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PhD Dissertation Defense

Entitled

STRUCTURAL IMPACT OF EPSTEIN-BARR VIRUS-ENCODED SMALL RNA-1 ON ITS TRANSPORT AND FUNCTION

by

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Abstract

Epstein-Barr virus (EBV)-encoded RNAs (EBERs) are two small, noncoding, structurally conserved transcripts that are expressed in millions of copies in all stages of EBV infection. Despite their abundant expression, the role of EBERs in EBV biology or pathogenesis is not well defined. However, they are associated with driving cell proliferation and improving intercellular communication via their secretion in exosomes. In spite of the pathogenic potentials of EBERs, the mechanisms involved in their transport and function remain to be elucidated. The aim of this PhD dissertation was to investigate the structural impact of EBER1 in its transport and function. The conserved structure of EBER1 was disrupted by deleting sequences corresponding to stem-loop (SL) 1, 3 and 4 of the RNA, creating three mutants: ΔSL1, ΔSL3, and ΔSL4. These mutants were cloned onto pHebo plasmid and transfected into Jurkat and HEK293T cell lines. Cells transfected with wildtype EBER1 and pHebo were used as the positive and negative control, respectively. Cell proliferation was investigated by microscopy, flow cytometry, microarray, qRT-PCR, and Western blotting. EBER1 transport was studied by quantifying EBER1 expression in the whole cell, nuclear, cytoplasmic and exosomal fractions by qRT-PCR in the presence of physiological expression of RPL22 and La, and after their silencing using siRNA technique. Furthermore, intracellular Ca^{2+} was measured by microscopy and Western blotting. There was a significantly higher proliferation in cells transfected with wildtype EBER1 compared to pHebo, ΔSL1 and ΔSL3 but not ΔSL4 mutant. Similarly, markers of S-phase and G2/M phase were significantly upregulated in wildtype EBER1 and ΔSL4 mutant. Moreover, CDT1 was upregulated in these cells. In ΔSL1 mutant, however, CDT1 was significantly downregulated and translocated to the cytoplasm. Statistically, the structure of EBER1 had a significant impact on its induced cell proliferation (p=0.045). Similarly, EBER1 structure had an impact on its transport. Compared to wildtype, the level of EBER1 expression in all mutants was significantly lower in the whole cell, cytoplasmic and exosomal fractions. ΔSL3 mutant showed significant nuclear retention. Silencing RPL22 resulted in increased nuclear-cytoplasmic trafficking of EBER1. Nonetheless, silencing La protein did not affect the secretion of EBER1. As an alternative mechanism of EBER1 secretion, the intracellular Ca^{2+} signalling mediated by store-operated Ca^{2+} entry was found to be proportional to EBER1 expression in exosomes. To summarise, stem-loops 1 and 3 appeared to be involved in EBER1-induce proliferation and cellular transport, respectively. The molecular mechanisms of EBER1-induced cell proliferation involve upregulating and retaining the nuclear expression of CDT1. EBER1-RPL22 interaction is implicated in the nuclear localisation of the RNA. Finally, this study hypothesised that cytosolic Ca^{2+} could play a role in the secretion of EBER1 into exosomes.

Keywords: EBER1, Function, Proliferation, Structure, Transport.