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Human papillomavirus promotes Epstein-Barr virus maintenance and lytic reactivation in immortalized oral keratinocytes

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ABSTRACT

Epstein-Barr virus and human papillomaviruses are human tumor viruses that infect and replicate in upper aerodigestive tract epithelia and cause head and neck cancers. The productive phases of both viruses are tied to stratified epithelia highlighting the possibility that these viruses may affect each other's life cycles. Our lab has established an *in vitro* model system to test the effects of EBV and HPV co-infection in stratified squamous oral epithelial cells. Our results indicate that HPV increases maintenance of the EBV genome in the co-infected cells and promotes lytic reactivation of EBV in upper layers of stratified epithelium. Expression of the HPV oncogenes E6 and E7 were found to be necessary and sufficient to account for HPV-mediated lytic reactivation of EBV. Our findings indicate that HPV increases the capacity of epithelial cells to support the EBV life cycle, which could in turn increase EBV-mediated pathogenesis in the oral cavity.

1. Introduction

Epstein-Barr virus (EBV) and human papillomaviruses (HPVs) are human tumor viruses that cause head and neck cancers. EBV is an enveloped, double-stranded DNA virus with tropism for epithelial cells and resting B lymphocytes. EBV was the first virus to be associated with human cancers; it is associated with several lymphoid and epithelial cancers including Burkitt's lymphoma, Hodgkin disease, nasopharyngeal carcinoma, and gastric cancers. HPVs are small, non-enveloped, double-stranded DNA viruses with a tropism for epithelial cells. High-risk HPVs are associated with 5% of human cancers including cervical and other anogenital cancers as well as a growing fraction of head and neck cancers, all epithelial in origin.

HPV and EBV both infect and replicate in upper aerodigestive tract epithelia (Herrero, 2003; Vincent-Bugnas et al., 2013). EBV infects the oral epithelia causing oral hairy leukoplakia on the tongue which is characterized by its productive infection (Greenspan et al., 1985) in addition to its association with nasopharyngeal cancers (zur Hausen et al., 1970). Likewise, HPV infects oral keratinocytes and the high-risk subtypes are associated with an increasing percentage of oropharyngeal cancers (Gillison et al., 2000; Stein et al., 2014). The life cycles of these two tumor viruses also share a relationship with the stratified epithelium in that the productive phase of the HPV life cycle and lytic reactivation of EBV are both induced upon differentiation of epithelial cells. The productive phase of the HPV life cycle, which includes late protein expression and genome amplification, occurs in the suprabasal compartment of stratified epithelium (Howley and Lowy, 2001). Similarly, EBV immediate-early protein expression and genome amplification, two hallmarks of EBV lytic reactivation, occur in suprabasal epithelial layers (Nawandar et al., 2015; Temple et al., 2014).

Given the tropism of both HPV and EBV for epithelial cells and the parallels in regard to their life cycles being tied to the differentiation of stratified epithelium, the question arises as to whether these viruses influence each other's life cycles under conditions in which co-infection may occur in the upper aerodigestive tract. We have established an *in vitro* model system for studying the

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influence of high-risk HPV and EBV on each other in monolayer oral epithelial cells as well as in stratified oral epithelial cells using organotypic (raft) cultures harboring these viruses alone and together. We found that the presence of HPV18 stabilizes the long-term maintenance of EBV genomes in monolayer culture and promotes EBV lytic reactivation in the suprabasal compartment of raft cultures. In addition, HPV oncogenes E6 and E7 are necessary and sufficient to promote HPV-mediated EBV lytic reactivation in suprabasal layers of oral stratified epithelium.

