学 位 論 文 の 要 旨

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学 位 論 文 名　A Single Nucleotide Polymorphism in the BART Promoter Region of Epstein-Barr Virus Isolated From Nasopharyngeal Cancer Cells

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論 文 内 容 の 要 旨

INTRODUCTION

Epstein-Barr virus (EBV) is a ubiquitous gamma herpes virus that is associated with nonmalignant diseases, such as infectious mononucleosis, and malignant diseases, such as nasopharyngeal carcinoma (NPC) and EBV-associated gastric cancer (EBVaGC). The occurrence of NPC shows ethnic and geographical distributional variations with high prevalence in southern China and Southeast Asia. Previous observations suggest a possibility that genomic variations in EBV might contribute to the pathogenesis of various human cancers at different geographical locations.

EBV encodes microRNAs (miRNAs), called BART miRNAs, are abundantly expressed in epithelial malignancies in comparison to their expression in B lymphomas and contribute to pathologies, such as tumor formation, in the host. However, the reason behind the high expression of BART miRNAs in NPC and EBVaGC has not been investigated extensively. We analyzed variation in the BART promoter region of six completely sequenced EBV strains: Akata, YCCEL1, SNU719, C666-1, Mutu I, and M81. A single-nucleotide polymorphism (SNP) located in downstream region of P1 was identified as being able to affect BART miRNA expression.

MATERIALS AND METHODS

We used human gastric carcinoma cell lines AGS and MKN28, and human NPC HONE1 cells that were infected with the Akata-EBV recombinant expressing enhanced green fluorescent

protein and a neomycin resistance gene. The BART promoter region was amplified from the genomic DNA of each cell line and amplified fragments were cloned into the pGL4.18 vector. Deletion and insertion mutations in the BART promoter regions were introduced via polymerase chain reaction amplification using the original plasmid DNA as a template. Cells were transfected with the pGL4.18 plasmid containing a luciferase gene driven by the BART promoter and the pGL4.74 Renilla luciferase gene. Luciferase activity was measured and was normalized using *Renilla* activity 48 h after transfection.

A total of 170 EBV-isolated sequences from NPC tissue samples and cell lines, and GC tissue samples, were retrieved from the NCBI database. The differences in the distributions of phenotypes and SNP G138557- presence among the different types of cancer, defined by gender and age, were evaluated using the Chi-squared or Fisher's exact test. In addition, odds ratios (ORs) for NPC associated with SNP G138557- were calculated. The distributions of the other variants of the BART promoter gene between EBV-infected NPC and EBVaGC were also evaluated using the Chi-squared or Fisher's exact test. All statistical analyses were operated using an R 3.5.1 with the 2-tailed significance level set at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The EBV strains, Akata, YCCEL1, Mutu I, and M81, shared exactly the same sequences, while two SNPs were found in the 591 nucleotide-long BART promoter region of these strains. SNU719 EBV had a point mutation at nucleotide 180 and C666-1 EBV had a deletion at nucleotide 513. The EBV strains, Akata, YCCEL1, Mutu I, and M81, shared exactly the same sequences, while two SNPs were found in the 591 nucleotide-long BART promoter region of these strains. SNU719 EBV had a point mutation at nucleotide 180 and C666-1 EBV had a deletion at nucleotide 513. To investigate whether each SNP affected the BART promoter activity, BART promoter regions of the different EBV strains, Akata, C666-1, and SNU719, were introduced into the pGL4.18 vector. The BART promoter activity was higher in EBV-positive cells compared to EBV-negative cells. Additionally, the promoter activity of cells transfected with the BART promoter C666-1 EBV was significantly higher than that of cells transfected with the Akata or SNU719 EBV BART promoters, which had similar promoter activities. These results suggest that the SNP at nucleotide 513 regulates the BART promoter activity.

Two recombinant pGL4.18 vectors containing BART promoter mutants, with mutations at nucleotide 513 of the BART promoter region, were constructed. One mutation was a G deletion from the Akata EBV BART promoter region (Akata_P G del.), whereas, the other was a C insertion into the C666-1 EBV (C666_P G>C) BART promoter region. The four pGL4.18 vectors with BART promoter sequences were transfected into the EBV-negative and positive AGS and HONE1 cells. Akata_P G del. showed a significant increase in promoter activity

compared to the original construct. However, G to C substitution at 513 in the BART promoter construct of the C666-1 sequence did not alter the promoter activity. There was no significant difference in promoter activity among Akata_P G del., C666_P, and C666_P G>C.

The frequencies of SNP G138557- were counted and compared among NPC and non-NPC isolates. High frequencies of SNP G138557- were detected in the BART promoter region of EBV from both NPC endemic and non-endemic areas (83.10%), whereas the SNP was scarcely found in EBV sequences from EBVaGC. In addition, G138557C substitution was detected in two cases from NPC tissues (2/125; 1.6%), but was not detected in EBVaGC. These results indicated that the frequency of G138557- was associated with NPC incidence. Moreover, such an accumulation of SNPs that was observable in the BART promoter region of EBV was hardly found in any other site of the EBV genomic sequence. A strong association was observed between SNP G138557- and a high risk of NPC ($P < 0.001$, OR = 5.67., 95% CI 2.39-13.44), suggesting that BART promoter sequences of EBV might be specifically associated with the occurrence of NPC.

CONCLUSION

In this study, we identified a novel functional SNP that increases BART promoter activity downstream of the P1 region. A deletion form of the SNP in the BART promoter region was strongly associated with the incidence of NPC in patients, and increased BART promoter activity in EBV derived from NPC cell lines. Our findings likely explain the reason for the increased amount of BART miRNA observed in NPCs. Since BART miRNAs are known to suppress the expression of apoptosis-promoting factors, innate immunity genes, and acquired immunity-related genes, the abundance of BART miRNAs will promote progression of NPC.