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A single nucleotide polymorphism in the BART promoter region of Epstein-Barr virus isolated from nasopharyngeal cancer cells

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ABSTRACT

Epstein-Barr virus (EBV) encodes BamHIA rightward transcript (BART) microRNAs (miRNAs). These miRNAs are expressed at high levels in epithelial tumors, such as nasopharyngeal carcinoma (NPC). BART miRNAs play important roles in EBV-associated malignancies, however, the reason for their high expression in NPC is unclear. We performed multiple sequence alignment of six completely sequenced EBV strains: Akata, YCCEL1, SNU719, C666-1, Mutu I, and M81. A single-nucleotide deletion was identified at the promoter region of BART. The luciferase assay suggested that this single-nucleotide polymorphism (SNP) significantly increased BART promoter activity. In addition to deletion, substitution at the same site also increased BART promoter activity. Analysis of the 170 EBV genome sequences from NPC and EBV-associated gastric cancers revealed that the frequency of this SNP was associated with NPC incidence and this SNP was found to be accumulated in the BART promoter region. Overall, our results suggested that this SNP should enhance BART promoter activity and thus, might contribute to the development of EBV-associated epithelial malignancies.

1. 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. In contrast, EBV is associated with about 10% of gastric carcinoma cases and the prevalence of EBVaGC is similar in America, Europe, and Asia. It is well known that epithelial tumor cells are clonal proliferations of cells latently infected with EBV. And EBV episomes within the tumor are derived from the same ancestral virus that originally established latent infection [2,3]. These observations suggest a possibility that genomic variations in EBV might contribute to the pathogenesis of various human cancers at different geographical locations [4].

EBV encodes about 40 microRNAs (miRNA) as part of the *Bam*HI A rightward transcripts (BARTs). They are known as BART miRNAs and expressed in all types of latent and lytic infections [1]. The miRNA binds to the 3′-UTR of its target mRNA by forming a RNA-induced silencing complex (RISC) and reduces gene expression, either by repressing translation or degrading the target mRNA [5]. 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 [6]. Using a mouse xenograft model, BART miRNAs have been shown to potentiate the seeding and growth of EBV associated tumors *in vivo* [7]. Moreover, BART microRNAs released into exosomes from C666-1 NPC cells were transferred to human endothelial cells [8].

BART expression is regulated by two TATA-less promoters, P1 and P2. P1 is active in both epithelial cells and B cells, but is downregulated by IRF5 and IRF7. P2 is more active in epithelial cells than in B cells and is upregulated by members of the c-Myc and C/EBP gene families [9]. 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

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identified as being able to affect BART miRNA expression.

2. Material and methods

2.1. BART promoter sequence alignment

The BART promoter sequences of Akata, YCCEL1 [10], SNU719 [11], C666-1 [12], Mutu I [13], and M81 [14] were extracted from the National Center for Biotechnology Information (NCBI). The sequences were aligned and analyzed using the MView program (EMBL, https://www.ebi.ac.uk/Tools/msa/mview/).

2.2. Cell culture

Human gastric carcinoma cell lines AGS and MKN28, and human NPC HONE1 cells were infected with Akata-EBV recombinant, expressing enhanced green fluorescent protein and a neomycin resistance gene [15]. Human gastric carcinoma cell line SNU719 cells naturally infected by EBV were also used for experimentation. Cells were cultured in Roswell Park Memorial Institute-1640 medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin in a 5% CO_2 incubator. Cells infected with EBV recombinants were selected by cultivating them in media containing 500 μg/mL (AGS, MKN28 and HONE1 cells) of G418 (Promega, Madison, WI).

2.3. Plasmid constructs

The BART promoter region was amplified from the genomic DNA of each cell line using KOD Plus Neo DNA polymerase (TOYOBO, Osaka, Japan) and the following primers: 5′-CTAGGCTAGCCCATT CACAGGGGTATCCAG-3′ and 5′-AGCTAAGCTTGAGGAACAACTTT GGCCTGA-3′. The amplified fragments were cloned into the pGL4.18 vector (Promega).

