ISOFORM-SPECIFIC FUNCTIONS OF THE HOST TRANSCRIPTION FACTOR, LYMPHOID ENHANCER FACTOR 1 (LEF1) IN EBV LATENCY AND REACTIVATION

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Lymphoid enhancer factor 1 (LEF1) is a transcription factor of the Wnt signaling pathway with increased expression observed in EBV-associated nasopharyngeal carcinoma and Burkitt's Lymphoma (BL). LEF1 transcriptional activities depends on interactions with either β-catenin for activation or with Groucho/transducin-like enhancer of split for repression. Previously, we demonstrated EBV infection and establishment of latent EBV correlated with increased expression of LEF1 in hTERT-immortalized normal oral keratinocytes (NOK), but how LEF1 influences EBV latency has not been examined. The EBV genome encodes up to 20 putative wnt response elements (WRE) suggesting that LEF1 may directly engage the EBV genome. Chromatin immunoprecipitation coupled quantitative PCR and "Cut-and-Run" sequencing analysis confirmed LEF1 binding at several WRE motifs on the EBV genome. To determine if LEF1 restricts EBV reactivation, latently infected NOK were transfected with siRNA that either targeted all LEF1 isoforms or only the long LEF1 isoforms without affecting expression of the short, N-terminally truncated isoform (delNLEF1). The delNLEF1 isoform lacks the b-catenin binding domain and shown to have distinct functions compared to long LEF1 isoforms. EBV reactivation in latently infected NOK cells was stimulated through calcium-induced differentiation. Under these conditions, we observed that targeting all LEF1 isoforms enhanced lytic gene induction over non-target control. In contrast, knockdown of only the long isoforms repressed EBV lytic gene expression, suggesting that the delNLEF1 isoform is involved in the maintenance of latency. In addition, viral reactivation in EBV-positive Akata BL showed loss of LEF1 protein through proteasomal degradation, with the truncated delNLEF1 protein isoforms showing the greatest decrease. The enhanced LEF1 degradation was specific to EBV-positive Akata BL and was not observed in EBV-negative Akata BL. These observations implicate a viral effector in the degradation of LEF1 during EBV reactivation and suggest a novel isoform-dependent role for LEF1 in regulating EBV latency and reactivation.