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UNDERSTANDING THE ROLE OF EPSTEIN-BARR VIRUS ENCODED RNAs (EBERS) IN EBV BIOLOGY

by

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Abstract

Epstein Barr virus (EBV) is an oncogenic herpesvirus aetiologically linked to several human malignancies of epithelial and lymphoid origins. An accumulating body of data indicates that EBV infected cells release specific viral components in nano-vesicles called exosomes. Epstein-Barr virus encoded RNAs (EBER1 and EBER2) are two highly abundant, non-protein coding RNAs that are consistently expressed in all EBV latency programs. Conventional *in situ* hybridization (ISH) studies have indicated that these RNAs are typically found in the nucleus of EBV infected cells. Recently, it was reported that EBERs are released out of the infected cells. However, the mechanism by which these highly abundant EBV products are transported from the nucleus to the cytoplasm and from there to outside of the cell remains unknown. In this thesis, we studied if EBER1 and EBER2 are excreted out from the infected cells via the exosomal pathway. Using differential ultracentrifugation, we isolated exosomes from EBV infected, non-infected and EBER1 transfected cell lines. The identity of purified exosomes was confirmed by electron microscopy and western blotting for exosomal markers. The presence of EBERs in cells, culture supernatant and purified exosomal fractions was determined using RT PCR. Both EBER1 and EBER2 were observed to be present not only in the culture supernatants but also in purified exosomes from all EBV infected cell lines. The EBER binding protein La was also observed to be present in the purified exosomal fractions. To directly track the journey of these small RNAs, we developed a novel electron microscopy based technique. Our results showed for the first time that at least a proportion of EBERs are transported from the nucleus to the cytoplasm, where they appear to be loaded into multi-vesicular bodies for eventual excretion via exosomes. Furthermore, the EBER binding protein La, was also localized in the same exosomal fractions suggesting that EBERs might be released from the EBV infected cell lines in the form of EBER-La complex. Moreover, when the purified exosomes from both type I and type III EBV latently infected cell lines were exposed to non-infected cells, they resulted in inducing apoptosis in the recipient cells in a time and dose dependent manner. Furthermore, the fluorescently labeled exosomes isolated from EBV infected cell lines were taken up by non-infected cell lines, where they induced apoptosis via the extrinsic pathway of cell death. The blocking of caspase 3/7/8 pathways resulted in inhibition of exosome mediated apoptosis. Molecular analysis of the exosomes from EBV infected cell lines showed the presence of Fas ligand (FasL) in the exosomal fractions. Our data indicated that exosomes from EBV infected cell lines induced apoptosis via the FasL mediated extrinsic pathway and incubation of exosomes with anti-FasL antibody resulted in reduction of apoptosis in the recipient cells. Together, our data support the view that EBV can hijack the cellular exosomal pathway to excrete specific viral and cellular components to modulate the microenvironment.

Keywords: Epstein-Barr virus encoded RNAs (EBER1 and EBER2), exosomes, microenvironment, electron microscopy, La protein, apoptosis, Fas ligand