2.4. Luciferase reporter assay

Cells were transfected with the pGL4.18 plasmid (Promega) containing a luciferase gene driven by the BART promoter and the pGL4.74 *Renilla* luciferase gene. Lipofectamine™ 2000 reagent (Invitrogen, Carlsbad, CA) was used for the transfections. Luciferase activity was measured in a Lumat^3 LB 9508 Single Tube Luminometer (Berthold Technologies, Bad Wildbad, Germany) using the Dual-Glo® Luciferase Assay (Promega) 48 h post-transfection. For each reaction, the *firefly* luciferase activity was normalized to *Renilla* luciferase activity.

2.5. Site-directed mutagenesis

Deletion and insertion mutations were introduced via polymerase chain reaction (PCR) amplification using the original plasmid DNA as a template. The sequences of the primers used for each mutation were as follows: Akata _P G del., 5′-GAAGGAGCT-GACACGAGTGCG-3′ and 5′-CCCCACCCGCCGTGTCTG-3′; C666_P G > C., 5′-AAGGAGCTGACACGAGTGCGTAGAAAGGG-3′ and 5′-GCCCCCACCCGCCGTGTCTG-3′. The PCR products were treated with *Dpn*I restriction endonuclease (TaKaRa Bio, Shiga, Japan) and then circularized to construct the mutated plasmid [16]. The introduction of each mutation was verified by sequencing.

2.6. Determination of SNPs in the BART promoter region of EBV-isolated sequences

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 $\alpha = 0.05$.

2.7. Accession number(s)

Sequence data for the EBV genomes (1624806787 and 1624813027) were downloaded from the GenBank PopSet database under the following accession numbers: AB850643.1 to AB850660.1 (EBV-NPC), KJ411974.1 (C666-1-2), LN827525.1 (C666-1-3), KC617875.1 (C666-1-4), KF373730.1 (M81), EU828625.1 (C15), EU828626.1 (C17), EU828627.1 (C18) MG021314 to MG021306 (EBV-GC), AP015015.1 (SNU719), and AP015016.1 (YCCEL1).

2.8. Statistical analyses

The Student's t-test was used for all data analyses. Curve fitting and analyses were performed using GraphPad Prism software (GraphPad Software, San Diego, CA). A probability (P) value of <0.05 was considered statistically significant. All results are expressed as the mean ± standard deviation.

3. Results

3.1. Sequence variation at the BART promoter region

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 (Fig. 1A). 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. Together with pGL4.74 *Renilla* luciferase expression vector, each pGL4.18 BART promoter vector was transfected into EBV-negative and positive AGS, MKN28, SNU719, and HONE1 cells. 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 (Fig. 1B). These results suggest that the SNP at nucleotide 513 regulates the BART promoter activity.

3.2. Functional SNP in BART promoter region

Site-directed mutagenesis was performed at nucleotide 513 with the BART promoter construct of Akata and C666-1 EBV sequences to verify functional SNPs in the BART promoter. 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

