

**Whole genome sequencing reveals a widely expressed mutation (MYD88 L265P) with oncogenic activity in Waldenstrom's Macroglobulinemia.**

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We performed whole genome sequencing (WGS) of lymphoplasmacytic cells from 30 Waldenstrom's Macroglobulinemia (WM) patients, with paired normal tissue sequencing for 10 patients. Tumor and normal genomes were both sequenced to an average of 66X coverage of mapped individual reads. A recurring sequence variant at position 38182641 in chromosome 3p22.2 was identified which resulted in a single nucleotide change from T→C in the myeloid differentiation primary response (MYD88) gene, and a predicted non-synonymous change at amino acid position 265 from leucine to proline (L265P). This variant was the most common of a median of 3,419 (range 2,540-4,011) somatic variants identified by WGS in paired patients, and was present in tumor cells from all 10 paired patients, and 16 of 20 unpaired patients. For 22 of 26 patients, the MYD88 L265P variant was heterozygous, whereas in 4 patients an acquired UPD event at 3p22.2 resulted in homozygous presence of the variant in at least a subset of tumor cells. Sanger sequencing confirmed the presence of the MYD88 L265P variant in all 26 patient tumor samples revealed by WGS, as well as in one additional patient's tumor sample that was not identified by the variant calling algorithms but for whom 12% of the WGS read level mappings showed the MYD88 L265P variant. In contrast, the MYD88 L265P variant was absent in normal paired tissues. Sanger sequencing therefore confirmed the somatic presence of the MYD88 L265P variant in tumor cells from 27 of 30 (90%) WM patients, and also identified this variant in BCWM.1 and MWCL-1 WM cells. In contrast, the MYD88 L265P variant was absent in CD138<sup>+</sup> selected tumor cells from 8 of 8 multiple myeloma (MM) patients, and CD19<sup>+</sup> cells from 12 of 12 healthy individuals, as well as in 7 of 8 patients with IgM monoclonal gammopathy of unknown significance (MGUS), in whom absence of the MYD88 L265P variant was further confirmed by TA cloning and sequencing of at least 100 clones. In the sole IgM MGUS patient in whom the MYD88 L265P mutation was detected, subsequent disease evolution occurred. Importantly, knock-down of MYD88 expression by lentiviral transduction led to loss of NF-κβ signaling and apoptosis of both BCWM.1 and MWCL-1 WM cells, with enhanced survival observed by complementary transduction with MYD88 L265P versus MYD88 wild type. The results of these studies therefore demonstrate a widely expressed somatic variant (MYD88 L265P) in malignant LPC of WM, whose presence confers oncogenic activity, and which may help distinguish WM disease from IgM MGUS or MM.