

Waldenström Macroglobulinemia-Specific Mechanism of BLIMP1-Mediated Cell Cycle Dynamics.

Stamatia-Maria Rapti, Dána Ágústsdóttir, Zarko Urosevic, Erna Magnúsdóttir

Department of Life Sciences, Faculty of Medicine, University of Iceland, Vatnsmýrarvegur 16, 101 Reykjavík, Iceland

Introduction: We have previously shown that the transcriptional repressor BLIMP1 is a strong mediator of survival in WM cells, but the mechanism of BLIMP1 action for the survival of WM cells has not been fully delineated. Recently, novel molecular tools for protein expression modulation have become available, allowing the rapid and precise control of protein levels. One such system is the FKBP12-derived destabilization domain (DD) degron that mediates constitutive degradation of proteins with which it is fused until a small synthetic molecule, Shield-1, is administered to rapidly stabilize DD fusion proteins.

Methods: To study BLIMP1's effect on cell cycle dynamics, we engineered RPCI-WM1 cells to express a doxycycline-inducible miR targeting the *PRDM1* (encoding BLIMP1) coding region, or a non-targeting control miR. Because miR-mediated depletion is slow, cells additionally expressed either a miR-resistant DD-tagged EGFP-BLIMP1 fusion (DDE-BLIMP1) or a DD-tagged EGFP control (DDE) that are constitutively degraded.

Following BLIMP1 knockdown with doxycycline and Shield-1-mediated stabilization for 24 h, cells were pulsed with EdU for 2 h, and click chemistry was used to label S-phase cells; DNA content and EdU were then analyzed by flow cytometry. To relate BLIMP1 levels, cell cycle phase, and double-strand breaks (DSBs), we combined EdU labeling with immunofluorescence staining for γ H2AX.

Direct BLIMP1 targets were identified by nascent RNA-seq using 5-ethynyl-uridine (EU) labeling. Endogenous BLIMP1 was knocked down by miR for 24 h while DDE-BLIMP1 was stabilized with Shield-1. Shield-1 was then withdrawn for 4 h to trigger rapid BLIMP1 degradation, with EU added for the final 2 h; control cells kept in Shield-1 and doxycycline were labeled in parallel. RNA was isolated, EU-labeled transcripts were biotinylated by click chemistry and captured on streptavidin, and the enriched RNA was used for short-read Illumina library preparation and sequencing.

Results: Our experiments reveal an inverse correlation between BLIMP1 levels and double-strand breaks (DSB) in RPCI-WM1 cells, particularly outside S-phase. DDE-BLIMP1 stabilization reduced DSBs by 12.5% compared to control cells with endogenous BLIMP1 levels, whereas miR-mediated BLIMP1 KD increased DSBs by 10.5%. Accordingly, BLIMP1 KD led to a G1 arrest and an increase in cell death. DDE-BLIMP1 stabilization had the opposite effect, with a 26% increase in cells in S-phase compared to control. However, chronic overexpression of DDE-BLIMP1 did not result in enhanced proliferation compared to control cells. Nascent RNA-sequencing pointed to novel targets of BLIMP1-mediated G1-to S-phase progression, potentially implicating the DSB repair machinery.

Conclusions: Our results show a novel mode of BLIMP1-mediated cell cycle dynamics in WM cells, where BLIMP1 drives G1-to S-phase progression through the modulation of genes driving cell cycle progression. The mode of BLIMP1-mediated S-phase entry appears in part to be mediated by the protection from DSB induction, thereby allowing cells to progress beyond the G1-S boundary. This is in contrast with previous observations where BLIMP1 was found to arrest cells in G1.

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