THE EPSTEIN-BARR VIRUS DEUBIQUITINATING ENZYME BPLF1 SUPPORTS CELL SURVIVAL AND VIRUS PRODUCTION BY REGULATING THE ACTIVITY OF TOPOISOMERASE II

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Cellular topoisomerases and helicases are essential for the replication of herpesviruses, but the mechanisms by which the viruses hijack the cellular enzymes are largely unknown. We found that the Epstein-Barr virus (EBV) encoded ubiquitin deconjugase BPLF1, selectively inhibits the degradation of topoisomerase-II (TOP2) in cells treated with the TOP2 poison Etoposide. Using transiently transfected and stable cell lines that express catalytically active or inactive BPLF1, we found that BPLF1 interacts with both TOP2α and TOP2β in co-immunoprecipitation and in *in vitro* pull-down assays and the active enzyme stabilizes TOP2 trapped in TOP2ccs, promoting a shift towards TOP2 SUMOylation. This hinders the activation of DNA-damage responses (DDR) and reduces the toxicity of Etoposide. The physiological relevance of this finding was validated using pairs of EBV carrying HEK-293T cells and EBV immortalized lymphoblastoid cell lines (LCLs) expressing the wild type or catalytic mutant enzyme. Induction of the productive virus cycle in cell lines encoding the active enzyme was accompanied by TOP2 deubiquitination, accumulation of TOP2ccs and a significantly weaker activation of the DDR, as assessed by the intensity of γH2AX specific bands in western blots. Cells carrying EBV that express catalytically active BPLF1 produced higher amounts of virion. In addition, the BPLF1 expressed during productive cycle increased the resistance of cells to Etoposide toxicity. Using knockout LCLs, we found that the capacity of BPLF1 to rescue cells during productive virus cycle from the toxicity of Etoposide is dependent on the expression of tyrosyl-DNA phosphodiesterase 2 (TDP2) that releases DNA-trapped TOP2 and promotes error-free DNA repair. These findings highlight a previously unrecognized function of BPLF1 in supporting a non-proteolytic pathway for TOP2ccs debulking that favors cell survival and virus production.