Gastroenterology and Hepatology From Bed to Bench.

ORIGINAL ARTICLE

Sequence variations of Epstein–Barr virus LMP1 gene in gastric cancer and chronic gastritis isolates from Iranian patients

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ABSTRACT

Aim: The current study aimed to investigate sequence variations in the C-terminus of latent membrane protein 1 (LMP1) in Epstein-Barr virus (EBV) isolates from Iranian patients with chronic gastritis or gastric cancer (GC).

Background: LMP1, an essential viral oncoprotein, is the critical element in the immortalization of B cells. It contains a small twenty-four amino acid cytoplasmic N-terminal region, six transmembrane segments, and a two hundred amino acid cytoplasmic C-terminal domain. Most LMP1-mediated signal transduction events are moderated by some functional parts of the cytoplasmic C-terminal domain.

Methods: Thirty-two EBV-positive biopsy tissues were obtained from patients with gastric cancer and patients with chronic gastritis. The C-terminal nucleotide sequences of LMP1 were amplified using nested-PCR and analyzed by DNA sequencing.

Results Four to eight copies of the 11 repeat elements (codon 254–302) were observed in the carboxyl-terminal site of patients, but no relationship was found between the number of repeat sequences and disease status. The 30-bp deletion corresponding to codon 345–354 of the B95-8 strain was observed in 34% of isolates, and the remaining samples were non-deleted. In the gastric cancer group, a higher number of 33-bp repeats (≥5 repeats) was observed in 30-bp-deletion (100%) than in non-deleted (42%) isolates, and the difference was statistically significant. Analysis revealed that a gastritis isolate may be the result of recombination between Alaskan and China1 strains.

Conclusion: Overall, the current results showed no association between C-terminal sequence variations of LMP1 and malignant or non-malignant isolate origin.

Keywords: EBV, Latent membrane protein 1, Sequence variations; Repeat elements

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Introduction

Epstein–Barr virus (EBV) is a ubiquitous infectious pathogen that infects more than 90% of the world's population (1). Primary infection with EBV often occurs in early childhood and is typically asymptomatic. In contrast, if the primary infection is delayed until adolescence, it often leads to infectious mononucleosis (IM) (2). EBV is also associated with a wide variety of malignancies, including Burkitt's lymphoma (BL), Hodgkin's lymphoma (HL), non-

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Hodgkin's lymphomas (NHL), post-transplant lymphoproliferative carcinoma (PTLD), nasopharyngeal carcinoma (NPC), and gastric cancer (GC) (3). EBV-associated gastric carcinoma (GC) is a non-endemic malignancy distributed throughout the world and accounts for about 4–18% of all reported GC cases (4). During latent infection in gastric cancer, EBV expresses a restricted set of genes, including EBNA1 (98.1%) and LMP2A (53.8%). LMP1 and LMP2B were also detected in 10% of cases (5). Based on variations of the LMP-1 gene, EBV is classified into 7 EBV strains: Alaskan (AL), China1, China2, China3, B95.8, Mediterranean (Med +/-), and North Carolina (NC) (6). So far, studies have found no evidence of an association between a distinct LMP1 strain of EBV and driven cancers. Findings support the geographical tropism of the LMP1 strains (7, 8). Therefore, it seems that examining other variations in the LMP1 sequence may provide a better understanding of the relationship between EBV-related cancers and LMP1 polymorphisms. Sequence variations in the C-terminus of LMP1 include 11 aa repeat sequences, the 30-bp deletion (30-bp-del) region, and other nucleotide and amino acid (aa) changes (8). The C-terminal cytoplasmic region of LMP1 involves three essential activating domains: CTAR1 (aa 191–232), which is essential for EBV-associated transformation of B-cells; CTAR2 (aa 351–386), a necessary factor for EBV-infected cell growth; and CTAR3 (aa 275–330), which, using JAK3, results in STAT3 activation (9). The 30 bp deletion (del-LMP1) in the carboxy-terminal of LMP-1, which corresponds to the loss of ten amino acids (codons 345–354) and occurs in the CTAR-2 region, is indicated as leading to the higher tumorigenic activity of EBV (10). The region between CTAR-1 and -2 (aa 233–350) in the LMP1 C-terminus contains four 11-aa repeat (QDPDNTDDNGP) sequences and a disruption of 5 aa (HDPLP: 275–279), with a different copy number between human isolates (11). To determine whether these polymorphisms are correlated with gastric cancer or gastritis, we analyzed the sequence of the C-terminal region of LMP1 from EBV-positive gastric carcinoma and chronic gastritis biopsies in Iranian patients.