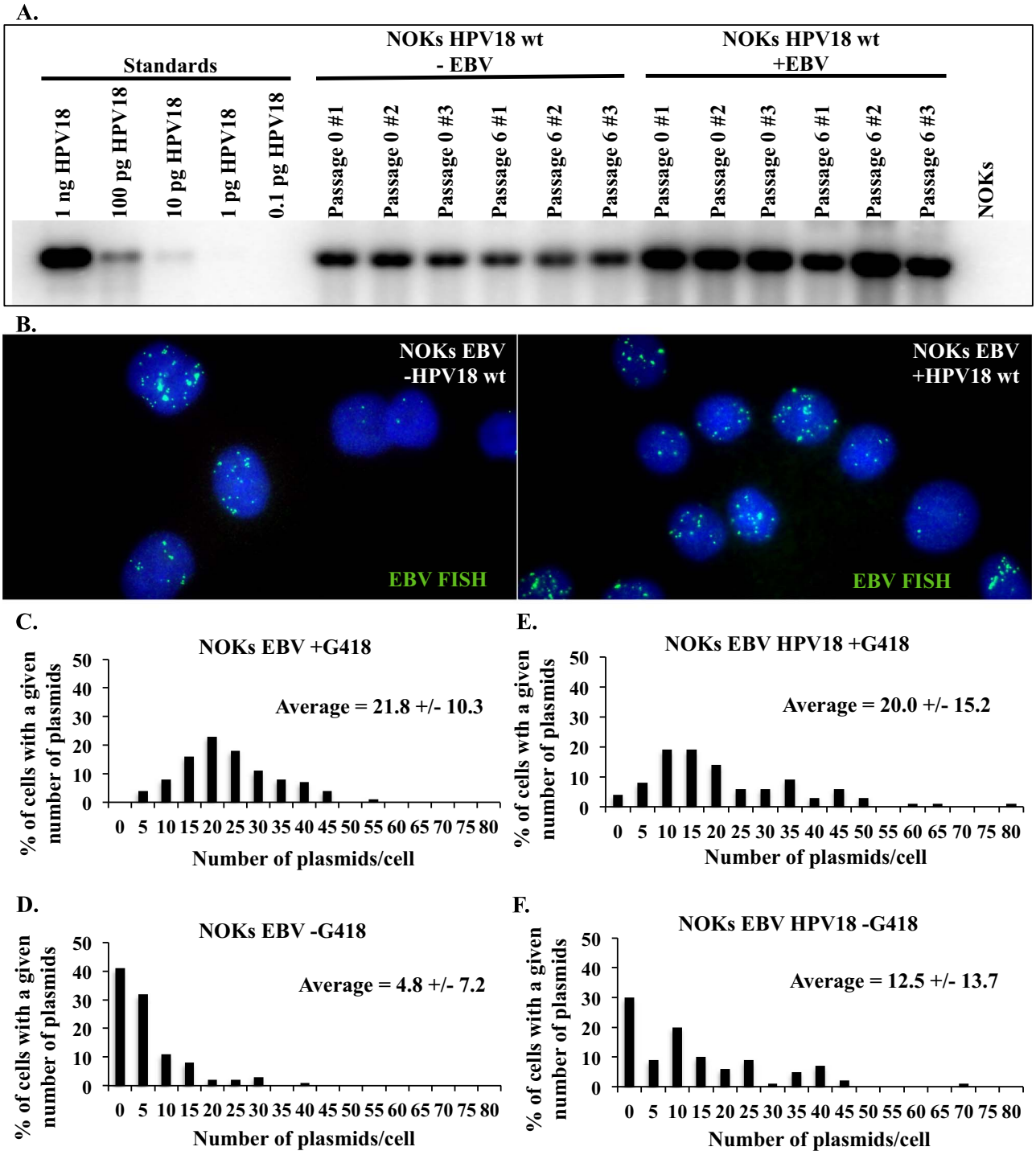

Fig. 1. HPV18 and EBV genomes are maintained alone and together in NOKs cells over several passages. (A) The HPV18 genome is maintained in NOKs cells ± EBV over six passages. Total genomic DNA (7 μg) isolated from three independent replicate populations (labeled as #1, #2, and #3) of NOKs cells ± EBV harboring HPV18 wild-type genomes at passage 0 and 6 post-HPV transfection was double digested with a single cutter of the HPV18 genome (Nco1) and Dpn1 (an enzyme that cuts only bacterially-replicated input DNA). The digested DNA (2.5 μg) was separated using a 0.8% agarose gel and transferred to a Hybond N+ membrane followed by HPV18-specific Southern blot analysis. (B) The EBV genome is maintained over several passages (> 15) in NOKs cells without HPV18 (left panel) and with HPV18 (right panel). The number of EBV genomes per cell was quantified in EBV-infected NOKs cells grown for 12 passages in the presence (C) or absence (D) of selection for EBV, highlighting the loss of EBV genomes in the absence of selection. The number of EBV genomes per cell was also quantified in EBV-infected NOKs cells harboring HPV18 grown for 12 passages in the presence (E) or absence (F) of selection for EBV.