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Sequence Variation of Epstein-Barr Virus (EBV)-encoded *BARF1* Promoter in EBV-Associated Gastric Carcinoma

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Abstract

Epstein-Barr virus (EBV)-encoded BARF1 is suspected to play an important role in development of EBV-associated gastric carcinoma (EBV-GC). The present study examined the sequence variation of *BARF1*-promoter region (−488/+87) of EBV genomes detected in 22 Colombian and 17 Japanese EBV-GCs. In addition, the EBV genomes in throat washing samples from 11 Colombian and 9 Japanese healthy donors (controls) were examined. All the EBV strains isolated from healthy donors had the same *BARF1*-promoter-region sequence as the prototype strain B95-8. In contrast, the EBV-GCs showed the following 8 point mutations in comparison with the B95-8 strain: G→C at −367 in 2 Colombian EBV-GC cases; T→A at −356 in 1 Colombian EBV-GC case; C→G at +15 in 1 Colombian EBV-GC case; C→T at +24 in 5 Colombian EBV-GC cases; T→G at +26 in 3 Colombian EBV-GC cases; T→C at +29 in 7 and 2 Colombian and Japanese EBV-GC cases, respectively; T→A at +44 in 5 Colombian EBV-GC cases; and G→A at +46 in 1 Japanese EBV-GC case. The observed case-control difference at position +29 was statistically significant (p=0.022, Fisher's exact test). Although the frequency of this point mutation in Colombian EBV-GCs was higher than that in Japanese EBV-GCs, the difference was not statistically significant (p=0.251). In summary, the present study, examining the *BARF1*-promoter region of EBV genomes detected in 39 EBV-GCs and throat washing specimens from 20 healthy donors, found a statistically significant increase of the point mutation of T→C at position +29 in EBV-GC. Further studies seem warranted to clarify the etiological significance of this finding.

Key words: Epstein-Barr virus, gastric carcinoma, BARF1 promoter, viral oncogene.

Introduction

Epstein-Barr virus (EBV) is associated with epithelial malignancies, including undifferentiated nasopharyngeal carcinoma (NPC)[1] and a part of gastric carcinoma (EBV-GC)[2, 3], and lymphoid malignancies such as Burkitt's lymphoma (BL)[4], Hodgkin's lymphoma [5], posttransplant lymphoproliferative disease [6], and nasal NK/T cell lymphomas [7].

In EBV-GC, the virus exists in a latent state and at least five EBV genes are expressed in the carcinoma: *EBERs, EBNA1, LMP2, BARF0,* and *BARF1* [8, 9]. Although EBV is able to immortalize human gastric primary epithelial cells [10], *LMP1*, a well-known viral oncogene in EBV-related lymphomas and NPC, is not expressed in EBV-GC [11], and critical viral oncogene in EBV-GC has not been established yet.

The *BARF1* (or p31) gene is able to induce malignant transformation and immortalization in a cell-type specific manner in cell culture systems [12–17]. Furthermore, BARF1 is a functional receptor for the human colony-stimulating factor [18], and it is able to inhibit interferon-alpha secretion from mononuclear cells [19], which indicates that BARF1 has not only oncogenic potentials but also may play a role in immunomodulation [20]. Expression of *BARF1* at mRNA level is frequently detected in a high proportion (up to 90%) of EBV-GC [8, 21], suggesting that BARF1 might be involved in EBV-GC development [8, 22, 23]. Recently, BARF1 was suggested to be an anti-apoptotic factor in gastric cancer cells [24].

Although *BARF1* is considered an early gene of lytic infection in B-lymphocytes [25], the transforming *BARF1* is exclusively transcribed as a latent gene in NPC and EBV-GC [8, 26]. In addition, *BARF1* is expressed in the absence of lytic gene expression in NPC and EBV-GC specimens, suggesting that *BARF1* acts as a latent gene in epithelial malignancies [21]. Thus, BARF1 exerts different functions in lymphoid and epithelial cells: BARF1 might be involved in the lytic cycle, acting as an early protein in lymphoid cells, whereas it has immortalizing / transforming capacities in epithelial cells [8, 27].

