MYD88 L265P as a response marker in Waldenstrom's macroglobulinemia

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Waldenström's macroglobulinemia (WM) is a B-cell malignancy characterized by bone marrow (BM) infiltration with lymphoplasmacytic cells and production of an IgM paraprotein. By whole genome sequencing, we recently identified a somatic mutation (L265P) in the MYD88 gene in 90% WM patients. To expand this finding for possible diagnostic testing, we developed an allele-specific (AS) PCR assay to allow reliable and quantitative assessments of MYD88 L265P. The AS-PCR primers showed high specificity of amplification in the agarose gel-based AS-PCR assay with a threshold of detection of 0.1% for the MYD88 L265P mutation. To quantify the levels of MYD88 L265P, a SYBR green-based real-time AS-PCR was developed with a threshold of detection of 0.08%. DNA from 97 patients with the clinicopathological diagnosis of WM and 40 healthy donors was analyzed by gel-based and real-time AS-PCR and Sanger sequencing to assess the sensitivity and reliability of the AS-PCR assays. A high concordance between the methods was observed. By either the gel-based or real-time AS-PCR, 87/97 (89.7%) WM patients were positive for MYD88 L265P. Using non-parametric ANOVA, MYD88 L265P positive patients showed greater BM involvement ($p=0.001$), lower serum IgA ($p<0.001$), lower serum IgG ($p=0.011$), and higher serum IgM ($p=0.007$) versus MYD88 L265P negative patients. To explore the potential of using the real-time AS-PCR method to determine therapeutic effect, BM biopsies were assessed for seven patients before and after treatment. The changes of BM involvement and levels of mutant MYD88 L265P were compared. A high correlation between the two assessments was observed ($R^2=0.90$). In conclusion, we developed a sensitive and inexpensive real-time AS-PCR method that allows reliable and quantitative detection of MYD88 L265P in WM.