学 位 論 文 の 要 旨

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学 位 論 文 名 Persistent Epstein-Barr Virus Infection of Epithelial Cells Leads to APOBEC3C Expression and Induces Mitochondrial DNA Mutations

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論 文 内 容 の 要 旨

INTRODUCTION

Epstein-Barr virus (EBV) infects lymphocytes and epithelial cells and is associated with the formation of tumors such as gastric cancer and nasopharyngeal cancer. These EBV-associated tumors arise from cells latently infected with EBV, and persistent expression of viral genes promotes tumor formation. In tumor cells of EBV-associated gastric cancer (EBVaGC), which accounts for approximately 10% of all gastric cancers. EBV shows type I latent infection, and only a few EBV genes are expressed, including EBV nuclear antigen 1, latent membrane protein 2A (LMP2A), EBV-encoded small RNAs, and *Bam*HI-A-fragment rightward transcript.

Apolipoprotein B mRNA Editing Enzyme, Catalytic Polypeptide-Like 3 (APOBEC3) proteins are cytidine deaminases that convert cytosine to uracil (C-to-U mutation) in host and viral genes. The APOBEC3 gene group was first reported to be upregulated as a defense factor for host cells against infection by viruses, particularly retroviruses, and to introduce genetic mutations into viral genomes. In addition, it has been suggested that APOBEC3 may introduce mutations not only into the viral genome but also into the host genome and mitochondrial DNA (mtDNA). However, it was unclear that which APOBEC3 induces mtDNA mutilation in EBVaGC. Here, I report that EBV infection induces the expression of the APOBEC3 family in gastric epithelial cell lines, and that APOBEC3C in particular causes severe damage to host mitochondria.

MATERIALS AND METHODS

Cells were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, 100 units/mL of penicillin, and 100 μg/mL of streptomycin at 37°C in a 5% CO_2 incubator. Human gastric cancer cell lines AGS and MKN28 were used. AGS and MKN28 cells were persistently infected with EBV carrying the enhanced green fluorescent protein gene integrated into the encodes the viral thymidine kinase. AGS cells persistently infected with LMP2A-deficient EBV were also used. MRNA expression levels were measured by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). And C-to-T mutation was detected by differential denaturation DNA PCR. Protein expression levels were detected by immunostaining and Western blotting. MtDNA copy number was measured by qPCR. APOBEC3C KO cell line was established by CRISPR system.

RESULTS AND DISCUSSION

I observed that in gastric epithelial cells persistently infected with EBV, the expression of APOBEC3 family genes increased, C-to-T mutations in the D-loop region of mtDNA increased. In addition, mtDNA copy number decreased in EBV infected cells. By introducing and expressing individual APOBEC3 family genes, APOBBEC3A, APOBEC3B, APOBEC3C, and APOBEC3D were increased C-to-T mutations in mtDNA. APOBEC3C was particularly expressed in the cytoplasm and decreased mtDNA copy number. Furthermore, I confirmed that APOBEC3C colocalized with mitochondria in EBV-infected cells. In contrast, persistent EBV infection increased mitochondrial copy number in APBEC3C-deficient cells.

Expression of the EBV latent gene LMP2A increased APOBEC3C expression. Conversely, APOBEC3C expression was reduced in LMP2A-deficient EBV-infected cells compared to wild-type EBV-infected cells.

The mtDNA D-loop is a non-coding region rich in A and T nucleotide sequences, containing many target motifs for APOBEC3. These mutations impair mitochondrial replication and function, promoting glycolysis and the Warburg effect, which may support viral persistence and tumorigenesis. Unlike APOBEC3A and APOBEC3B, APOBEC3C localizes to mitochondria despite lacking migration signals, causing significant mitochondrial damage. EBV genes such as LMP2A further enhance APOBEC3C expression, contributing to mtDNA mutations and altered metabolism.

These findings suggest that EBV disrupts mitochondrial integrity to create a tumor-favorable environment, but further studies are needed to clarify the functional consequences.

CONCLUSION

Persistent EBV infection in gastric epithelial cells induces APOBEC3C expression, leading to C-to-T mutations in the mitochondrial D-loop and a reduction in mtDNA copy number.