The present study determines the sequence variation of the *BARF1* promoter and compares the frequency of the sequence variations in EBV-GC cases and healthy controls, in order to shed light on the etiological significance of BARF1 in EBV-GC development. In addition, the sequence variations of the *BARF1* promoter in EBV-GCs were also compared between two countries where different EBV-GC frequencies have been reported: Colombia (13%) and Japan (6%). Previous studies indicated that the proportion of EBV-GC varies geographically, ranging from 2 to 17% [28], which might be partially explained by the variation of prevailing EBV genotypes.

Materials and methods

Specimens. Paraffin-embedded tumor samples of 22 Colombian EBV-GC cases and 17 Japanese EBV-GC cases were examined. The EBV presence in these EBV-GC cases was examined using the *in situ* hybridization assay described before [29]. Throat washing samples from 85 healthy donors from Colombia and 118 healthy donors from Japan were used as controls. Throat washing samples were collected by gargling with 15 mL of phosphate-buffered saline and stored at −20°C until use. This study was approved by the ethics committee of Kagoshima University Graduate School of Medical and Dental Sciences.

Cell lines. The B95-8 cell line was used as a reference in this study. The cell line was originally obtained by exposing marmoset blood leukocytes to EBV which was derived from peripheral blood leukocytes of a Caucasian patient with infectious mononucleosis (883L cell line)[30]. The B95-8 cell line has been used as a prototype in previous studies of EBV sequence analyses, because it produces infectious EBV and it is biologically and antigenically indistinguishable from other EBV isolates [31]. EBV-positive Akata cell line, derived from a Japanese case of BL [32, 33], was kindly provided by Dr. Kenzo Takada (Hokkaido University, Japan). SNU719, a naturally derived EBV-positive gastric cancer cell line from a Korean patient [34], was kindly provided by Dr. Woo Ho Kim (Seoul National University, Korea). All cells were cultured in RPMI-1640 (Gibco, BRL) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone, Logan, UT, USA) at 37°C in a humidified 5% CO_2 incubator.

DNA extraction. DNAs were extracted from the cell lines, using the DNA isolation kit (Roche, IN). Paraffin-embedded tissue specimens were cut into 10-μm thick slices, and DNA was extracted using the WaxFree DNA extractor kit (TrimGen Corp., MD) according to manufacturer's instructions. Cellular fractions of throat washing specimens were collected by centrifugation at 16,000xg for 50 min, and the collected pellet was re-suspended in 100 μL of extraction buffer (TE buffer; 10 mM Tris-HCl and 1mM EDTA, pH8.0). The pellet was treated with proteinase K (200 μg/mL) at 37℃ overnight, followed by phenol/chloroform extraction and ethanol precipitation. Finally, the extracted DNA sample was dissolved in 50 μL of TE buffer.

Polymerase Chain Reaction (PCR). After DNA quantification, a 544-bp fragment corresponding to housekeeping *GAPDH* gene was confirmed by PCR using a specific primer set [35] to evaluate the quality of the DNA samples. The *BARF1* promoter sequence from −488 to +87 (575 bp) was amplified by PCR using 10 ng of DNA in a 25 μL reaction mixture containing 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 2.0 mM $MgCl_2$, 250 μM dNTP, 2 μM of each primer, and 1.25 U Taq polymerase (Invitrogen Corp., CA). The amplification profile was 1 cycle at 96°C for 5 min, followed by 45 cycles of 95C° for 1 min, 46°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 10 min. Primer sequences used in this study are listed in Table 1. The PCR products were visualized on 1% agarose gel electrophoresis using ethidium bromide (0.5 μg/mL).

