Epstein-Barr virus co-opts TFIIH component XPB to specifically activate essential viral lytic promoters

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SPR inhibits virus production in a non tumor-derived B cell line.

Previously, we have shown that SPR inhibits EBV production in EBV infected gastric carcinoma and B lymphoma cells. In order to determine whether SPR had antiviral activity in an EBV infected cell type that is not derived from a tumor, we tested the effect of SPR on virus production in normal B cells transformed and immortalized with EBV: lymphoblastoid cell line (LCL) 3BLCL-ZHT (1). 3BLCL-ZHT cells have been stably transduced with the EBV Zta transactivator gene fused to the hormone binding domain of tamoxifen receptor, allowing EBV lytic replication upon tamoxifen treatment. EBV replication in 3BLCL-ZHT cells was induced by adding tamoxifen and the effect of SPR on virus production was assessed. Infectious viral particles were measured in cell supernatants by a GFP transduction assay as described above. As shown in Figure S1A, SPR treatment led to inhibition of infectious virion production (~47%), demonstrating the antiviral activity of SPR in B cells.

In order to determine whether antiviral activity of SPR was also correlated to XPB protein degradation in LCLs, we measured XPB protein abundance by immunoblotting with and without SPR treatment (Figure S1B). As expected, SPR treatment induced XPB protein degradation, indicating that the antiviral activity of SPR is related to XPB degradation. Consistent with its identification as an SM inhibitory agent, the effect of SPR on EBV late gene expression correlates well with the effect of knocking down SM expression or genetically deleting SM in recombinant EBV (2). SM's effect on EBV gene expression is highly gene specific, enhancing mRNA accumulation from fifteen late lytic genes that are essential for various aspects of virion structural assembly and infectivity (3).The pattern of EBV gene expression in the presence of SPR parallels that of SM knockdown or deletion (2). We therefore asked whether the effect of SPR on EBV gene expression in physiologically infected B cells is SM-specific. We compared the effect of SPR on SM dependent and SM independent EBV gene expression. SPR strongly inhibited SM-dependent gene expression (BILF2 and BDLF1) but had no effect on SM-independent gene expression (BALF2 and BDLF4). These data confirmed that the antiviral activity of SPR is linked to XPB protein degradation and that SPR specifically blocks SM function by inhibiting SM dependent EBV gene expression both in normal and cancer cells infected with EBV.

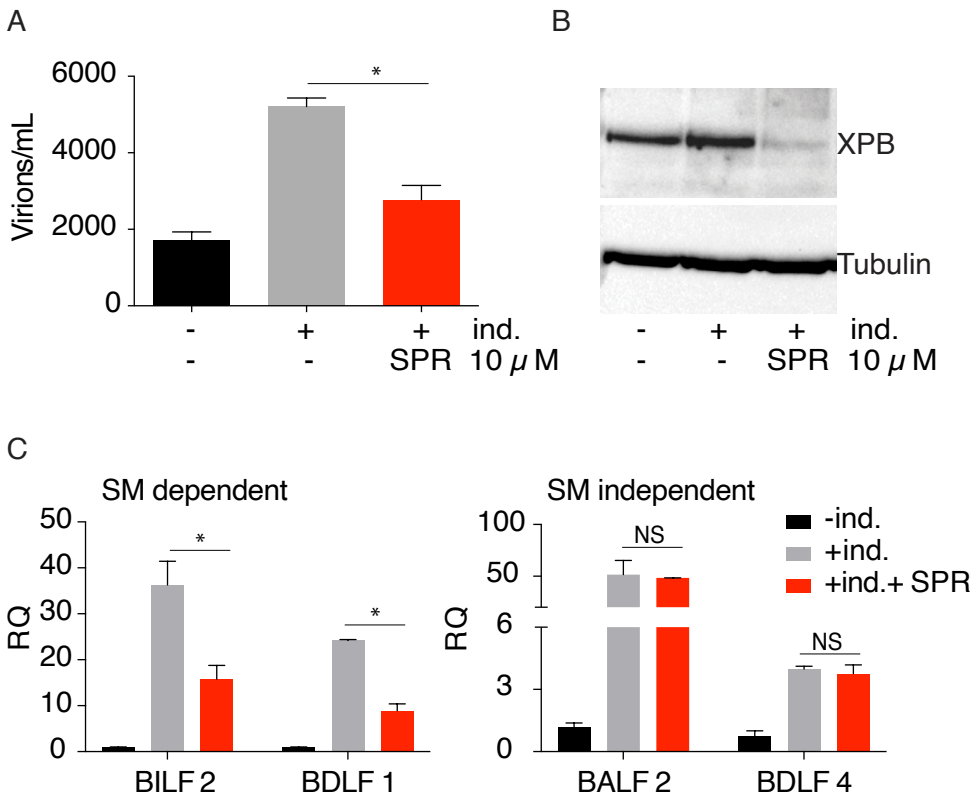

Figure S1: Antiviral and gene-specific effect of SPR in an EBV-transformed B lymphoblastoid cell line (LCL). (A) SPR Inhibits virus production in LCL. EBV-GFP infected 3BLCL-ZHT cells were treated with tamoxifen to induce EBV lytic replication (+ ind.) and treated or mock-treated with SPR in parallel. Virion production was measured by infecting Raji cells with induced cell supernatants followed by flow cytometry as described above. (B) SPR induces XPB degradation in LCL. Protein lysates from cells in (A) were immunoblotted using anti-XPB. Blot were stripped and re-probed with anti-tubulin antibody as a loading control. (C) Gene specific effect of SPR on EBV lytic RNA expression. RNA was isolated at 48 hr after EBV lytic replication and SPR treatment. RT-qPCR was performed to measure the effect of SPR on SM dependent (BILF2 and BDLF1) and SM independent RNAs (BALF2 and BDLF4). The error bars indicate the SEM from three replicates. *, p = 0.005-0.03, NS, p = 0.67-0.83.

