Title: ***In vitro*** **migration and cellular invasion of human cells expressing variants of the Epstein-Barr virus LMP1 oncoprotein**

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Abstract

The Epstein-Barr virus (EBV) latently infects more than 90% of human adults. Viral infection is associated to the development of some cancers, including nasopharyngeal carcinoma. Oncogenic potential of the virus is usually studied in terms of its capacity to transform the infected cells nevertheless some studies suggest that the EBV infection may also contribute to immune evasion, and cancer progression. Several latent membrane protein 1 (LMP1) variants are described (B95.8, Alaskan, Med+, China 1, and China 2), discriminated mainly by variations in its C-terminal and the transmembrane domains of the protein. LMP1 C-terminal domain activate intracellular signaling pathways that regulated cell migration; moreover, LMP1 upregulate the expression of cell proteins with roles in extracellular matrix remodeling and angiogenesis. Currently it is unknown whether different LMP1 variants possess distinct properties regarding biological phenomena relevant to immune evasion, and cancer progression. Thus, in the present study we evaluated the *in vitro* migration and invasiveness, and immunomodulation of HLA-ABC, HLA-DR, CD80, CD83, CD54, CD40, and PD-L1 of HEK293T cells, and NP69 cells. For this evaluation, HEK293T, and NP69 cells, transfected with pBabe vectors containing LMP1 variants, were analyzed regarding cell migration using scratch wound healing assay 24h post-transfection. Next, we used these cells to verify cell invasion by transwell invasion assay 48h post-transfection. Chemotaxis migration assay was also performed in these cells 24h post-transfection, using transwell inserts, and EGF as chemoattractant agent. In order to verify LMP1 immunomodulation, we performed flow cytometry analysis in these cells. We observed that Alaskan, Med+, China 1, and China 2 stimulates distinctly migration of NP69 when compared to cell without LMP1. We also saw that HEK293T-China 2 had a pronounced cellular invasion when compared to HEK293T. In regard of expression of molecules involved in immune response modulation, we observed that LMP1 enhanced expression of CD80, and CD83 in NP69-Alaskan cells; and CD54, and PD-L1 in a similar level in NP69-LMP1 cells. In summary, LMP1 promote distinct cellular migration in NP69-LMP1 cells, and HEK293T-China 2 invasion, and is capable of modulate molecules involved in immune evasion.

Keywords: EBV, LMP1, NPC, Cellular migration, and cellular invasion.

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