REDUCED IRF4 LEVEL CONTRIBUTES TO LYTIC PHENOTYPE OF TYPE 2 EBV INFECTED B CELLS

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Type 1 (T1) EBV and Type 2 (T2) EBV differ substantially in their EBNA2 and EBNA 3A/B/C latency proteins and behave differently in infected B cells. T2 EBV transforms B cells less efficiently than T1 EBV *in vitro,* and T2 EBV-infected B cells are more lytic. We previously showed that Increased NFATc1/c2 activity, and the presence of an NFAT-binding motif within the Zp-V3 form of the BZLF1 immediate-early promoter contained in all T2 EBV strains, both contribute to enhanced lytic infection in T2 EBV-infected B cells. Here we have compared cellular and viral gene expression in early-passage lymphoblastoid cell lines (LCLs) infected with T2 versus T1 EBV strains using bulk RNA-seq as well as single-cell RNA-seq analyses. We find that T2 and T1 LCLs are readily distinguishable, with approximately 600 differentially expressed cellular genes. Gene Set Enrichment Analysis (GSEA) suggests increased B-cell receptor (BCR) signaling, NFAT activation, and enhanced expression of epithelial-mesenchymal-transition-associated genes in T2 EBV-infected versus T1 EBV-infected LCLs. We show that T2 LCLs also have decreased RNA and protein expression of IRF4, a cellular protein required for survival of T1 LCLs and plasma cell differentiation. Importantly, IRF4 has also been reported to decrease BCR signaling, and low levels of IRF4 promote the development of the BCR-dependent tumor, CLL, in mouse models and in humans. We demonstrate that knock-down of IRF4 in a T1 LCL (infected with the Zp-V3-containing Akata strain) induces lytic reactivation, and conversely, over-expression of IRF4 in Akata Burkitt lymphoma cells inhibits both NFATc1 and NFATc2 expression and lytic EBV reactivation. Single-cell RNA-seq confirmed that T2 LCLs have many more lytic cells compared to T1 LCLs and showed that lytically infected cells have both decreased IRF4, and increased NFATc1, in comparison to latently infected cells. These studies reveal numerous differences in cellular gene expression in B cells infected with T2 versus T1 EBV and show that differences in IRF4 expression contribute to differences in both the latent and lytic phenotypes in B cells infected with T1 versus T2 EBV.

Topic: EBV latency program and reactivation