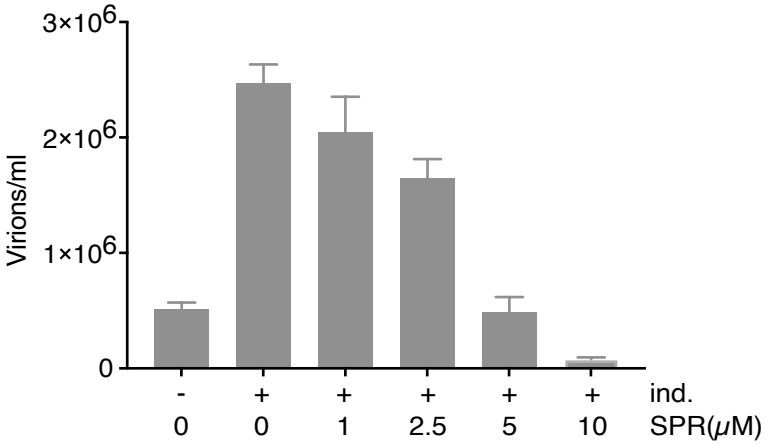

Figure S2: Dose-response of SPR effect on EBV virion production. SPR Inhibits virus production in AGSiZ cells in a concentration dependent manner. EBV-GFP infected AGSiZ cells were treated with doxycyline to induce EBV lytic replication (+ ind.) and treated or mock-treated with various concentrations of SPR in parallel. Virion production was measured by infecting 293 cells with cell supernatants followed by flow cytometry to detect cells newly infected by GFP-expressing EBV.

XPB	5'CCACTGTAAGCTGGGTTTGAC3' 5'CATCCAGTTGGCTTCGTAGAG3'
BILF2	5'GGGAAGAAGACGACCAATAC3' 5'TTGTGGTGTGGGAGACTAATG3'
BDLF1	5'TGGATGAGGTTAGCGTGGACAGTT3' 5'TCTAACTTCACGGTGGCATGCTCT3'
BALF2	5'GTGAGCTACGCACCCGCCAT3' 5'CTGACCGGTTGACTTCG3'
BDLF4	5'CCACCTGTGCTCGTATAGTAAG3' 5'GATCCATCACATACGTGGTACTC3'
BcLF1	5'GTGGATCAGGCCGTTATTGA3' 5'CCTCAAACCCGTGGATCATA3'
BGLF1	5'CACCTCCTACTCCCGTATCTAT3' 5'CCCAACAACTTTCCCAACTAAC3'
BBRF2	5'GGTCGACCACCTCAACATATTC3' 5'GCACATCTCCATAAGGTTCACA3'
β-actin	5'TCAAGATCATTGCTCCTCCTGAG3' 5'ACATCTGCTGGAAGGTGGACA3'
GAPDH	5'AGGGTCATCATCTCTGCCCCCTC3' 5'TGTGGTCATGAGTCCTTCCACGAT3'
BDLF1 TSS	5'TGATAATTGTCAATAAAGATGGATTTGAAA3' 5'GAGGAGAGAGACACTACCACTT3'
BZLF2 TSS	5'TTGAAGTGGTTGCCATGGT3' 5'GCACCCTCACCTGCTTAAAT3'
BDLF4 TSS	BDLF4 TSS 5'TGGCCGGGGTGGAATGT3' 5'GGAGGCTCAATCGGCCTTG3'
BGLF1 TSS	5'GATGCTGTAGCCAAACGCA3' 5'TGTGGACATCCATAGCTCTCTC3'

Table S1. Primers used for each EBV and cellular gene for RT qPCR.

BcLF1p	5'CATCTCCCTCTTACCTTGTGTC3' 5'GGC ATCCACCGTCAGATAG3'
BZLF2p	5'TATCTTCCTGTCGGCTCTCT3' 5'AAGTGCGATGGCGGTAAA3'
BDLF1p	5'CGCCGGTACGAAGAATATTAAAG3' 5'GGTATACAGACGAGAGGAGAGA3'
BBRF2	5'TGGCCATCTACATCGAGGA3' 5'ACGCGCAGGCTTACTTT3'
BALF2p	5'ATACCTTGGGCATCATGCAG3' 5'TAGATGTAGCCGCACGGA3'
NFKBIAp	5'CTCATCGCAGGGAGTTTCT3' 5'ACTGCTGTGGGCTCTGCA3'
IL-6p	5'TAGAGCTTCTCTTTCGTTCCCGGT3' 5'TGTGTCTTGCGATGCTAAAGGACG3'
GAPDHp	5'TACTAGCGGTTTTACGGGCG3' 5'TCGAACAGGAGGAGCAGAGAGCGA3'
EBV Cp	5'TTCGCCCACGACTTGAAA3' 5'CTTCGGTGTCCTTGTCTCTATG3'

Table S2. Primers used for ChIP assays.

SI References

1. E. Johannsen *et al.*, Proteins of purified Epstein-Barr virus. *Proc Natl Acad Sci U S A* **101**, 16286-16291 (2004).
2. D. Verma, J. Thompson, S. Swaminathan, Spironolactone blocks Epstein-Barr virus production by inhibiting EBV SM protein function. *Proc Natl Acad Sci U S A* **113**, 3609-3614 (2016).
3. J. Thompson, D. Verma, D. Li, T. Mosbruger, S. Swaminathan, Identification and Characterization of the Physiological Gene Targets of the Essential Lytic Replicative Epstein-Barr Virus SM Protein. *J Virol* **90**, 1206-1221 (2016).