EBV BORF2 PROMOTES G1/S CELL CYCLE ARREST THROUGH INTERACTIONS WITH P53 AND APOBEC3B

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In lytic infection by herpesviruses, cells are arrested at the G1/S phase of the cell cycle, but the proteins and mechanisms involved are not well understood. We previously screened proteins from three herpesviruses (HSV1, EBV, and CMV) for the ability to induce G1/S arrest in FUCCI cells, which express fluorescently tagged proteins at different stages of the cell cycle allowing for easy identification of G1/S cells [1]. EBV BORF2 was identified as one such protein and was further shown to induce p53 levels without inducing the p53 target protein p21, suggesting that p53 was inactivated by BORF2. To better understand these observations, we performed affinity purification coupled to mass spectrometry (AP-MS) with BORF2, revealing specific interactions with the host proteins p53 and APOBEC3B (A3B). A3B is a nuclear cytosine deaminase that can mutate ssDNA during replication, therefore, has the potential to inhibit viral infection. We previously showed that, in lytic EBV infection, BORF2 relocalizes A3B from the nucleus to perinuclear bodies containing BORF2, thereby protecting EBV genomes from A3B mutations [2]. Investigation of the interactions of BORF2 with A3B and p53 showed that, while these interactions did not depend on the same BORF2 amino acids, A3B still inhibited BORF2 binding to p53, presumably because BORF2 relocalized to perinuclear bodies in the presence of A3B. Cell cycle analysis showed that G1/S induction by BORF2 was abrogated when either p53 or A3B was silenced or when an A3B-binding mutant of BORF2 was used, suggesting that BORF2 binding to A3B was critical to induce G1/S arrest. This hypothesis was further supported by our findings that the BORF2 homologue from HSV1 (UL39), which can also interact with and relocalize A3B, also induced G1/S arrest, while the homologue from CMV (UL45), which does not interact with A3B, did not induce G1/S [3]. Like BORF2, UL39 was shown to induce p53, and UL39-induced G1/S arrest was dependent on p53 and A3B. A role for A3B in G1/S arrest in EBV lytic infection was further supported by the finding that silencing A3B resulted in more cell proliferation after EBV reactivation. Together the data support a model in which the p53 that is induced by BORF2 is inactive when it is bound by BORF2, but becomes active to induce G1/S arrest when A3B is present because A3B sequesters BORF2 in perinuclear bodies where it is unavailable to bind p53. Our study provides a new mechanism by which G1/S arrest can be induced in herpesvirus lytic infection and identifies an additional role for A3B in herpesvirus infection.

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