Does aberrant BCR signaling contribute to WM survival?

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Neoplastic transformation in Waldenström's Macroglobulinemia (WM) occurs in the context of hyperactive MYD88 and CXCR4 signaling. Nevertheless, the presence of chronic active B-cell receptor (BCR) signaling, a feature of multiple IgM+ lymphomas remains poorly elucidated in WM. We investigated the activity of BCR signaling in primary WM cells, by interrogating multiple BCR-related phospho-proteins in a resting and *ex-vivo* stimulated state and evaluated features of network remodeling through multiparametric phosphoflow cytometry. WM cells exhibited (a) at variable degrees, significantly higher levels of constitutive signaling activation compared to normal peripheral blood and bone marrow mature B-cells, (b) high sensitivity to proximal kinase inhibition and (c)significantly augmented signaling amplitude, sensitivity andprolongation through SFK, SYK, BLNK, PLCγ2 and ERK upon BCR crosslinking.In order to approach intra-WM signaling heterogeneity, we generated BCR phosphosignatures comprised of the highly variable phosphoresponses that patients demonstrated for pSFK, pSYK, pBLNK, pPLCγ2, pERK and pAKT. Agglomerative clustering analysis partitioned our cohort into a "high" and a "healthy-like" signaling profile, with the latter being linked to a significantly more indolent clinical phenotype.Interestingly, higher surface IgM expression correlated with the "high" signaling group, while phosphatase activity was similarly low between the two groups, showing that phosphatase loss in not sufficient to fully potentiate signaling. Moreover, no correlation was observed between the BCR signaling potential and the MYD88, CD79A/B or IGHV mutation status of these patients.Single cell analysis revealed that WM cells exhibit high levels of intra-clonal heterogeneity in regards to their BCR signaling potential compared to normal B cells. Ongoing Mass Cytometry (CyTOF) studies assessing 31 B-cell-centered immunophenotypic, signaling and proliferation parameters have provided novel insight

about the clonal WM architecture, highlightingsubclones with a distinct immunophenotype, anergic signaling featuresanda high proliferative index compared to the majority of WM clones. The findings of this study provide the first evidence of augmented BCR signaling in WM cells, in the absence of BCR associated mutations, and provide new hypotheses about the presence of antigenic or non-antigenic BCR directed signals as potent WM drivers in a clonal and subclonal level.