Methods

Patients

This study examined EBV-DNA-positive tissue samples from 21 patients with gastric cancer and 11 patients with chronic gastritis at the Research Center for Gastroenterology and Liver Diseases, Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran, over a period of two years (2019 –2021). DNA was extracted and purified from tissue samples using the QIAamp DNA Mini Kit, according to the standard tissue protocol. The Concentration and purity of DNA were measured through absorbance ratio at 260/280 using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, United States).

PCR for detection of LMP1

Nested PCR was performed to amplify the C-terminus of the LMP1 gene. The first round of amplification was done using the outer primers of LMP1-F1 (5'-GCTAAGGCATTCCCAGTAAA-3') and LMP1-R1 (5'-GATGAACACCACCACGATG -3') in a total volume of 25 ul containing 1× PCR reaction buffer, 100 ng of DNAs extracted from tissues, 50 pmol of each primer, 1.5 mM MgCl 2, 0.4 mM each dNTP and 1U Taq polymerase (amplicon). The cycling protocol was started with an initial denaturation for 5 min at 95 ºC, then 35 cycles of denaturation for 30 seconds at 94 °C, annealing for 30 seconds at 55 °C and extension for 1 min at 72 °C. A final elongation step for 4 min at 72 °C was then conducted. For the second round of PCR, we employed the inner primers of LMP1-F2 (5'-CGGAACCAGAAGAACCCA-3') and LMP1-R2 (5'- TCCCGCACCCTCAACAAG-3'); then 1 ml of the PCR product was used as the template. The same experimental conditions were utilized for the second round except for the annealing temperature which was 60 °C. In each experiment, the B95-8 cell line and sterile water were used as the positive and negative controls, respectively. Afterward, five microliters of the second-round PCR products were analyzed by electrophoresis in 1.2% agarose gel.

DNA sequencing

For sequencing, the nested PCR amplicons were purified using a QIAGEN MinElute Purification Kit (QIAGEN, Hilden, Germany), according to the

manufacturer's instructions. Cleaned PCR products were sequenced using internal PCR primers and the BigDye terminator cycle sequencing kit (Applied Biosystems). Cycle sequencing was done in an ABI 3130 automated sequencer (ABI PRISM 3130 Genetic Analyzer; Applied Biosystems). Both LMP1strands (sense and antisense) were sequenced and compared.

Identification of nucleotide and amino acid changes

Nucleotide sequences obtained from samples from this study were translated into amino acid (aa) sequences and compared with reference sequences from the GenBank database in BioEdit software. Mutations of isolated sequences were identified using the same software.

Statistical analysis

Data was evaluated using GraphPad Prism software, version 9.0 (GraphPad Software, Inc., San Diego, CA), and a p-value< 0.05 was considered statistically significant. The Fisher's exact test was performed to compare distribution differences of the LMP1 variations.

Results

Sequence alternations in the carboxyl-terminus region

The amino acid change of C-terminus-activating region 1 (CTAR1), codon 194-232, at amino acid position 212 (Gly →Ser/Arg/Ala/Asp) was detected in almost all samples, regardless of disease status. Moreover, the amino acid change at position 229 (Ser→Thr) was observed in all of the Med strain isolates. In the C-terminus-activating region 2 (CTAR2), codon 351–386, the presence of the 30-bp deletion at codon 345–354 was observed in 11/32 isolates, and the amino acid change at position 352 (His→Arg/Asp) was identified in approximately all remaining isolates. In the C-terminal activating region 3 (CTAR3), codon 275–330, the 5 amino acid insertion at codons 275–280 between the 2nd and 3r repeat or between the 3rd and 4th repeat, was detected in 6/32 isolates. The changes at amino acid positions 309 (Ser→Asn/Asp) and 322 (Gln →Glu/Asn/Asp/Thr) were observed in all except B95-8 strain isolates, in which the amino acid change was only at codon 328 (Glu→Gln) (Figure 1).