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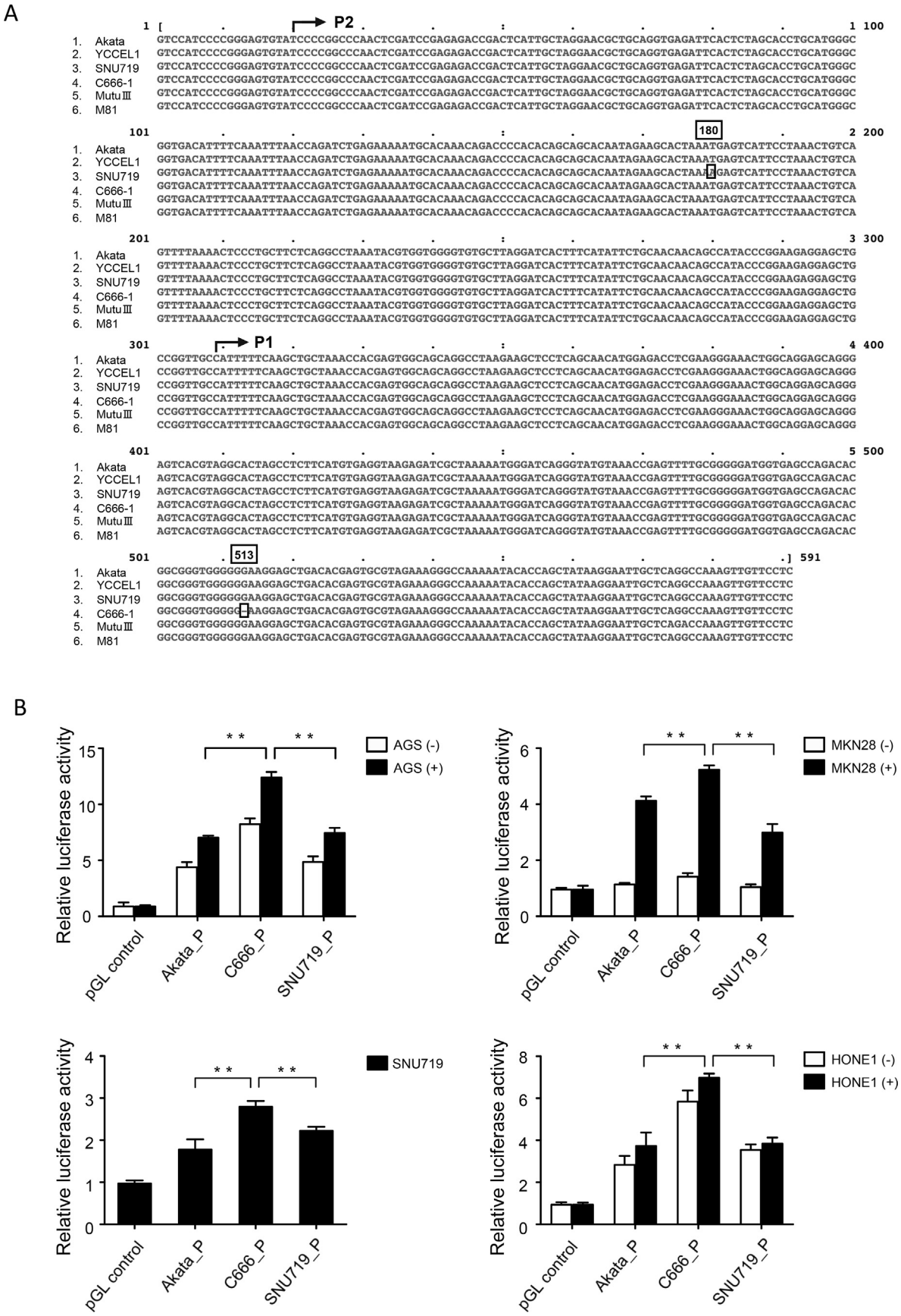

Fig. 1. Alignment of BART promoter sequences from six EBV strains. (A) Multiple-sequence alignment of sequences from six EBV strains. Sequence variations are indicated alongside nucleotide numbers. BART promoters P1 and P2 are schematically illustrated in the sequence alignment, respectively. The boxed columns indicate different nucleotide sequences among the six virus strains, and each nucleotide number is enclosed in a square and shown above the line. (B) EBV-negative and -positive cells were transfected with each BART promoter vector, together with the pGL4.74 *Renilla* luciferase expression vector. Error bars indicate SD. $^{**}P < 0.01$. pGL control was set at 1 and indicates pGL4.18. White and black columns indicate EBV negative and positive cells, respectively. Akata_P, C666_P, and SNU719_P indicate pGL4.18-EBV Akata strain, pGL4.18-EBV C666-1 strain, and pGL4.18-EBV SNU719 strain BART promoters, respectively.

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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 (Fig. 2).

3.3. Relationship between SNP in BART promoter region and the risk of NPC and other epithelial malignancies

To identify if the variations in the EBV BART promoter region was related to the NPC disease phenotype, we analyzed 170 publicly accessible EBV sequences from NPC and GCs (Table 1). 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 (46.43%; Table 2). 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. Logistic regression analysis for G138557-was conducted in these isolates for further confirmation. 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; Table 2), suggesting that BART promoter sequences of EBV might be specifically associated with the occurrence of NPC.