DNA sequencing. The PCR-amplified fragments of the *BARF1* promoter (575 bp) were extracted from the agarose gel using the QIAEXII gel extractor kit (Qiagen, Chats worth, CA). Then, 20 ng of DNA were sequenced by the dideoxynucleotide chain terminator method, using the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer Co. Ltd.) according to the manufacturer's instructions. The sequence was resolved on an ABI Prism 310 Genetic Analyzer (Perkin Elmer Co.

Table 1. Sequences and coordinates of primers used in this study.

Primer	Oligonucleotide sequence	B95-8 coordinates
***BARF1* promoter†**		
Primer -488 (F)	5'-GGTCATCCAGGTAGTTTCGC-3'	165016-165035
Primer +87 (R)	5'-GACTCGCTCACCCAAGAAAG-3'	165590-165571
GAPDH*		
Primer (F)	5'-GCCTCCTGCACCACCAACTG-3'	
Primer (R)	5'- CGACGCCTGCTTCACCACCTTCT-3'	

† Primers were designed using a primer design software (CLC genomics).
* Primers used by Nanbo et al. [35].

Ltd.). All sequences were confirmed by duplicate analysis.

Sequence Analysis. B95-8 sequence was used as a prototype strain to define nucleotide variations (GenBank accession number V01555). AG876 and GD1 strains were used as EBV2 and NPC references, respectively (GenBank accession numbers DQ279927, AY961628). In addition, the EBV-positive Akata strain derived from Japanese BL and the SNU719 strain derived from a Korean EBV-GC were also analyzed. The MatInspector 2.2 software (Genomatix) was used for promoter analysis based on transcription factor binding sites [36].

Statistical analysis. The Fisher's exact test was conducted using STATA software, version 9.2. (STATA Corp, Lakeway Drive, College station USA). All the *P* values presented are two sided.

Results

Sequence variation of the *BARF1* promoter in EBV-GC and healthy controls

We first verified the DNA quality of the throat washing samples by PCR amplification of *GAPDH* (544 bp). This region was amplifiable in 70 out of 85 (82%) and 63 out of 118 (53%) of throat washing samples from Colombia and Japan, respectively. In 11 (16%) out of those 70 Colombian specimens and 9 (14%) out of 63 throat Japanese specimens, *BARF1*-promoter region (-488/+87, 575 bp) could be amplified. In all formalin-fixed paraffin-embedded blocks of EBV-GCs from Colombia and Japan, the same *BARF1*-promoter region and the *GAPDH* gene were amplifiable. In addition, sequences of the same *BARF1*-promoter region of Akata and SNU719 cell lines were also determined (Fig. 1). There was no sequence variation of this region among these cell lines when compared to the prototype B95-8 (EBV type 1), AG876 (EBV type 2), and GD1 (Chinese NPC).

The results of sequence variation between EBV-GC cases and healthy controls are summarized in Fig. 1. All the healthy donors had the same sequences of *BARF1* promoter region (–488/+87) as B95-8. On the other hand, the EBV-GC cases showed the following 8 point mutations in comparison with the B95-8 strain: G→C at –367 in 2 cases; T→A at –356 in 1 case; C→G at +15 in 1 case; C→T at +24 in 5 cases; T→G at +26 in 3 cases; T→C at +29

Figure 1. Sequence variation of the *BARF1* promoter in EBV-GC cases and healthy controls. Sequences were analyzed in 39 EBV-GC cases and 20 throat washing samples from healthy donors. The results were compared to the prototype strain and another reported sequences. *Prototype EBV B95.8 strain (GenBank accession number V01555). †AG876 is an EBV type-2 strain (GenBank accession number DQ279927). ‡GD1 strain is derived from Chinese NPC (GenBank accession number AY961628). GC: gastric carcinoma. TW: throat washing samples from healthy donors.

in 9 cases; T→A at +44 in 5 cases; and G→A at +46 in 1 case (Table 2). The observed case-control difference at position +29 was statistically significant (p=0.022, Fisher's exact test).

