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# DAY 3

# EBV biology & infection

# Epstein-Barr virus tegument proteins BPLF1 and BGLF2 are viral antagonists of interferon production and signaling

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Epstein-Barr virus (EBV) successfully establishes a persistent infection in more than 95% of all adults but is etiologically associated with the development of lymphoid and epithelial malignancies, such as Burkitt's lymphoma, Hodgkin's lymphoma, nasopharyngeal carcinoma and gastric carcinoma, in a small subset of subjects latently infected with EBV. EBV has evolved to evade interferon (IFN)-dependent innate immune response using multiple counter-strategies. Here, we perform functional screens with an expression library of EBV-encoded proteins to search for interferon (IFN) antagonists. Two tegument proteins BPLF1 and BGLF2 are found to be IFN-antagonizing proteins that potently suppress IFN production and signaling. BPLF1 is a large tegument protein with deubiquitinase activity. When type I IFN production is induced by DNA or RNA through their sensors either cGAS plus STING or RIG-I plus MAVS, BPLF1 exhibits a strong and deubiquitinase-dependent suppressive effect on the induction. BPLF1-mediated deubiquitination shows no ubiquitin linkage preference, with equally strong activity on both K63- and K48-linked ubiquitintion of STING and TBK1. Enforced expression of BPLF1 through CRISPRa in cells infected with EBV results in remarkable suppression of IFN production induced by DNA or RNA. We have also identified another EBV tegument protein BGLF2 as a suppressor of IFN signaling. The suppression effect is medicated though multiple targets including STAT1, STAT2 and JAK1. BGLF2 interacts with STAT2 to induce its K48-linked polyubiquitination and consequent proteasomal degradation. Additionally, BGLF2 inhibits tyrosine phosphorylation of JAK1 and STAT1 through recruitment of tyrosine phosphatase SHP1. IFN signaling is more robustly activated by a BGLF2-deficient EBV. Combined, we have delineated the IFN antagonism of EBV tegument proteins BPLF1 and BGLF2, which target ubiquitination and deubiquitination of key signal transducers to circumvent IFN production and signaling in EBV-infected cells. Supported by HMRF 17160822, HMRF 18170942 and RGC C7027-16G.

# Elucidating Epstein-Barr virus molecular pathogenesis in the nasopharynx by 3-D air-liquid interface culture models and single cell RNA-sequencing

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In the nasopharyngeal epithelium, latent Epstein-Barr virus (EBV) infection is associated with the cancer nasopharyngeal carcinoma (NPC). Despite the longstanding association of clonal EBV infection in NPC tumors, the molecular pathogenesis of EBV in the nasopharyngeal epithelium has remained enigmatic. It is not clear what cell types in the nasopharynx are susceptible to EBV infection or what restriction factors influence EBV susceptibility and the type of resultant EBV infection (latent or lytic). Conventional 2-D cell culture cannot recapitulate the differentiation-dependent induction of the lytic cycle. Instead, 3-D cell culture models of the nasopharyngeal epithelium have emerged as a new way to address EBV molecular pathogenesis. The nasopharynx is composed of pseudostratified (respiratory) and stratified squamous epithelium. These two types of epithelium can be modeled by culturing nasopharyngeal cell lines or primary (conditionally reprogrammed) cells in defined conditions at the air-liquid interface (ALI). Such ALI cultures modeling EBV infection in the nasopharynx can be analyzed by EBV molecular diagnostics as well as the emerging technology of single cell RNA-sequencing that require unique bioinformatics to interpret the co-terminal transcripts of gamma-herpesvirus genomes. In this seminar, nasopharyngeal 3-D culture models as applied to studies of EBV reactivation and EBV susceptibility will be presented.

# Impacts of strain variation on EBV virology

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The Epstein-Barr virus (EBV) is a ubiquitous tumor virus that infects the majority of human population. EBV is also endowed with strong transforming properties and is etiologically linked with many human lymphomas and carcinomas. Nevertheless, the incidence of EBV-associated malignancies varies drastically from all geographical areas. Similar to the scenario of human papilloma virus, the existence of certain “pathogenic EBV strains” might be an explanation whereas the complexity and giant EBV genome elevated the difficulty for exploring such hypothesis. Thanks for the advanced sequencing technology and recombinant techniques in the past decade, more evidences indicating that EBV-associating diseases, specially carcinomas, might be highly contributed by certain pathogenic EBV strains. By phenotypic characterization of different EBV strains and genetic knockout or exchanges, we are allowed to understand the pathogenic roles of EBV in human diseases that might lead to the development of proper vaccines and drugs against specific EBV-associated diseases in the future.