4. Discussion

Some EBV-associated diseases have shown very different incidence patterns depending on factors, such as geographic region, population, host genetics and ethnicity [17]. The variation in the EBV genome sequence might contribute to the distribution of EBV-associated diseases. A number of studies have reported significant variations in the EBV genome sequence in relation to cancers. The SNP G155391A in the RPMS1 open reading frame is specifically and significantly associated with a high risk of NPC in southern China [18]. EBV-encoded RNA (EBER) polymorphisms were identified in people with NPC from north China and these NPC isolates showed different variation patterns from those of EBVaGCs and healthy donors [19]. The V3 polymorphism in the Zp promoter enhances EBV lytic reactivation [20]. This polymorphism is frequently found in African BL [20] and NPC cases from Hong Kong and Indonesia [21]. 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 [22], the abundance of BART miRNAs will promote progression of NPC. Though EBV is detected at early stages of NPC in 100% of known cases, expression of the viral oncogene, LMP1, is limited to only 60% [23]. On the other hand, BART miRNAs are expressed at high levels in all NPCs. Thus, the upregulation of BART miRNAs should have important roles in the development of NPC.

The C666-1 BART promoter showed 1.7-fold-higher activity than the Akata and SNU719 promoters in all EBV-positive and -negative cells (Fig. 1B). In many cases, there were several miRNA binding sites in the 3′UTR region of the target gene [24]. In addition, secreted miRNAs will be transmitted to adjacent cells via extracellular vesicles to become functional [8,25]. Finally, hundreds of EBV copies exist per cell in early passage of NPC tumor cell [26]. Hence, we assume that the 1.7-fold upregulation in the promoter activity of C666-1 must correspond to significantly high BART miRNA levels.