Sequence variation of the *BARF1* promoter in EBV-GC from Colombia and Japan

Since the prevailing EBV genotype and the EBV-GC frequency vary geographically, the sequence variations of the *BARF1*-promoter region were compared between 22 Colombian EBV-GCs and 17 Japanese EBV-GCs (Table 3). Although the frequency of the point mutation at position +29 in Colombian EBV-GCs was higher than that in Japanese EBV-GCs, the difference was not statistically significant (p=0.251, Fisher's exact test). Regarding the rest positions, the frequency of each mutation in Colombian EBV-GCs was higher than that of Japanese EBV-GCs at all positions except at +46.

Identification of potential transcription factors binding to the *BARF1* promoter

In addition to the core promoter of *BARF1* (starting from position −34), sequences encompassing the proximal and distal promoter may contain primary and additional regulatory elements that level BARF1 transcriptional activation. In order to identify potential transcription factors and elements required in mediating transcription activation of the *BARF1* promoter, the *BARF1* promoter region (−488/+87) of B95-8 was additionally analyzed bioinformatically using the MatInspector 2.2 [37]. Twelve matches were found in this sequence for ubiquitous transcription factors, including E2F-myc activator/cell cycle regulator, p53 tumor suppressor, E-box binding factors, and activator protein 2 (AP-2) (Table 4). Furthermore, 25 matches for transcription factors of the digestive system were identified (Table 5).

Discussion

In the present study, the *BARF1* promoter region (−488/+87) of EBV genome obtained from 39 EBV-GCs and 20 healthy donors was examined. There was a statistically significant increase of the point mutation of T→C at position +29 in EBV-GC cases (p=0.022). This point mutation corresponds to the amino acid change of L→P. However, the frequency of this mutation showed no significant difference between Colombian and Japanese EBV-GC cases. In addition, the SNU719 cell line, which

Table 2. Mutations in the *BARF 1* promoter (−488/+87) in EBV-GC and healthy controls.

B95.8 coordinate	Location	Mutation	Codon	Aminoacid change	Number of EBV-GC cases	Number of controls	P value
165136	-367	G→C	-	-	2/39	0/20	0.544
165146	-356	T→A	-	-	1/39	0/20	1.000
165518	+15	C→G	5	I→M	1/39	0/20	1.000
165527	+24	C→T	8*	-	5/39	0/20	0.156
165529	+26	T→G	9	L→R	3/39	0/20	0.544
165532	+29	T→C	10	L→P	9/39	0/20	0.022
165547	+44	T→A	15	V→E	5/39	0/20	0.156
165549	+46	G→A	16	A→T	1/39	0/20	1.000

*Silent mutation at codon 8.

Table 3. Mutations in the *BARF 1* promoter (−488/+87) in EBV-GC from Colombia and Japan.

B95.8 coordinate	Location	Mutation	Codon	Aminoacid change	EBV-GC Colombia	EBV-GC Japan	P value
165136	-367	G→C	-	-	2/22	0/17	0.495
165146	-356	T→A	-	-	1/22	0/17	1.000
165518	+15	C→G	5	I→M	1/22	0/17	1.000
165527	+24	C→T	8*	-	5/22	0/17	0.057
165529	+26	T→G	9	L→R	3/22	0/17	0.243
165532	+29	T→C	10	L→P	7/22	2/17	0.251
165547	+44	T→A	15	V→E	5/22	0/17	0.057
165549	+46	G→A	16	A→T	0/22	1/17	0.436

*Silent mutation at codon 8.

Table 4. Potential transcription factors binding to the *BARF 1* promoter (ubiquitous).

