Eprobe mediated RT-qPCR for the detection of leukemia-associated translocations

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Eprobe Leukemia Assay with melting curve analysis has potential as a versatile method for rapid screening of the fusion gene at initial diagnosis and for the quantification of minimal residual disease monitoring

BACKGROUND & OBJECTIVES
A novel Eprobe-based one-step RT-qPCR assay (Eprobe Leukemia Assay) has been developed.
1. Eprobe Leukemia Assay possesses the synthetic quantitative standard RNAs that guarantee the routine performance with strict quality control.
2. Eprobe Leukemia Assay is combined with melting curve analysis.

The aim of this study is to evaluate the efficacy of the Eprobe Leukemia Assay focusing on the common leukemia associated fusion genes; Major and minor BCR-ABL1, RUNX1-RUNX1T1, PML-RARA (bcrl, 2, 3) and cABL as an internal control.

MATERIALS & METHODS

Materials:
Patient samples: A total of 67 primary leukemia patient samples (31 molecularly positive and 36 negative), which were predetected by the existing laboratory-developed TaqMan RT-qPCR assays.
Control RNA isolated from human cell line: Total RNA of major and major BCR-ABL1-negative HL60 cell line was utilized to evaluate the minor BCR-ABL1 primer sets.

Synthetic RNA:
A synthetic DNA molecule was designed and synthesized.

Methods:
• The Eprobe leukemia assay is comprised of Eprobes, which are excit-on-controlled hybridization-sensitive fluorescent oligonucleotides.
• Melting curve analysis was performed on synthetic quantitative standard RNAs with strict quality control.
• Quantification capacity was evaluated by comparison with TaqMan RT-qPCR using 67 primary samples.

RESULTS

Monitoring by Eprobe and TaqMan RT-qPCR

Eprobe Exciton-controlled hybridization-sensitive fluorescent oligonucleotide

Melting curve analysis of amplification products

Correlation of major and minor BCR/ABL1 mRNA expression levels in double-positive CML

New primer candidates of minor BCR-ABL1 for Eprobe RT-qPCR

Best primer pair: F96-R06

Improved Eprobe Leukemia Assay for abnormally low sloped amplification curve lines of low-level minor BCR-ABL1 transcripts

FUTURE PLAN
• Expand the study to incorporate more data from multiple centers for future studies.
• An "Eprobe leukemia panel kit" based on this Eprobe mediated RT-qPCR assay, equipped with synthetic quantitative standard RNAs and additional targets including CBFB-MYH11, ETV6-AML1, MLL-4AF4, and MLL-AF9 rearrangements is currently under development.

Establishment of the accurate Eprobe RT-qPCR assay for samples with low transcript levels of minor BCR-ABL1

Simultaneous minor BCR-ABL1 was detected in 11 (59%) of 19 primary samples of major BCR-ABL1-positive CML cases.

Low-level minor BCR-ABL1 1, I in major and minor BCR-ABL1 dual-positive CML primary samples (pt #20 and #21)

Low-level minor BCR-ABL1, I in major and minor BCR-ABL1 dual-positive CML primary samples (pt #20 and #21)

Low-level minor BCR-ABL1, dual-positive CML primary samples (pt #20 and #21)

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