The SNP site in the BART promoter region is expected to be a

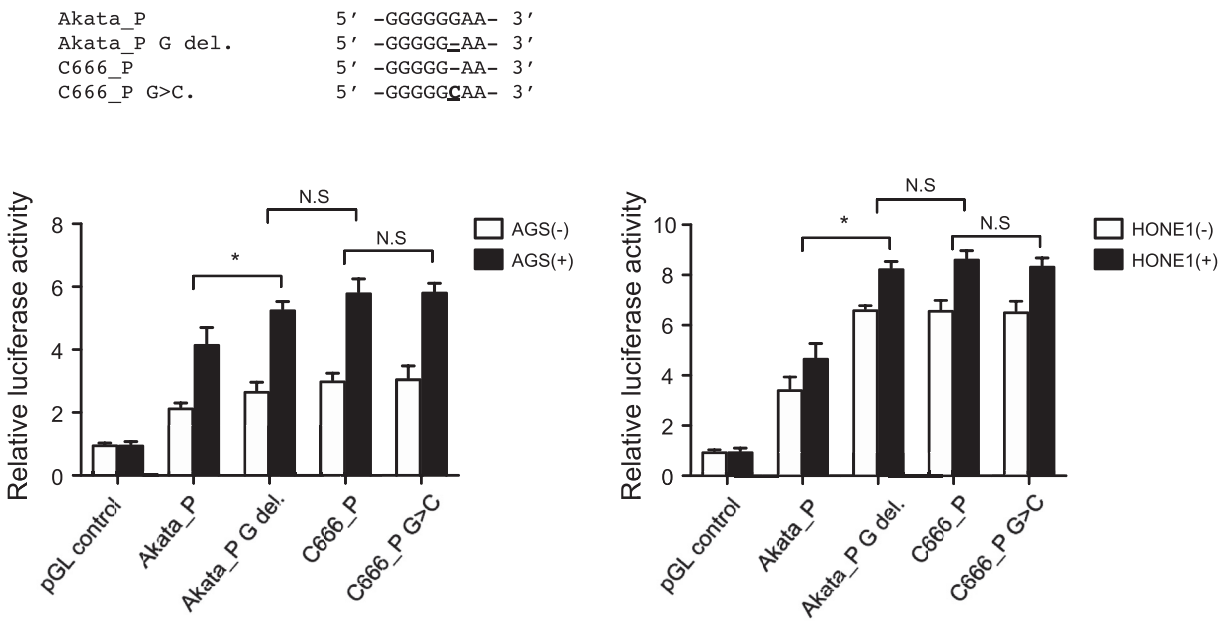

Fig. 2. Identification of functional SNP in BART promoter region. The upper panel represents mutation sites in each BART promoter region. EBV-negative and -positive AGS cells and EBV-negative and -positive HONE1 cells are designated as AGS (−), AGS (+), HONE1(−), and HONE1(+), respectively. These cells were transfected with the original or mutant BART promoter together with the pGL4.74 *Renilla* luciferase vector. Error bars indicate SD. *$P < 0.05$. pGL control was set at 1 and indicates pGL4.18. White and black columns indicate EBV-negative and -positive cells, respectively. Akata_P and C666_P indicate pGL4.18-EBV Akata strain and pGL4.18-EBV C666-1 strain BART promoters, respectively. Akata_P G del indicates pGL4.18-EBV Akata strain BART promoter SNP G138557-. C666_P G > C indicates pGL4.18-EBV C666-1 strain BART promoter SNP G138557C.

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Table 1
Summary of geographic origin and phenotypes of publicly accessed EBV isolated sequences in this study.

	Reference	Africa	Western	Endemic area (China)	Non-endemic area (East Asia)	Total
NPC						
Tumor tissue(s)	[27]			99	17	**116**
Tumor tissue(s)	K. Xiao et al., unpublished, 2015			18		**18**
Cell line(s)						
C666-1-1	[27]			1		**1**
C666-1-2	[28]			1		**1**
C666-1-3	[29]			1		**1**
C666-1-4	[30]			1		**1**
M81	[14]			1		**1**
C15	[31]			1		**1**
C17	[31]			1		**1**
C18	[31]			1		**1**
EBVaGC						
Tumor tissue(s)	[27]				13	**13**
Tumor tissue(s)	[32]				13	**13**
Cell line(s)						
SNU-719	[33]				1	**1**
YCCEL1	[33]				1	**1**
Total				**104**	**45**	**170**

Table 2
Association of BART SNP G138557-with the risk of NPC and other malignancies.

Position	Reference/alternative genotype	Alternative genotype frequency in NPC	Alternative genotype frequency in EBVaGC	OR (95%CI)	*P*	Z score	Annotation
138557	G/-	83.10%	46.43%	5.67 (2.39 −13.44)	<0.001	3.94	high activity of BART promoter

repressor binding site induced by EBV infection, because the BART promoter assay always shows higher activity in EBV-positive cells than in EBV-negative cells (Figs. 1B and 2). Computational analysis predicted that ETS family genes might interact with this site. It would be interesting to investigate the role of ETS transcription factors in EBV-related tumorigenesis. Future research should focus on understanding the contribution of EBV genome variations in EBV-associated epithelial malignancies.

Author contributions

HK, HI, and HY conceived and designed the experiments; HK, YK, and AB performed the experiments; HK and AB analyzed the data; HK, AB, and HY wrote the manuscript; HI reviewed the manuscript. All authors proofread and approved the final manuscript.

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Declaration of competing interest

The authors declare no competing interests.