Family	Detailed family information	B95.8 coordinates	Promoter location	Opt.*	Core sim.†	Matrix sim.‡	Sequence (core sequence is underlined)
E2FF	E2F-myc activator/cell cycle regulator	165026-165042	-478/-462	0.84	1.000	0.849	GTAGTTTCGCACCGCAA
P53F	p53 tumor suppressor	165236-165258	-268/-246	0.92	0.828	0.934	CAGGGCTGGCAAAGGCAGGTCTT
P53F	p53 tumor suppressor	165237-165259	-267/-245	0.73	0.800	0.750	AGGGCTGGCAAAGGCAGGTCTTT
E2FF	E2F-myc activator/cell cycle regulator	165335-165351	-169/-153	0.85	1.000	0.869	CGAGGGCGCGACCCACG
TF2D	General transcription factor IID, GTF2D	165345-165383	-159/-121	0.69	0.847	0.690	ACCCACGCCTCGACCGGGGTCCTCACAAACACAGAATCT
VTBP	Vertebrate TATA binding protein factor	165427-165443	-77/-61	0.82	1.000	0.830	GCTTCAGGCTTATATGA
EBOX	E-box binding factors	165448-165460	-56/-44	0.93	1.000	0.942	ATGGGCGTGGCAG
VTBP	Vertebrate TATA binding protein factor	165463-165479	-41/-25	0.82	1.000	0.830	TAGTATAAGACGCGAGG
MTEN	Core promoter motif ten elements	165508-165528	+5/+25	0.88	0.838	0.907	CCAGGTTCATCGCTCAGCTCC
EBOX	E-box binding factors	165540-165512	+37/+49	0.93	1.000	0.948	TCCTGTGTGGCCG
AP2F	Activator protein 2	165546-165560	+43/+57	0.90	0.881	0.913	GTGGCCGCCGGCCAG
AP2F	Activator protein 2	165546-165560	+43/+57	0.90	0.881	0.946	GTGGCCGCCGGCCAG

*Opt: optimized value defined in a way that a minimum number of matches is found in non-regulatory test sequences. †Core similarity: The "core sequence" of a matrix is defined as the (usually 4) consecutive highest conserved positions of the matrix. ‡Matrix similarity: A perfect match to the matrix gets a score of 1.00 (each sequence position corresponds to the highest conserved nucleotide at that position in the matrix).

Table 5. Potential transcription factors binding to the BARF 1 promoter (digestive system).

