DIFFERENTIATION-INDUCED SYNTHESIS OF BLIMP1 LEADS TO XBP-1s SYNERGIZING WITH b-ZIP PROTEINS TO PROMOTE LYTIC EBV REACTIVATION IN EPITHELIAL CELLS

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The life cycle of Epstein-Barr virus (EBV) is unique in that it can establish latency and replicate in both lymphotropic and epithelial cells. Regardless of cell type, differentiation induces expression of B lymphocyte-induced maturation protein-1 (Blimp1) and accumulation of unprocessed proteins, resulting in endoplasmic reticulum (ER) stress. In response, the cell activates via phosphorylation the transmembrane kinase/endoribonuclease inositol-requiring enzyme, IRE-1α. Phosphorylated IRE-1α then splices XBP-1 transcripts, leading to synthesis of the stress response factor XBP-1s, a b-ZIP transcription factor. Prior reports indicated XBP-1s can activate transcription from the immediate-early promoter Zp and induce synthesis of lytic EBV antigens in EBV-positive lymphoid cell lines [1,2]. Unclear was what happens in epithelial cells.

We previously reported that Blimp1 induces lytic EBV reactivation in both EBV-positive epithelial and B cell lines, doing so by activating transcription from Zp and Rp [3]. Here, we set out to determine the primary mechanism by which Blimp1 expression leads to Zp activation in epithelial cells. We found that normal oral keratinocytes latently infected with the Akata strain of EBV (NOK-Akata) when differentiated by culturing as organotypic rafts synthesized Blimp1, phosphorylated IRE-1α, and accumulated XBP-1s along with lytic EBV antigens. Utilizing Zp-luciferase reporter plasmids, we mapped Blimp1-response elements to b-ZIP response elements located within the ZII element as well as a consensus XBP-1s response element (XRE) located at ~nt. -80. Blimp1 induced synthesis of XBP-1s by signaling phosphorylation of IRE-1α via the MAPK pathway. Drug-directed inhibition of Blimp1-induced IRE1α activity partially blocked both Zp-driven luciferase activity and expression of lytic EBV antigens in gastric cancer-derived AGS-Akata cells. Mutation of the nt. -80 XRE within the context of a whole EBV genome led to inability of Blimp1 to induce lytic EBV antigens in infected AGS cells. ChIP analysis indicated that presence of this XRE was necessary for XBP-1s binding to Zp. Induction of lytic EBV antigens by tunicamycin, a known inducer of ER stress and XBP-1s synthesis, or the phorbol ester TPA also required presence of this Zp XRE. The XRE we had previously mapped in Rp [3] also looks like a composite XBP-1s/b-ZIP binding element. Thus, we conclude that synergy among b-ZIP transcription factors, including XBP-1s bound to the XRE and ZII elements within Zp, probably regulates differentiation-dependent reactivation of EBV in oral keratinocytes as well as plasma cells.

1. Bhende et al. 2007 J. Virol. 81:7363-7370.
2. Sun and Thorley-Lawson. 2007 J. Virol. 81:13566-13577.
3. Reusch et al. 2015 J. Virol. 89:1731-1743.