Measurement of ZAP-70 Expression in CLL Using an Optimized Flow Cytometric Assay for ZAP-70 Protein Levels in Whole Blood Samples

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Abstract

ZAP-70 expression can be assessed by the detection of specific antibodies that recognize an activated T cell receptor (TCR) or B cell receptor (BCR). The presence of ZAP-70 expression is usually associated with increased activity of the BCR signaling pathway in chronic lymphocytic leukemia (CLL). In this study, we evaluated a new flow cytometric assay for the detection of ZAP-70 expression in whole blood samples from patients with CLL. The assay was optimized to improve sensitivity and specificity compared to previous methods. The results showed that the new assay was able to detect ZAP-70 expression in a broader range of patient samples, with a lower background signal and increased sensitivity for detecting low levels of ZAP-70 expression.

Materials

ZAP-70 antibodies were purchased from Dako, Inc. Antibody dilutions were prepared by adding the appropriate concentration of antibody to phosphate-buffered saline (PBS) and incubating at room temperature for 30 minutes. Whole blood samples were collected from patients with CLL and stained with FITC-conjugated anti-ZAP-70 antibody. The samples were analyzed using a flow cytometer, and the data were analyzed using FlowJo software.

Methods

Optimized Flow Cytometric Assay

1. Whole blood samples were collected from patients with CLL and stained with FITC-conjugated anti-ZAP-70 antibody.
2. The samples were analyzed using a flow cytometer, and the data were analyzed using FlowJo software.
3. The optimized assay was able to detect ZAP-70 expression in a broader range of patient samples, with a lower background signal and increased sensitivity for detecting low levels of ZAP-70 expression.

Results

ZAP-70 Antibody and Methods Comparisons

(a) Formaldehyde Fixation

(b) Optimal Fixation/Fluor 5-100

(c) Flow Results

(d) Image Analysis

(e) Image Cytometry

(f) Data Analysis

Conclusions

- The performance characterization of different antibody clones/conjugates demonstrate variability in the detection of ZAP-70 protein expression in B, T, and NK cells.
- The optimized Formaldehyde/Fluor 5-100 method for whole blood fixation and permeabilization provides an improved signal-to-noise ratio in all cells except for data in T cells.
- ZAP-70 protein expression in normal T lymphocytes degrades logarithmically over time, with a mean decrease of 13% in expression occurring within the first 24 hours.
- The constitutive levels of expression of ZAP-70 in T, NK, and B cells populations (defined by the 4 surface markers) provides a method to quantify ZAP-70 expression in the CLL population as a relative MFI readout. This approach allows standardization across laboratories and multiple instrument platforms.

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