Variation in the 11 amino acids repeat element in LMP1

Within the C-terminal region of LMP1, there are diverse redundant segments, including an 11 amino-acid (aa) repeat element between the 253rd and 306th amino acids, which has been shown to sort independently of each isolate. In the C-terminal domain of the B95-8 strain exist four perfect 11 amino acid (QDPDNTDDNGP) repeats, with a disruption of 5 aa (HDPLP: 275–279) after the second repeat. The five amino acid sequence is also located after the last repeat (aa: 302–306). In gastric cancer and chronic gastritis samples of this study, the number of repeat elements varied from 4 to 8. There was no correlation between the number of repeat sequences and disease status (Figure 2). The same repeat sequence of B95-8 was also found in 3 gastric cancer samples. Seven gastric cancer and six gastritis samples had 5 perfect repeats of the 11 amino acid sequence, and they had the 5 amino acid insertion only after the last repeat. Five gastric cancer and one gastritis sample had four 11 amino acid repeat elements, and they also had the 5 amino acid insertion after the last repeat.

One GC isolate had 5 perfect repeats of the 11 amino acid and 5 amino acid sequences between the 2nd and 3rd repeat and the 5 amino acid sequence at the end. Two gastritis isolates had 5 perfect repeats of the 11 amino acid and 5 amino acid sequences between the 3rd and 4th repeat and the 5 amino acid sequence at the end. Four GC isolates and two gastritis isolates had 6 perfect repeats of the amino acid sequence, but not the 5 amino acid insertion, as well as the 5 amino acid sequence at the end. A GC isolate had 8 complete replicates of the amino acid sequence but not the 5 amino acid insertion, with the 5 amino acid sequence at the end. Except for the B95-8 isolates, this 11-amino-acid repeat region had changed to QGPDNTDDNGP after the second 11-AA in most cases. B95-8 isolates had a mean of 4.5 repeats (4 to 5 copies). Med isolates had a mean of 5 repeats (4 to 8 copies). Both China 1 isolates carried 6 repeats. North Carolina and Alaskan isolates carried 6 and 5 repeats, respectively.

The LMP1 polymorphisms (deleted and non-deleted) were compared to the presence of (4 or ≥5) 33-bp repeats. In the gastric cancer group, a statistically significant difference was observed in the distribution of the number of repeat elements (4 or ≥5) among LMP1 polymorphisms ($p<0.018$) (Table 1).