Family	Detailed family information	B95.8 coordinates	Promoter location	Opt.*	Core sim.†	Matrix sim.‡	Sequence (core sequence is underlined)
FKHD	Fork head domain factors	165034-165050	-470/-454	0.98	1.000	0.995	GCACCGCAAACACCACT
RXRF	RXR heterodimer binding sites	165066-165090	-438/-414	0.78	0.833	0.800	ACCCTGAGCCGCGACCAGTAGTCGT
AHRR	AHR-arnt heterodimers and AHR-related factors	165128-165152	-375/-350	0.77	1.000	0.780	AGCCGTACGTGCGTGTCTTTGCCCC
NR2F	Nuclear receptor subfamily 2 factors	165134-165158	-369/-345	0.82	1.000	0.823	ACGTGCGTGTCTTTGCCCCCGATGT
PAX6	PAX-4/PAX-6 paired domain binding sites	165226-165244	-278/-260	0.87	1.000	0.879	GGCAGAGGACCAGGGCTGG
RXRF	RXR heterodimer binding sites	165258-165282	-246/-222	0.79	0.761	0.818	TTCTCATCCCGGGTGAACACCGCGT
AHRR	AHR-arnt heterodimers and AHR-related factors	165270-165294	-234/-210	0.77	1.000	0.772	GGTGAACACCGCGTACATGGCCCTG
AHRR	AHR-arnt heterodimers and AHR-related factors	165339-16564	-165/-139	0.90	1.000	0.904	GGGCGCGACCCACGCCTCGACCGGGG
TF2D	General transcription factor IID, GTF2D	165346-165383	-158/-120	0.69	0.847	0.690	ACCCACGCCTCGACCGGGGTCCTCACAAACACAGAATCT
RXRF	RXR heterodimer binding sites	165344-165405	-122/-98	0.75	0.750	0.803	TCTGTAGACTTGGCTGGCCTCATGG
RXRF	RXR heterodimer binding sites	165398-165422	-105/-81	0.75	1.000	0.750	CCTCATGGTCTCGTCAGGCCAGCTC
VTBP	Vertebrate TATA binding protein factor	165427-165443	-77/-61	0.82	1.000	0.830	GCTTCAGGCTTATATGA
PAX6	PAX-4/PAX-6 paired domain binding sites	165427-165445	-77/-59	0.75	0.754	0.778	GCTTCAGGCTTATATGATA
CDXF	Vertebrate caudal related homeodomain protein	165436-165454	-68/-50	0.84	1.000	0.853	TTATATGATAAAATGGGCG
KLFS	Krueppel like transcription factors	165445-165463	-59/-41	0.98	1.000	0.984	AAAATGGGCGTGGCAGAAT
VTBP	Vertebrate TATA binding protein factor	165463-165479	-41/-25	0.82	1.000	0.830	TAGTATAAGACGCGAGG
RXRF	RXR heterodimer binding sites	165478-165502	-26/-2	0.80	0.790	0.901	GGCCTGGGTGAGGAGAGTCCAGAGC
NR2F	Nuclear receptor subfamily 2 factors	165490-165516	-12/+13	0.83	0.796	0.831	GAGTCCAGAGCAATGGCCAGGTTCA
SORY	SOX/SRY-sex/testis determinig and related HMG box factors	165494-165510	-8/+9	0.90	1.000	0.910	CCAGAGCAATGGCCAGG
RXRF	RXR heterodimer binding sites	165501-165525	-1/+24	0.78	0.833	0.831	AATGGCCAGGTTCATCGCTCAGCTC
NR2F	Nuclear receptor subfamily 2 factors	165503-165527	+2/+26	0.82	0.809	0.847	TGGCCAGGTTCATCGCTCAGCTCCT
MTEN	Core promoter motif ten elements	165508-165528	+5/+25	0.88	0.838	0.907	CCAGGTTCATCGCTCAGCTCC
CHRE	Carbohydrate response elements, consist of two E box motifs separated by 5 bp	165525-165544	+22/+41	0.82	0.800	0.828	CTCCTCCTGTTGGCCTCCTG
NF1F	Nuclear factor 1	165543-165563	+40/+60	0.81	1.000	0.855	TGTGTGGCCGCCGGCCAGGCT
NF1F	Nuclear factor 1	165543-165563	+40/+60	0.81	1.000	0.835	TGTGTGGCCGCCGGCCAGGCT

*Opt: optimized value defined in a way that a minimum number of matches is found in non-regulatory test sequences. †Core similarity: The "core sequence" of a matrix is defined as the (usually 4) consecutive highest conserved positions of the matrix. ‡Matrix similarity: A perfect match to the matrix gets a score of 1.00 (each sequence position corresponds to the highest conserved nucleotide at that position in the matrix).

has been established from Korean EBV-GC, did not show this mutation. Although other 7 mutations were also found in EBV-GC cases but not in controls, there was no statistically significant case-control difference.

The TATA sequence was identified at -34 upstream of the *BARF1* Open Reading Frame by Zhang *et al.* [38], and an early promoter TATAAGA EDR1 at position 165466 was also reported by Baer *et al.* [39]. Since proximal and / or distal encompassing region of the core BARF1 promoter may contain potential binding sites of primary and / or additional regulatory elements that activate BARF1 transcriptional level, it would be ideal to extend the region for sequence analysis. However, the maximum range for the sequence analysis was around 600bp since clinical specimens used in the present study were paraffin-embedded tissues and the specimens were limited. Primers from −488 to +87 were selected using a primer design software, CLC genomics.

