnosis, targeted therapy and minimal residual disease (MRD) monitoring in leukemia.

1. Introduction

Identification of specific recurrent genetic abnormalities is critical for disease evaluation, optimal risk stratification, and treatment planning, which is recommended by the USA National Comprehensive Cancer Network [1]. The Philadelphia chromosome (Ph) is a chromosome 22 derivative resulting from a translocation between chromosomes 9 and 22 causing the BCR-ABL fusion [2]. BCR-ABL fusions are present in approximately 95% of chronic myelogenous leukemia (CML), and 25–30% of adult acute lymphoblastic leukemia (ALL) cases [3]. They can also be detected in 2–4% of children with ALL [4]. The Philadelphia chromosome and a specific BCR-ABL transcript are also reported in Chronic neutrophil leukemia (CNL) [5]. Therefore, detection of the ratio of BCR-ABL fusion versus a reference control can be used for diagnosis, prognosis, and monitoring of ongoing therapies in leukemia patients.

Currently, several types of methods are used for clinical detection of BCR-ABL fusion genes, which include chromosome analysis, fluorescence in situ hybridization cytogenetic testing (FISH) [6], quantitative real-time PCR (qPCR) [7] and reverse transcription quantitative PCR (RT-qPCR) [8]. The RT-qPCR method is the most sensitive assay available for the measurement of BCR-ABL chimeric mRNA by detecting either of the breakpoints in the fusion gene. A major advantage of the RT-qPCR assay is the strong correlation between the results obtained from the peripheral blood and the bone marrow, which allows molecular monitoring without the necessity of obtaining bone marrow aspirations. In addition, the RT-qPCR assay is the only tool capable of monitoring responses after the patient has achieved a Complete Cytogenetic Response (CCyR).

There are more than 19 isoforms of BCR-ABL fusion gene, including major (M-bcr) e13a2, e14a2, e13a3 and e14a3 [9–15], minor bcr (m-bcr) e1a2 and e1a3 [16–18], micro bcr (μ-bcr) e19a2 and e19a3 [19–21], and nano bcr (n-bcr) e6a2 and e6a3 [22–24] (Fig. 1A and 1B). It has been reported that BCR-ABL fusion gene isoforms have been screened for using the RT-qPCR method, but only a few isoforms such as e13a2, e14a2 and e1a2 have been detected at a time in one tube.