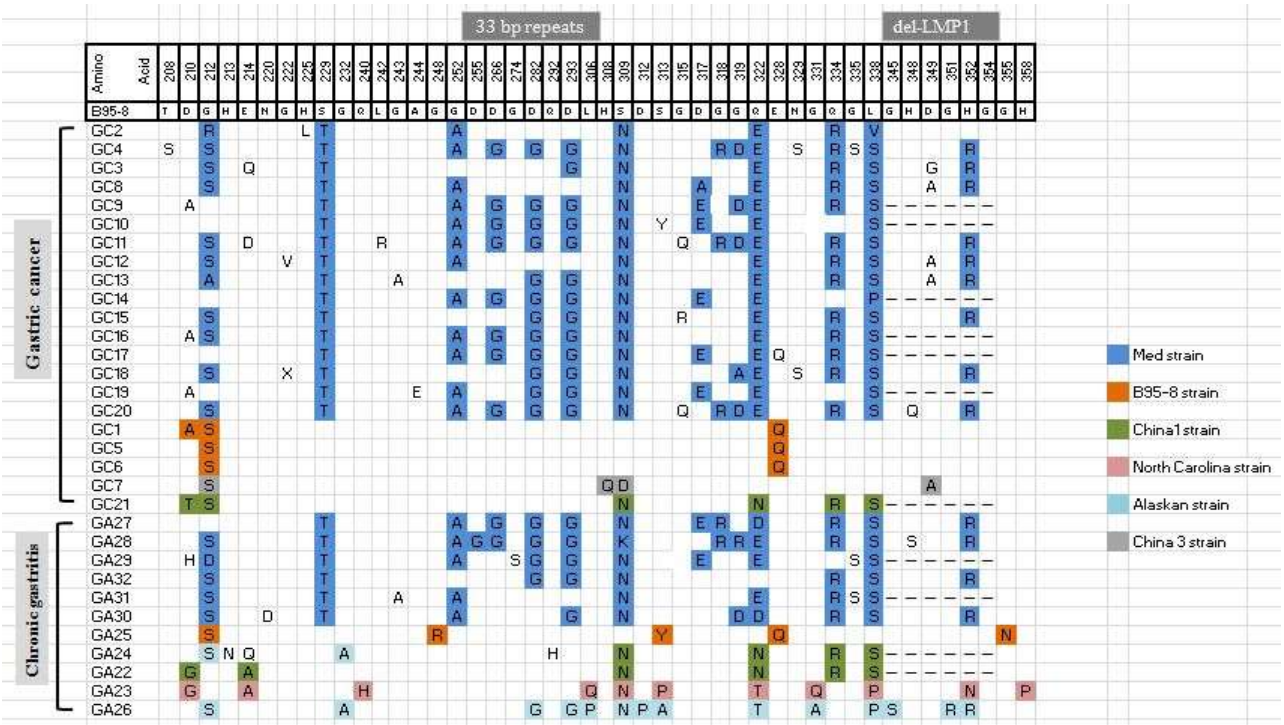

Figure 1. Amino acid variation in the C-terminal of Iranian LMP1 isolates. Numbers across the top correspond to amino acid positions under which the B95-8 strain amino acid sequence is listed. The positions of the 33-bp repeats and the 30-bp deletion are denoted across the top, changes in the amino acids are denoted by letter changes, and a dash indicates a deletion of this amino acid position. Names in the left column refer to the individual isolates, and the capital letter GC represents a gastric cancer specimen, while the capital letter GA represents a gastritis specimen. The strain-identifying changes are shaded, patterned, and arranged by groups. Recombinant isolate is characterized by more than one strain identifying pattern and shading.

