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Waldenström Macroglobulinemia and Blimp1 involvement in Double Strand Breaks and cell cycle.

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It is well established that Waldenström Macroglobulinemia (WM), an incurable B-cell lymphoma, exhibits significant phenotypic heterogeneity ranging from B-cells to Plasma cells, demonstrating a lymphoplasmacytic nature. Furthermore, BLIMP1 is a well-known transcription factor and a crucial regulator in the plasma cell differentiation program, facilitating the transition from B-cell to plasma cell lineage. BLIMP1 exerts its function by directly binding to the DNA and modulating transcription.

To investigate BLIMP1's role in WM, we used the RPCI-WM1, a well-established published WM cell line, which we further engineered to express a doxycycline-induced microRNA targeting endogenous BLIMP1 for knockdown (KD). We further engineered the KD cell line to overexpress exogenous BLIMP1 via a lentivirally delivered Destabilization Domain EGFP-BLIMP1, which is continuously degraded unless protected by the Shield-1 molecule. Furthermore, we have developed an experimental protocol to rapidly return from overexpression to normal BLIMP1 levels.

Our experiments reveal a negative correlation between BLIMP1 levels and double-strand breaks (DSB), particularly outside the S-Phase. We measured DSBs by measuring 53bp1 foci after 48hr BLIMP1 KD and overexpression induction. BLIMP1 overexpression has 12.5% fewer DSBs than normal levels, while KD has 10.5% more DSB than normal levels, with KD leading to cell death by day six.

To understand BLIMP1's impact on the cell cycle, we use EdU, a thymidine analog incorporated into newly synthesized DNA during replication and then stained with an azide fluorochrome which binds to the EdU and DAPI for the DNA content. After 48 hours of BLIMP1 KD induction, we have 15% more cells in G1 and 2.5 times fewer cells in the S phase, and three times more apoptotic cells when compared to control cells, specifically: 62%±3.6 of cells are in G1, 11.4±1.4% are in S phase, and 20%±1.5 are apoptotic when we KD BLIMP1, compared to 54%±2.5, 29.4±2.57 and 5.9%±0.1 respectively in control cells. We can observe that when we KD BLIMP1, the cells seem to arrest in G1 and are slowly driven to cell death. Inversely, when we are overexpressing BLIMP1, there are 22% fewer cells in G1 and 26% more cells in the S phase compared to the control results; specifically, there are 43.4±4.9% cells in G1 and 42.5%±5 of cells in the S-phase when we overexpress BLIMP1 versus 54.03±2.47% and 29%±2.6 respectively in controls. These results show that BLIMP1 is needed to get through the cell cycle. However, when we continuously overexpress BLIMP1, it appears to slow down the cell cycle compared to the control cell line.

Our results suggest that BLIMP1 is essential in cell cycle maintenance and survival as it inhibits proteins that increase DSB and lead to cell death. Although the high number of cells in the S-phase suggests a higher replication rate, continuous overexpression of BLIMP1 leads to decreased cell count compared to control. This result is in accordance with BLIMP1's role in plasmablasts as an inhibitor of cell proliferation. Together, our results suggest that BLIMP1 is important for the cell cycle in many ways. This property is something we want to explore further in WM.