LMP1 PROMOTES PROLIFERATION, AND INHIBITS DIFFERENTIATION, IN EBV-INFECTED TELOMERASE-IMMORTALIZED NORMAL ORAL KERATINOCYTES (NOKS) BY ACTIVATING THE HIPPO PATHWAY EFFECTORS, YAP AND TAZ

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Although carcinomas such as nasopharyngeal carcinoma (NPC) account for almost 90% of EBV-associated human cancers, progress in examining EBV's role in their pathogenesis has been limited by the difficulty in establishing persistent latent infection in non-transformed epithelial cells, and because EBV infection of primary epithelial cells *in vitro* does not by itself transform these cells. We have recently established an *in vitro* model system to examine how EBV contributes to NPC using a telomerase-immortalized normal oral keratinocyte (NOKs) cell line which can be stably infected with EBV and retains the ability to differentiate. Although we previously showed that EBV infection of NOKs cells increases cellular proliferation, and inhibits differentiation, when NOKs cells are grown on "raft" cultures, the viral genes and/or RNAs required for this effect remain unknown. Here we have used a newly constructed LMP1 mutant virus (in the context of the AG876 virus genome) to show that the latent EBV protein, LMP1, is both required, and sufficient, to induce cellular proliferation in NOKs monolayer cell culture when growth factors are limiting. In addition, we show that LMP1 is both required, and sufficient, to inhibit spontaneous NOKs cell differentiation induced by growth factor deprivation in monolayer culture, or by methylcellulose suspension. Further, we show that these LMP1 effects are mediated by activation of the Hippo signaling effectors, YAP and TAZ. We find that LMP1 increases YAP and TAZ activity both by increasing Src-mediated tyrosine phosphorylation of YAP (which increases its activity), and by decreasing Hippo mediated serine phosphorylation of YAP and TAZ (which inhibits their activity). Importantly, knockdown of YAP and TAZ expression in EBV-infected NOKs cells is sufficient to reverse LMP1-mediated effects on cellular proliferation, differentiation, and EMT. Finally, we show that treatment of EBV-infected NOKs cells with Ibrutinib (a BTK inhibitor recently shown to block YAP and TAZ activity through an off-target effect) or Dasatinib (a Src inhibitor), reverse the effects of LMP1 on NOKs cell proliferation and differentiation using clinically relevant doses. These results are the first to show that LMP1 promotes proliferation and inhibits differentiation of telomerase- immortalized keratinocytes in the context of the intact viral genome, and the first to demonstrate that LMP1 activates the YAP activity in epithelial cells. Our findings suggest that drugs which inhibit YAP and/or TAZ activity will be useful for inhibiting LMP1-mediated transformation of epithelial cells in humans.

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