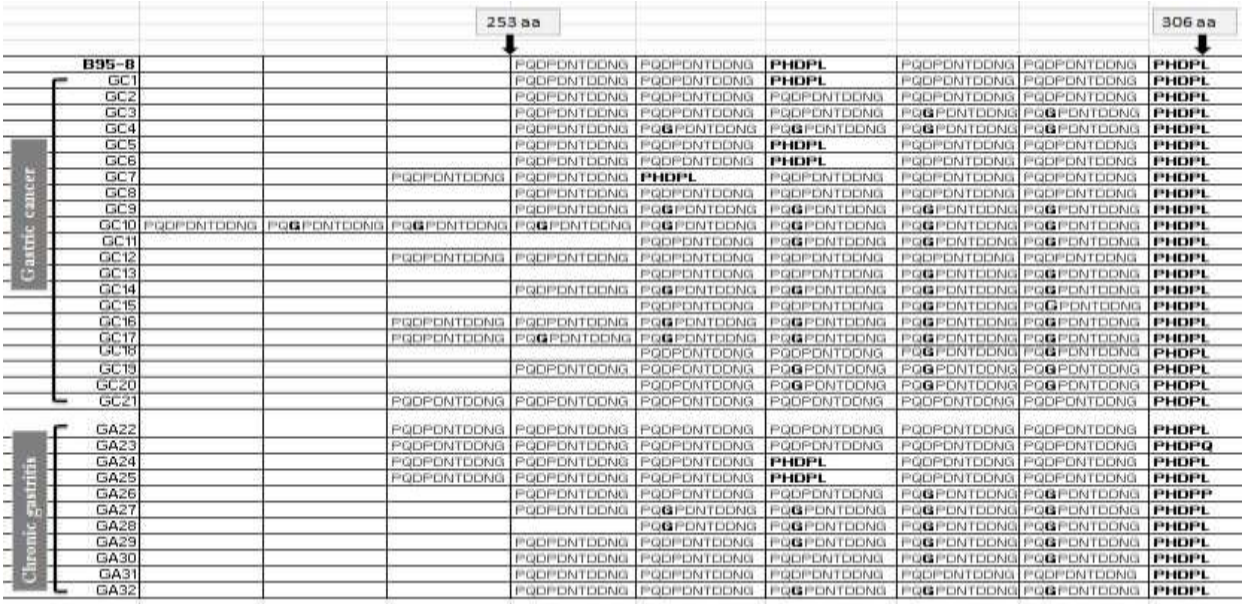

Figure 2. Variations in the 11 aa repeat units in the C-terminal of LMP1 isolates obtained from biopsy tissues of gastric cancer and chronic gastritis patients. Across the top, the pattern of 11 aa repeat units between aa 253 and 306 in the LMP1 C-terminal domain of B95.8 strain is shown with the LMP1 amino acid positions. The names in the left column refer to the individual isolates. PQDPDNTDDNG, 11 aa repeat sequence; PQGPDNTDDNG, 11 aa repeat sequence with point mutation D→G; PHDPL, 5 amino acid sequence.

Table 1. Distribution of LMP1 33-bp repeat elements and deletions between gastric cancer and chronic gastritis isolates

			Number of 33 bp tandem repeat units			
			4	5-8		Number of samples
Group	Origin	30-bp-deletion	Number of isolates		P value	tested
Gastric cancer	Tissue biopsy	30-bp-deletion	-	7	0.0180	21
		Non-deleted	8	6		
Chronic gastritis	Tissue biopsy	30-bp-deletion	-	4	>0.9999	11
		Non-deleted	1	6		

Inter-strain recombination

Analysis revealed that one of the isolates had a similar amino acid pattern to the two strains. It has been suggested that the 33-bp-repeats region in the C terminus of the LMP1 sequence can be a site for recombination during replication of the virus. Our analysis on the C-terminal domain indicated that the GA24 isolate had changes in the characteristics of the Alaskan strain on one side of the 11-amino acid repeats and some of the specific mutations of the China 1 strain on the other side of the repeats. The GA24 isolate also had the characteristic serine and alanine at amino acid positions 212 and 232 of the Alaskan strain, respectively, while the distal side of the 11 amino acid repeat element was marked by China 1 strain mutations, including asparagine, arginine, and serine at positions 322, 334, and 338, respectively. These findings indicate that this isolate has arisen from inter-strain recombination across the 33-bp repeats (Figure 1).

Discussion

It would be very interesting to determine whether distinct EBV strains link to EBV-carrying malignancies. So far, there is no clear link between a distinct LMP1 strain of the EBV and gastric cancer, and observations in this regard are controversial. According to previous reports that indicate the distribution of EBV strains in EBV-associated tumors follows geographical tropism (7, 8), these strains or types cannot be used as valuable markers for estimating the association with a particular type of EBV-associated tumors. Sequence variation analysis in other parts of LMP1, especially regions that have a significant effect on its biological function, may give us a clearer picture of the association of EBV polymorphisms with relevant malignancies. The 30 bp deletion is the most frequent polymorphism in the C-terminus LMP1 that is detected in EBV-associated malignancies. The tumorigenicity of this polymorphism has been described in several reports (12). The 30-bp deletion corresponding to 10 amino acids deletion (at codon 345–354) that is located in the NF-kB-activating region CTAR-2 can result in a more oncogenic variant (13). In addition, the del-LMP1 has previously been shown to have the potential to transform rodent fibroblast cell lines in vitro (14). The worldwide distribution of LMP1 30-bp deletion polymorphism has been reported by many studies (12). To date, several studies have been suggested that EBV-associated gastric cancer (EBVaGC) is associated with the del-LMP1 polymorphism (15-18). However, in the current study, no clear association was observed between gastric cancer and this polymorphism. These results are in agreement with some studies that have suggested deleted LMP1 as a race or geographic-linked variation (19-21). The literature has also shown the association between del-LMP1 and some amino acid substitutions, including changes at amino acid positions 132 (Arg→Gly) and 309 (Ser→Asn), which are important variations regarding the activity of del-LMP1 (19, 22, 23). In the present study, the change at amino acid position 309 (Ser→Asn/Asp) was observed for all isolates other than the B95-8 strain isolates. Here, serine 309, which is located in CTAR3, might lead to lowering the LMP1 half-life and, eventually, reducing the transformation potential of LMP1. Our findings confirmed previous reports (24) claiming that isolates of the B95-8 strain contain four perfect repeats of the 11 amino acid sequence (PQDPDNTDDNG) and five amino acid sequence (PHDPL) after the second repeat, except for one case (GA25) that had 5 perfect repeats with the 5 amino acid insertion located after the 3rd repeat. The presence of different numbers (4–8) of an 11 amino acid repeats element between amino acids 253 and 306 in the carboxy-terminal domain of LMP-1 was observed in this study. To clarify the relationship between the number of repeats and disease status, we compared the copy number of 33-bp repeats in two

