学 位 論 文 の 要 旨

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学 位 論 文 名	Exploring the Anti-EBV Potential of Suberoylanilide Hydroxamic Acid: Induction of Apoptosis in Infected Cells Through Suppressing BART Gene Expression and Inducing Lytic Infection
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論 文 内 容 の 要 旨

INTRODUCTION

Epstein-Barr virus (EBV) is associated with various cancers, including Burkitt lymphoma, natural killer/T-cell lymphoma, nasopharyngeal carcinoma, and EBV-associated gastric cancer (EBVaGC). In these EBV positive tumors, EBV persistently infect to cells and express limited number of viral genes as latent infection. However, drugs targeting EBV-positive tumors have yet to be developed. Among the approximately 10 latent EBV genes, *Bam*HI A Rightward Transcript (BART) miRNAs are consistently expressed regardless of the type of latency. BART microRNAs (miRNAs) constitute a cluster of 40 miRNAs located in the BART region of the genome. These BART miRNAs are known to play roles in cell proliferation, differentiation, apoptosis induction, immune evasion, and so on. In addition, BART miRNAs suppress EBV lytic infection, which induces apoptosis. In this study, we searched for drugs that suppress BART gene promoter activity and investigated their effects.

MATERIALS AND METHODS

A vector containing the secretory NanoLuc gene inserted downstream of the BART gene promoter was introduced into cells, and a stable expression line was established. The established cells were treated with a drug library, and NanoLuc activity was measured. EBV-positive cells were treated with drugs that reduced enzyme activity, and the expression level of BART miRNA was measured by RT-qPCR. Additionally, the cell proliferation ability of EBV-positive cells was examined using the CCK-8 assay. Cell apoptosis was analyzed by FACS. Furthermore, the expression of apoptosis-inducing genes targeted by BART miRNA was examined by RT-qPCR and Western blot. These experiments were also performed in cells infected with an EBV strain lacking the lytic infection gene BZLF1 (BZLF1-KO).

RESULTS AND DISCUSSION

We identified suberoylanilide hydroxamic acid (SAHA), an inhibitor of histone deacetylase enzymes (HDAC), as an agent that suppresses BART promoter activity and transcription of BART miRNAs. SAHA treatment demonstrated a more pronounced inhibition of cell proliferation in EBV-positive gastric cancer cells compared to EBV-negative cells, affecting both p53 wild-type and mutant gastric epithelial cells. SAHA treatment enhanced lytic infection in wild-type EBV-infected cells, while also enhancing cell death in EBV BZLF1-KO-infected cells. It reduced BART gene expression by 85% and increased the expression of proapoptotic factors targeted by BART miRNAs. Treatment of EBV-positive cells with SAHA activated caspase 3. A similar phenomenon was observed in cells infected with EBV BZLF1-KO. These findings suggest that SAHA not only induces lytic infection but also leads to cell death by suppressing BART miRNA transcription and promoting the apoptotic program.

We demonstrated a remarkable similarity in both the half maximal inhibitory concentration

(IC_{50}) value and the capacity to induce apoptosis between wild-type EBV- and EBV BZLF1-KO-infected cells when exposed to SAHA. However, SAHA enhanced apoptosis independently of EBV lytic infection. Furthermore, the rate of apoptosis in SAHA-treated both EBV-infected cells and EBV BZLF1-KO-infected cells was similar, so we believe that the anti-EBV effect of SAHA is likely mediated by the decrease in BART miRNA expression. Consequently, SAHA not only triggers cell death in EBV-positive cells through lytic infection, but also amplifies apoptosis through suppressing the expression of BART miRNAs. Variants of EBV lacking various viral lytic genes have been associated with numerous instances of lymphomas and epithelial cancers.

Consequently, SAHA is anticipated to exhibit potent antitumor effects against EBV-associated cancers in which it is difficult to induce apoptosis solely through inducing lytic infection.

CONCLUSION

This study demonstrated that the HDAC inhibitor SAHA induces cell death in EBV-positive cells both with and without leading to lytic infection. SAHA was also shown to have efficacy in p53 mutant EBV-positive gastric cancer cells. Given that certain EBV-related tumors lack p53 mutations and are infected with an EBV strain that never exhibits lytic infection, this drug could be effective for the treatment of various EBV-associated cancers.