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References

[1] L.S. Young, A.B. Rickinson, Epstein-Barr virus, 40 years on, Nat. Rev. Cancer 4 (2004) 757–768, https://doi.org/10.1038/nrc1452.
[2] S. Imai, S. Koizumi, M. Sugiura, et al., Gastric carcinoma: monoclonal epithelial malignant cells expressing Epstein-Barr virus latent infection protein, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 9131–9135, https://doi.org/10.1073/pnas.91.19.9131.
[3] N. Raab-Traub, K. Flynn, The structure of the termini of the Epstein-Barr virus as a marker of clonal cellular proliferation, Cell 47 (1986) 883–889, https://doi.org/10.1016/0092-8674(86)90803-2.
[4] M. Chiara, C. Manzari, C. Lionetti, et al., Geographic population structure in Epstein-Barr virus revealed by comparative genomics, Genome Biol. Evol. 8 (2016) 3284–3291, https://doi.org/10.1093/gbe/evw226.
[5] J. Winter, S. Jung, S. Keller, et al., Many roads to maturity: microRNA biogenesis pathways and their regulation, Nat. Cell Biol. 11 (2009), https://doi.org/10.1038/ncb0309-228, 228-2234.
[6] X. Cai, A. Schäfer, S. Lu, et al., Epstein-Barr virus microRNAs are evolutionarily conserved and differentially expressed, PLoS Pathog. 2 (2006) e23, https://doi.org/10.1371/journal.ppat.0020023.
[7] J. Qiu, P. Smith, L. Leahy, et al., The Epstein-Barr virus encoded BART miRNAs potentiate tumor growth *in vivo*, PLoS Pathog. 11 (2015), e1004561, https://doi.org/10.1371/journal.ppat.1004561.
[8] D.G. Meckes Jr., K.H. Shair, A.R. Marquitz, et al., Human tumor virus utilizes exosomes for intercellular communication, Proc. Natl. Acad. Sci. U. S. A 107 (2010) 20370–20375, https://doi.org/10.1373/pnas.1014194107.
[9] H. Chen, J. Huang, F.Y. Wu, et al., Regulation of expression of the Epstein-Barr virus BamHI-A rightward transcripts, J. Virol. 79 (2005) 1724–1733, https://doi.org/10.1128/JVI.79.3.1724-1733.2005.
[10] D.N. Kim, M.K. Seo, H. Choi, et al., Characterization of naturally Epstein-Barr virus-infected gastric carcinoma cell line YCCEL1, J. Gen. Virol. 94 (2013) 497–506, https://doi.org/10.1099/vir.0.045237-0.
[11] S.T. Oh, J.S. Seo, U.Y. Moon, et al., A naturally derived gastric cancer cell line shows latency I Epstein-Barr virus infection closely resembling EBV-associated gastric cancer, Virology 320 (2004) 330–336, https://doi.org/10.1016/j.virol.2003.12.005.
[12] S.T. Cheung, D.P. Huang, A.B. Hui, et al., Nasopharyngeal carcinoma cell line (C666-1) consistently harbouring Epstein-Barr virus, Int. J. Cancer 83 (1999) 121–126, https://doi.org/10.1002/(sici)1097-0215(19990924)83:1<121::aid-ijc21>3.0.co;2-f.
[13] A. Nanbo, E. Kawanishi, R. Yoshida, et al., Exosomes derived from Epstein-Barr virus-infected cells are internalized via caveola-dependent endocytosis and promote phenotypic modulation in target cells, J. Virol. 87 (2013) 10334–10347, https://doi.org/10.1128/JVI.01310-13.
[14] M.H. Tsai, A. Raykova, O. Klinke, et al., Spontaneous lytic replication and epitheliotropism define an Epstein-Barr virus strain found in carcinomas, Cell Rep. 5 (2013) 458–470, https://doi.org/10.1016/j.celrep.2013.09.012.
[15] S. Maruo, L. Yang, K. Takada, Roles of Epstein-Barr virus glycoproteins gp350

