LEVERAGING DIVERSE EPITHELIAL CELL LINE MODELS TO STUDY EBV LATENCY AND IDENTIFY CELLULAR RESTRICTION FACTORS OF INFECTION

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Epstein-Barr Virus (EBV) is a ubiquitous human herpesvirus that infects over 95% of the adult population as a latent infection present in memory B cells. While EBV infection is typically asymptomatic, it can cause various malignancies including B cell lymphomas, as well as epithelial cancers such as gastric carcinoma, nasopharyngeal carcinoma, and lymphoepithelioma-like carcinoma (LELC) found in diverse epithelial tissues. EBV-associated epithelial tumors are made up of monoclonally infected epithelial cells and often display dense lymphocyte infiltration [1,2]. During initial infection, highly differentiated epithelial cells within the oral cavity serve as lytic viral replication factories, and epithelial cells have canonically been thought of as refractory to latent infection. Indeed, most epithelial cells are highly resistant to EBV infection and latent outgrowth *in vitro*. We leveraged largescale genomic datasets that were publicly available to identify a unique subset of diverse epithelial cell lines that are highly susceptible to EBV infection, including the lung adenocarcinoma cell line A549 [3]. We used the co-culture method of infection [4] and found that 6 days post infection, between 50-70% of A549 cells expressed GFP indicating viral infection in comparison to the gastric cancer cell line AGS where we see only 5-15% of cells expressing GFP. Additionally, in a latency outgrowth assay, we found establishment of latent clones in A549 cells compared to other gastric cell lines GES1 and MKN1 was 180-fold more efficient [5]. It is thought that EBV infection alone cannot drive transformation and tumorigenesis of epithelial tumors and it has been hypothesized that prior genetic perturbations are required though these have yet to be identified. We aim to use the highly susceptible cell line A549 to study cellular restriction factors of EBV latency through a CRISPR/cas9-based screening approach.

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