W39: BLIMP1 as a pro-survival gene in Waldenström's Macroglobulinaemia

Kimberley Anderson, Árný Björg Ósvaldsdóttir, Erna Magnúsdóttir

Department of Anatomy and Department of Biomedical Sciences, Faculty of Medicine, University of Iceland, Vatnsmýrarvegur 16, 101 Reykjavík, Iceland

The transcription factor B-lymphocyte induced maturation protein-1 (BLIMP1) drives the maturation of naïve-B-cells to become antibody secreting plasma cells. It is encoded by the *PRDM1* gene positioned on chromosomal region 6q21-q22.1 which is recurrently deleted in Waldenström's Macroglobulinaemia (WM). BLIMP1 is furthermore known to constitute a tumor suppressor in diffuse large B-lymphocytic lymphoma, consistent with its role in preventing plasma cell proliferation.

Using cell culture model systems for WM we observed that BLIMP1 protein is expressed in RPCI-WM1, MWCL-1 and BCWM.1 cell lines, but to a varying degree, with relatively uniform but low expression in RPCI-WM1 cells as judged by antibody staining, and more heterogeneous expression levels in MWCL1 cells. We genetically engineered RPCI-WM1 cells to inducibly express two distinct micro-RNAs (miR) under a doxycycline (dox) responsive promoter. The depletion of BLIMP1 upon dox addition unexpectedly caused rapid cell death in RPCI-WM1 cells but a non targeting control (NTC) miR had no effect on cell survival. There was an increase in apoptosis at 48h post Dox as assayed by Annexin V staining, with no surviving cells 5 days after BLIMP1 depletion. Genetic complementation using lentivirally expressed *PRDM1* cDNA resistant to miR mediated knock-down rescued the cell death phenotype, thereby excluding miR mediated off-target effects. Genome wide expression profiling of RPCI-WM1 cells upon BLIMP1 depletion using RNA-seq revealed increased expression of B-cell transcription factors such as *CIITA, BCL6, PAX5* and *SPIB* that constitute canonical targets of BLIMP1 repression during plasma cell differentiation were increased. Concurrently there was a loss of plasma cell genes such as *XBP1* but unexpectedly *IRF4* was induced up on BLIMP1 depletion indicating a more complicated gene regulatory relationship than simply binary B-cell vs. plasma cell differentiation mediated by BLIMP1 levels. At the same time, pro-apoptosis genes such as *CASP3, CASP8, CASP9, FAS, MAP3K8* and *BAX* with a concurrent loss of the pro-survival gene *BCL2* expression, showing that a sensitization to apoptosis induction at the level of transcriptional regulation occurs downstream of BLIMP1 depletion.