INVESTIGATING THE STRUCTURAL IMPACT AND MOLECULAR MECHANISMS INVOLVED IN INTRACELLULAR TRANSPORT AND SECRETION OF EPSTEIN-BARR VIRUS-ENCODED RNA-1

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Abstract:

Statement of the Problem: *Epstein-Barr virus (EBV)-encoded RNAs (EBERs) are the most abundantly expressed transcripts of EBV. Their role in EBV biology and pathogenesis remains unclear. Nonetheless, they are believed to be involved in many pathogenic processes including the spread of the virus through improved cell-cell communication [1]. Although primarily localised in the nucleus, EBERs have been reported in other subcellular compartments such as cytoplasm and exosomes [1], [2]. The mechanisms by which EBERs are transported to the cytoplasm and subsequently secreted into exosomes are not known. However, there are reports implicating their secondary structure and their interaction with cellular proteins [1], [3], [4]. These proteins include ribosomal protein, L22 (RPL22) for nuclear-cytoplasmic trafficking [1], [5] and La antigens for secretion into exosomes [1], [2]. Therefore, the aim of this study was to investigate the structural impact and molecular mechanisms involved in the intracellular transport and secretion of EBER1.*

Materials and Methods: *Using site-directed mutagenesis, we generated three EBER1 mutants by deleting sequences corresponding to stem-loops (SL) 1, 3 and 4 of the RNA. These mutants, SL1, SL3 and SL4, and the wildtype EBER1 were individually cloned into pHebo plasmid. The plasmids were stably transfected into HEK293T cell lines. Exosomes from transfected cells were isolated and characterised by a Nanoparticle analyser. The expression of EBER1 in the cell, nucleus, cytoplasm and exosomes was quantified using qRT-PCR. Similarly, the expression of RPL22 and La antigen were determined in these fractions using qRT-PCR and western blotting. Cells transfected with wildtype EBER1 were used as reference (100%) while those transfected with empty pHebo (vector only) as the negative control. Additionally, siRNA silencing was employed to explore the effect of these proteins on the nuclear-cytoplasmic transport and secretion of EBER1.*

Findings: *Successful transfection was confirmed by cells' growth in Hygromycin-B-supplemented media and positive expression of the gene using RT-PCR. Furthermore, EBER1-specific in situ hybridisation was used to confirm EBER1 expression in cells transfected with wildtype EBER1 and mutants. EBV infected cell line, B95.8, was used as a positive control. The pHebo transfectants and untransfected cells were consistently negative for the expression of EBER1. Compared to cells transfected with wildtype EBER1, there were a significantly decreased expression of EBER1 from SL1 and SL4 mutants in the total cellular, nuclear, cytoplasmic and exosomal fractions. SL3 mutant showed significant increased nuclear expression. This mutant, in turn, had the least percentage of nuclear-cytoplasmic transport. There was no difference in the percentage secretion in all three mutants compared to the wildtype. There was no significant dysregulation in the expression of RPL22 and La. Silencing of RPL22 correlated to decreased expression of EBER1 in all transfectants when compared to its expression in the control cells. Furthermore, silencing the expression of RPL22 indicated less nuclear expression of both RPL22 and EBER1 genes in the wildtype and SL1 mutant with a corresponding higher cytoplasmic expression. In SL3 and SL4 mutants, however, both RPL22 and EBER1 were expressed higher in the nucleus. Nonetheless, in SL3 mutant, there was less nuclear retention of EBER1 when compared to the retention observed in the normal expression of RPL22 protein. Silencing of La protein, on the other*

hand, does not correspond to EBER1 expression at both cellular and exosomal levels.

***Conclusion & Significance**: Nuclear-cytoplasmic trafficking of EBER1 seems to be linked to its conserved structure and/or its RPL22-binding. This study, therefore, concludes that SL3 of EBER1 appeared to be crucial in its intracellular transport and RPL22 was implicated in the process. EBER1-La interaction might not be the sole/direct means through which EBER1 is secreted into exosomes. Since trafficking of EBERs is associated with improved cell-cell communication and the spread of EBV [1], unveiling the mechanisms involved in this transport is critical to understanding the biology of EBV and its associated diseases.*

Biography

Zubaida Hassan is a 4th year full-time PhD student in Prof. Gulfaraz Khan's lab at the College of Medicine and Health Sciences, United Arab Emirates University. The main theme of research in Prof. Khan's lab is EBV and its associated diseases, an area that he has been working on for over two decades. Zubaida's PhD project is on understanding the structural impact and molecular mechanisms involved in EBV-encoded RNA (EBER1)-induced proliferation, and trafficking. Zubaida has several publications in peer-reviewed journals, and she has presented her work at many conferences. Zubaida uses techniques in Molecular biology, Virology, Viral Pathology, Cell Biology, Bioinformatics, data analysis etc. Zubaida obtained her Master's degree from Universiti Putra Malaysia in 2015, and her Bachelor's degree from Federal University of Technology, Yola, Nigeria in 2011.

About Research Topic

Epstein-Barr virus (EBV)-encoded RNAs (EBERs) are small, noncoding, and structurally conserved RNA. They are expressed at >10^{6} copies per EBV-infected cell. Although primarily localised in the nucleus, EBERs have also been reported to be present in other cellular compartments. This study aimed to investigate the mechanisms of transport of EBER1. Understanding the mechanisms involved in the transport of EBER1 is crucial in understanding the biology of EBV and its associated diseases. The structural impact of EBER1 in the trafficking was explored by site-directed stem-loops (SL) deletions. While its protein interaction was investigated by silencing the cellular expression of the proteins.

***About Institution**:*

United Arab Emirates University (UAEU) is a public research university located in Al Ain, an oasis city in the emirate of Abu Dhabi, United Arab Emirates. It is the oldest university in the UAE established by the founding father of the country, the late Sheikh Zayed bin Sultan Al Nahyan. In the 2021, UAEU was ranked as the second top university in the UAE, 4th in the GCC, the 5th in the Arab region and 284th in the QS World University Rankings. UAEU has both undergraduate and postgraduate degree programs in nine colleges.

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