A novel method for inv(16);CBF-β-MYH11 fusion gene quantitation by real-time PCR using single-concentration calibration plasmids

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MATERIALS AND METHODS

Acute myeloid leukemia (AML) is a genetically heterogeneous clonal disease of the bone marrow characterized by the accumulation of acquired somatic aberrations in hematopoietic progenitor cells that alter normal cellular mechanisms. These aberrations, which range from single nucleotide changes to chromosomal rearrangements, often occur in genes encoding transcription factors or disrupt elements of the transcription pathway that regulate growth rate, survival, and differentiation or malignant transformation. Known genetic aberrations include chromosome translocations, five genes (NPM1, FLT3, CEBPA, MLL, Nras), among a variety of other genes, most often mutated.2,3 Chromosomal rearrangements frequently observed in AML cases include i(17)(q21); RUNX1-RUNX1T1 (t(15;17); PML-RARA, inv(16);CBF-MYH11 and others. Additionally, chromosome loss or gain is common, frequently involving chromosomes 7, 8, 21, X and Y. These varied and diverse genetic aberrations provide the basis for the wide array of phenotypic expression and prognosis seen within this disorder.

The inv(16);MYH11 abnormality is one of the more frequent chromosomal rearrangements found in de novo AML. The rearrangement generates a core binding factor β (CBFβ)-myosin 11 (MYH11) chimeric protein.3 The CBFβ-MYH11 chimeric protein blocks differentiation of myeloid leukemia cells. Over 24 different breakpoints have been identified for these gene rearrangements. All CBFβ breakpoints are highly conserved and result in an intron resulting in a mRNA transcript fusion of exon 5 with the MYH11 partner. The majority of MYH11 breakpoints (21/24) occur within a single intron. The remaining three breakpoints occur upstream. Approximately 98% of all breakpoints generate three different fusion gene products, types A, D and E (Figure 1). CBFβ-MYH11 type A is the most common fusion product, found in 86% of inv(16)-positive AML. Clinically, CBFβ-MYH11 is associated with the French-American-British (FAB) M2 subtype. Patients with CBFβ-MYH11 generally have good long-term prognosis and response rates to treatment, however relapses occur in 30-35% of cases.4,5

Testing for minimal residual disease using a sensitive quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) test provides a clinical benefit.1,6 We have previously described quantitative PCR assays that utilize a known ratio calibrator plasmid containing the targeted mutation and its wild-type form.4 We have applied the principles used in quantitative allele-specific PCR assays to the detection and quantitation of chromosomal translocations. By combining the translocation target and reference gene at a fixed ratio into a single plasmid we can normalize quantitation between sample testing and maximize patient testing to reduce costs while maintaining a highly specific and accurate assay.

Here we describe a qRT-PCR method that does not require standard curves to calculate CBFβ-MYH11 fusion transcription. The assay is specific to detect each of the CBFβ-MYH11 types A, D, and E and sensitive to allow for monitoring patient transcript levels over time. Relative quantitation using a single hydrolysis probe is ideal for qRT-PCR assay principles, a sensitive, accurate and cost effective quantification strategy that maximizes patient sample testing over absolute quantitative strategies that rely on standard curves. Here we describe a novel approach to quantitate CBFβ-MYH11 fusion transcripts using plasmid calibrators specific for the A, D, E types in a multiplex PCR format.

BACKGROUND

Acute myeloid leukemia (AML) is a genetically heterogeneous clonal disease of the bone marrow characterized by the accumulation of acquired somatic aberrations in hematopoietic progenitor cells that alter normal cellular mechanisms. These aberrations, which range from single nucleotide changes to chromosomal rearrangements, often occur in genes encoding transcription factors or disrupt elements of the transcription pathway that regulate growth rate, survival, and differentiation or malignant transformation. Known genetic aberrations include chromosome translocations, five genes (NPM1, FLT3, CEBPA, MLL, Nras), among a variety of other genes, most often mutated.2,3 Chromosomal rearrangements frequently observed in AML cases include i(17)(q21); RUNX1-RUNX1T1 (t(15;17); PML-RARA, inv(16);CBF-MYH11 and others. Additionally, chromosome loss or gain is common, frequently involving chromosomes 7, 8, 21, X and Y. These varied and diverse genetic aberrations provide the basis for the wide array of phenotypic expression and prognosis seen within this disorder.

RESULTS

Quantitation is linear in multiplex reaction

• Dilution series of CBFβ-MYH11 FusionQuant fusion gene standard in singlet (A) and multiplex reaction (B)
• PCR efficiency consistent in multiplex PCR
• Linear response over 5-log dilution

RESULTS

CONCLUSIONS

• We describe a sensitive, quantitative RT-PCR assay for detection and quantitation of inv(16);CBFβ-MYH11 fusion transcripts.
• The assay uses a calibrator plasmid containing inv(16) and ABL1 (pCBFβ-MYH11/ABL1) at a defined ratio.
• Calibrator is run at a single concentration.
• Distincts and quantitates inv(16);CBFβ-MYH11 types A, D, E.
• Single point calibrator quantitation yields similar values as data calculated using conventional standard curves.
• Does not require the generation or purchase of standards of known copy number.

REFERENCES