EBV-immortalized epithelial cells are not tumorigenic in nude mice [14], suggesting that interaction(s) between EBV and cellular genes will be critical for inducing malignant transformation of epithelial cells. In the case of *LMP1* transcription, additional viral and cellular transcription factors were identified in mediating

transcription activation of the *LMP1* promoters [40–44]. Other studies have identified cellular and viral factors that regulate the expression of EBV BARTs [45, 46], which recently garnered attention in NPC development. On the other hand, the regulatory mechanism of BARF1 expression is still unclear. Therefore, it would be worthwhile to identify elements that might regulate *BARF1* transcription to understand etiological roles of BARF1 in EBV-associated malignancies. The analysis of the *BARF1* promoter region (−488/+87) of B95-8 identified 12 matches in this sequence for ubiquitous transcription factors, including E2F-myc activator/cell cycle regulator, p53 tumor suppressor, E-box binding factors, and activator protein 2 (AP-2) (Table 4). Furthermore, 25 matches for transcription factors of the digestive system were identified (Table 5). Interestingly, the position at +29, where a significant case-control difference of the mutation frequency was observed, was comprised in one of the 25 matches, which is carbohydrate response elements (+22/+41) (Table 5). It would be important to examine: 1) the role of carbohydrate response elements on *BARF1*-promoter activation, and 2) the significance of the mutation in the regulation of *BARF1*-promoter activation.

Previous studies revealed that bcl-2 upregulation is induced by BARF1 in NPC [22, 46]. The N-terminal domain of *BARF1* gene (codons 1 to 54) was reported to be essential for malignant transformation of rodent fibroblasts and activation of bcl-2 [22, 47]. Regarding EBV-GC, however, there have been conflicting results in BARF1-induced upregulation of bcl-2 [48–50]. In the present study, five mutations in codons 5, 9, 10, 15, and 16 were found in EBV-GCs (Table 2). Recently, point mutations in codons 16, 20, and 29 were reported in NPCs from Hong Kong at frequencies of 1/50, 4/50, and 4/50, respectively [51, 52]. Interestingly, the same mutation in codon 29 (V→A) was also reported in NPCs from North Africa (8/8) and EBV-GCs from Hong Kong (2/10) [51]. However, this mutation was not found in any EBV-GCs from Colombia and Japan in this study.

The present study identified the sequence variations of the *BARF1* promoter of EBV detected in EBV-GCs and throat washing specimens obtained from healthy controls. Further studies are warranted in order to determine the significance of these mutations found in EBV-GC and the etiological significance of BARF1 in EBV-GC development.

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Epstein-Barrウイルス（EBV）関連胃がんにおける EBV-*BARF1*遺伝子配列の変異

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Epstein-Barrウイルス（EBV）遺伝子産物であるBARF1は，EBウイルス関連胃がんの発がん過程において重要な役割を果たしている可能性がある．本研究では，南米コロンビア（22例）と日本（17例）のEBウイルス関連胃がん症例から得られたがん組織を用いて，プロモーター領域を含むBARF1 遺伝子上流領域（開始コドン上流488位から下流87位まで）の遺伝子配列解析を行った．更に対照群として，健常人（コロンビア11名，日本９名）のうがい液から検出されたEBウイルスの解析も行った．その結果，対照群から検出されたEBウイルスの塩基配列はすべて，すでに報告されているB95-8の塩基配列と同じであった．一方，EBウイルス関連胃がん組織から検出されたウイルスの塩基配列では，次の８箇所の変異が確認された．開始コドン上流367位のＧ→Ｃ変異（コロンビア２例），上流356位のＴ→Ａ変異（コロンビア１例），下流15位のＣ→Ｇ変異（コロンビア１例），下流24位のＣ→Ｔ変異（コロンビア５例），下流26位のＴ→Ｇ変異（コロンビア３例），下流29位のＴ→Ｃ変異（コロンビア７例，日本２例），下流44位のＴ→Ａ変異（コロンビア５例）および下流46位のＧ→Ａ変異（日本１例）．対照群と比べて，EBウイルス関連胃がんで観察された29位の変異頻度は高く，統計学的に有意であった（p=0.022）．この部位の変異頻度は，日本よりもコロンビアのEBウイルス関連胃がん症例において多く認められたものの，統計学的有意差はなかった（p=0.251）．今後，EBウイルス関連胃がんと対照群において有意差を認めた+29におけるＴ→Ｃ変異の発がん過程における意義を明らかにする必要がある．