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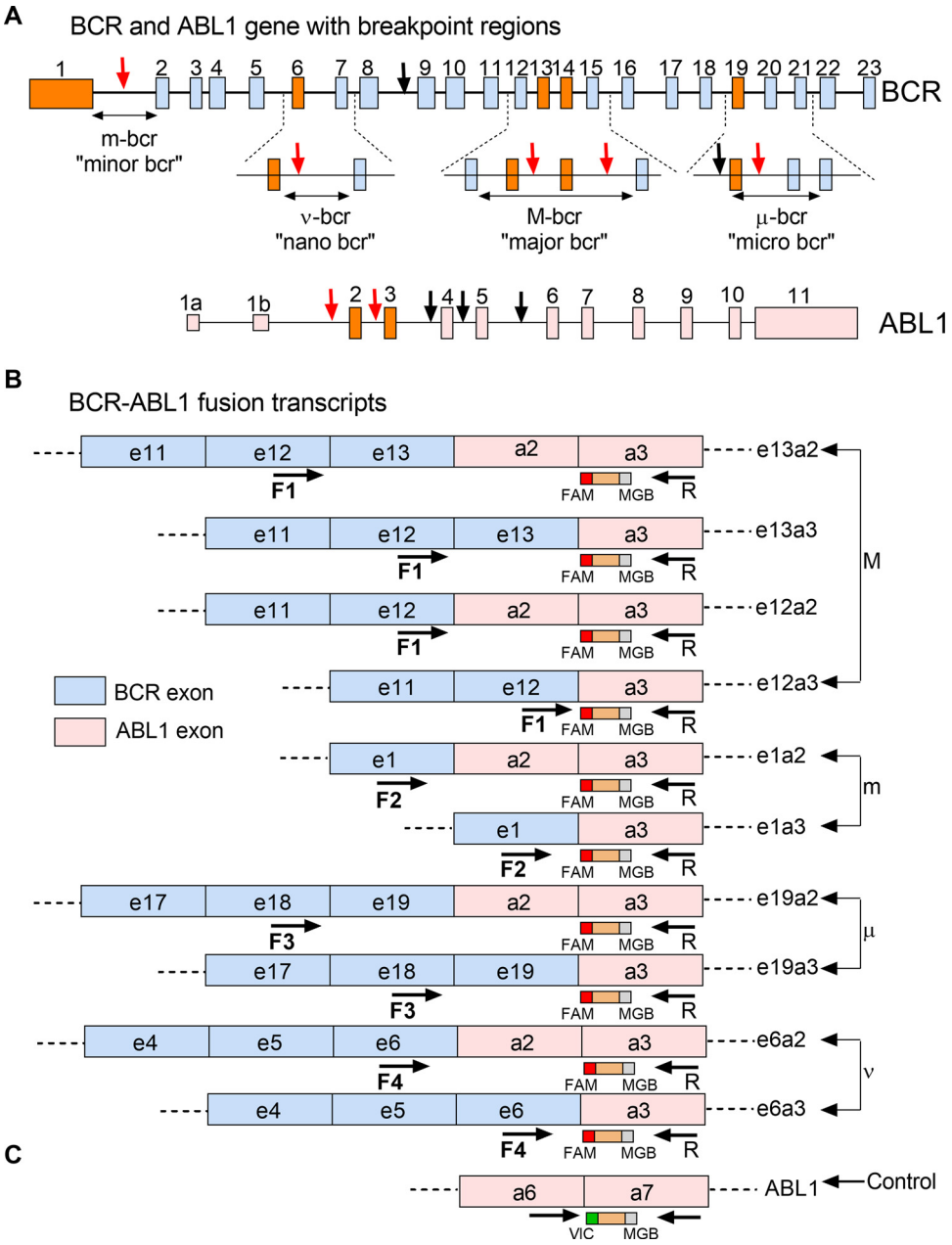

Fig. 1. Primers and probes design. (A) The gene structure of BCR and ABL, red arrow is a common breakpoint site; black arrow is a rare breakpoint site. (B) the transcripts for the BCR-ABL fusion isoforms and location sites of the primers and probes, M: major bcr, including e13a2, e13a3, e14a2 and e14a3; m: minor bcr, including e1a2 and e1a3; μ: micro bcr, including e19a2 and e19a3; *ν*: nano bcr, including e6a2 and e6a3. (C) Location sites for the primers and probe in reference gene ABL1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Jennings LJ et al. [25] reported detecting only e13a2 and e14a2 by using the Droplet Digital PCR method. Park KJ et al. [26] also only detected e13a2, e14a2 and e1a2 by using the multiplexing quantification PCR method. These reports suggest that these methods can only detect a limited number of the fusion gene isoforms in one reaction. The Multiplex RT-PCR reaction gives the possibility of detecting multiple variants of the fusion BCR-ABL gene in one reaction. Burmeister T et al. [21] reported that the multiplex RT-PCR method could detect eight BCR-ABL isoforms (e1a2, e1a3, e6a2, e13a2, e13a3, e14a2, e14a3, e19a2), and Link-Lenczowska D et al. [27] reported the detection of ten BCR-ABL isoforms (e13a2, e14a2, e1a2, e19a2, e13a3, e14a3, e6a2, e6a3, e1a3, e19a3) in one tube. However, the multiplex RT-PCR method also has problems including low sensitivity, being tedious, and ineffective for leukemia diagnosis and monitoring of minimal residual disease (MRD).

Here we report a new rapid method, Fluorescent Multiplex RT-qPCR, to detect a maximum number of BCR-ABL isoforms in one reaction. We also propose a scheme using this method for the diagnosis and monitoring of MRD in patients with CML with a rare fusion of the BCR and ABL genes, and for monitoring treatment response to tyrosine kinase inhibitors (TKIs) in these patients.

2. Materials and methods

2.1. Patients and tissue samples

Samples included a total of 85 bone marrow samples from newly diagnosed patients with BCR-ABL positive leukemia, and 31 bone marrow samples from newly diagnosed patients with BCR-ABL negative leukemia before therapy, confirmed by RT-qPCR Using the LightCycler