and gp25 in the infection of human epithelial cells, J. Gen. Virol. 82 (2001) 2373–2383, https://doi.org/10.1099/0022-1317-82-10-2373.
[16] A. Hemsley, N. Arnheim, M.D. Toney, et al., A simple method for site-directed mutagenesis using the polymerase chain reaction, Nucleic Acids Res. 17 (1989) 6545–6551, https://doi.org/10.1093/nar/17.16.6545.
[17] S. Correia, A. Palser, C. Elgueta Karstegl, et al., Natural variation of Epstein-Barr virus genes, proteins, and primary microRNA, J. Virol. 15 (2017), https://doi.org/10.1128/JVI.00375-17 e00375-00317.
[18] F.T. Feng, Q. Cui, W.S. Liu, et al., A single nucleotide polymorphism in the Epstein-Barr virus genome is strongly associated with a high risk of nasopharyngeal carcinoma, Chin. J. Canc. 34 (2015) 563–572, https://doi.org/10.1186/s40880-015-0073-z.
[19] Y. Wang, X. Zhang, Y. Chao, et al., New variations of Epstein-Barr virus-encoded small RNA genes in nasopharyngeal carcinomas, gastric carcinomas, and healthy donors in northern China, J. Med. Virol. 82 (2010) 829–836, https://doi.org/10.1002/jmv.21714.
[20] J.A. Bristol, R. Djavadian, E.R. Albright, et al., A cancer-associated Epstein-Barr virus BZLF1 promoter variant enhances lytic infection, PLoS Pathog. 14 (2018), e1007179, https://doi.org/10.1371/journal.ppat.1007179.
[21] S. Correia, R. Bridges, F. Wegner, et al., Sequence variation of Epstein-Barr virus viral types, geography, codon usage, and diseases, J. Virol. 92 (2018), https://doi.org/10.1128/JVI.01132-18 e01132-01118.
[22] X. Lin, M.H. Tsai, A. Shumilov, et al., The Epstein-Barr virus BART miRNA cluster of the M81 strain modulates multiple functions in primary B cells, PLoS Pathog. 11 (2015), e1005344, https://doi.org/10.1371/journal.ppat.1005344.
[23] Y. Zhao, Y. Wang, S. Zeng, et al., LMP1 expression is positively associated with metastasis of nasopharyngeal carcinoma: evidence from a meta-analysis, J. Clin. Pathol. 65 (2012) 41–45, https://doi.org/10.1136/jclinpath-2011-200198.
[24] G. Martello, A. Rosato, F. Ferrari, et al., A microRNA targeting Dicer for metastasis control, Cell 141 (2010) 1195–1207, https://doi.org/10.1016/j.cell.2010.05.017.
[25] N. Raab-Traub, D.P. Dittmer, Viral effects on the content and function of extracellular vesicles, Nat. Rev. Microbiol. 15 (2017) 559–572, https://doi.org/10.1038/nrmicro.2017.60.
[26] W. Lin, Y.L. Yip, J. Jia, et al., Establishment and characterization of new tumor xenografts and cancer cell lines from EBV-positive nasopharyngeal carcinoma, Nat. Commun. 9 (2018) 4663, https://doi.org/10.1038/s41467-018-06889-5.
[27] M. Xu, Y. Yao, H. Chen, et al., Genome sequencing analysis identifies Epstein-Barr virus subtypes associated with high risk of nasopharyngeal carcinoma, Nat. Genet. 51 (2019) 1131–1136, https://doi.org/10.1038/s41588-019-0436-5.
[28] H. Kwok, C.W. Wu, A.L. Palser, et al., Genomic diversity of Epstein-Barr virus genomes isolated from primary nasopharyngeal carcinoma biopsy samples, J. Virol. 88 (2014) 10662–10672, https://doi.org/10.1128/JVI.01665-14.
[29] A.L. Palser, N.E. Grayson, R.E. White, et al., Genome diversity of Epstein-Barr virus from multiple tumor types and normal infection, J. Virol. 89 (2015) 5222–5237, https://doi.org/10.1128/JVI.03614-14.
[30] K.K. Tso, K.Y. Yip, C.K. Mak, et al., Complete genomic sequence of Epstein-Barr virus in nasopharyngeal carcinoma cell line C666-1, Infect. Agents Cancer 8 (2013) 29, https://doi.org/10.1186/1750-9378-8-29.
[31] R.H. Edwards, A.R. Marquitz, N. Raab-Traub, Epstein-Barr virus BART microRNAs are produced from a large intron prior to splicing, J. Virol. 82 (2008) 9094–9106, https://doi.org/10.1128/JVI.00785-08.
[32] I. Borozan, M. Zapatka, L. Frappier, et al., Analysis of Epstein-Barr virus genomes and expression profiles in gastric adenocarcinoma, J. Virol. 92 (2018), https://doi.org/10.1128/JVI.01239-17 e01239-17.
[33] T. Kanda, Y. Furuse, H. Oshitani, et al., Highly efficient CRISPR/Cas9-mediated cloning and functional characterization of gastric cancer-derived Epstein-Barr virus strains, J. Virol. 90 (2016) 4383–4393, https://doi.org/10.1128/JVI.00060-16.

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