Detection of the MYD88 L265P Mutation in Waldenström’s Macroglobulinemia Using a Highly Sensitive Allele-Specific PCR Assay

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Abstract

Introduction: Waldenström’s macroglobulinemia (WM) is a B-cell malignancy characterized by bone marrow (BM) infiltration with lymphoplasma cytic cells and production of an IgM paraprotein. By whole genome sequencing, we recently identified a somatic mutation (L265P) in the MYD88 gene in 27/30 (90%) WM patients (Treon et al, ASH 2011). To expand this finding for possible diagnostic testing, we developed an allele-specific PCR assay for MYD88-L265P and evaluated this assay in a large cohort of WM patients.

Materials and methods: An allele-specific PCR assay was developed with a threshold of detection of 0.1% for detection of the MYD88-L265P mutation. DNA from bone marrow aspirates from 96 patients with the clinicopathological diagnosis of WM and 9 healthy controls was used for assessment of MYD88-L265P expression by both allele-specific PCR and Sanger sequencing. Findings were correlated with clinical parameters.

Results: We observed that 85/96 (89%) WM patients were positive for MYD88-L265P using the allele-specific PCR assay. Of the 85 allele-specific PCR positive patients, 80 demonstrated a detectable mutation peak by Sanger sequencing. All 11 allele-specific PCR negative patients remained negative by Sanger sequencing. By the allele-specific PCR assay, MYD88-L265P positive patients showed greater bone marrow involvement (p<0.001) and higher serum IgM (p<0.001) versus MYD88-L265P negative patients.

Conclusion: MYD88-L265P is highly expressed in BM samples of WM patients using an allele-specific PCR assay, and is associated with greater bone marrow disease burden and serum levels. Use of allele-specific PCR provides a simple and sensitive diagnostic tool for detection of the MYD88-L265P mutation.

Five WM patients showed negative MYD88-L265P by Sanger sequencing but weak positive by allele-specific real-time PCR. Correlation with clinical parameters was further analyzed.

PCR reaction was performed in volume of 25 ul with 50 nM of forward primers and reverse primers and 100 ng DNA. Thermal cycling conditions were 2 min at 94°C, followed by 40 cycles of 94°C for 30s, 57°C for 30s, and 68°C for 30s, with a final extension at 68°C for 5 min. Sensitivity was assessed by a serial dilution of the WM cell line DNA. PCR products were separated on 2% agarose gel.

A total of 96 untreated WM patients and 9 healthy controls were analyzed by real-time allele-specific PCR and Sanger sequencing.

We developed an allele-specific real-time PCR assay for detection of MYD88-L265P using commercially available PCR mix. This method provides necessary sensitivity and specificity and can be easily implemented in each laboratory with real-time PCR technology. Analysis of this larger cohort confirmed our previous finding that the MYD88-L265P allele is frequently mutated in WM patients and, in combination with clinical features, represents a reliable molecular marker for this disease. Interestingly, the WM patients with positive MYD88-L265P tended to have greater bone marrow disease burden and higher serum IgM compared to MYD88-L265P negative patients. Quantification of MYD88-L265P by real-time PCR allows the detection of minimal residual disease and monitoring therapeutic effect.