gastric cancer and gastritis groups. The results indicated that there is no association between the number of repeated sequences and the mentioned parameter. The point mutation D→G (PQDPDNTDDNG→PQGPDNTDDNG) was frequently found in gastric cancer (13/21, 61.9%) and gastritis (6/11, 54.5%) cases, although the difference between the two groups was not statistically significant. Interestingly, a higher number of 33-bp repeats (≥5 repeats) was detected in 30-bp-deletion (100%) isolates than in non-deleted (42%) isolates of gastric cancer, and the difference was statistically significant, as previously reported (25). However, in gastritis isolates, the difference was not significant. This phenomenon may be the outcome of recombination activity during lytic viral DNA replication and be formed after the occurrence of the 30 bp deletion (26). In the present study, one isolate (GA24) seems to be the consequence of recombination, which may have occurred from recombination through the 33-bp repeat region, as reported by previous studies (7).

Conclusion

Until now, the tumorigenic mechanism by which EBV leads to gastric cancer development has remained unclear. Given the ubiquitous nature of EBV and the different incidence rates of EBV-associated malignancies in different geographical areas, the development of these malignancies may be correlated with particular EBV polymorphisms. In summary, we found no association between gastric cancer and del-LMP1 polymorphism. In isolates of gastric cancer, a higher number of 33-bp repeats (≥5 repeats) was observed in deleted than in non-deleted strains, and this difference was statistically significant. Furthermore, one isolate was identified that seems to be a consequence of recombination between two strains.

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Conflict of interests

The authors declare that they have no conflict of interest.

References

1. Chen M-R. Epstein–Barr virus, the immune system, and associated diseases. Front Microbiol 2011;2:5.

2. Gequelin LCF, Riediger IN, Nakatani SM, Biondo AW, Bonfim CM. Epstein-Barr virus: General factors, virus-related diseases and measurement of viral load after transplant. Rev Bras Hematol Hemoter 2011;33:383-8.

3. Neparidze N, Lacy J. Malignancies associated with epstein-barr virus: pathobiology, clinical features, and evolving treatments. Clin Adv Hematol Oncol 2014;12:358-71.

4. Jia X, Guo T, Li Z, Zhang M, Feng Y, Dong B, et al. Clinicopathological and immunomicroenvironment characteristics of Epstein–Barr virus-associated gastric cancer in a chinese population. Front Oncol 2021;10:586752.

5. Ribeiro J, Oliveira C, Malta M, Sousa H. Epstein–Barr virus gene expression and latency pattern in gastric carcinomas: a systematic review. Future Oncol 2017;13:567-79.

6. Banko A, Miljanovic D, Lazarevic I, Cirkovic A. A systematic review of Epstein–Barr virus latent membrane protein 1 (LMP1) gene variants in nasopharyngeal carcinoma. Pathogens 2021;10:1057.

7. Edwards RH, Seillier-Moiseiwitsch F, Raab-Traub N. Signature amino acid changes in latent membrane protein 1 distinguish Epstein–Barr virus strains. Virology 1999;261:79-95.

8. Wang H-Y, Sun L, Li P, Liu W, Zhang Z-G, Luo B. Sequence variations of Epstein-Barr virus-encoded small noncoding rna and latent membrane protein 1 in hematologic tumors in northern china. Intervirology 2021;64:69-80.

9. Ranger-Rogez S. EBV genome mutations and malignant proliferations. In: Drouet, E. ed. Epstein-Barr Virus - New Trends [Internet]. London: IntechOpen; 2020. Available from: https://www.intechopen.com/chapters/73024.

10. Knecht H, Bachmann E, Brousset P, Sandvej K, Nadal D, Bachmann F, et al. Deletions within the LMP1 oncogene of Epstein-Barr virus are clustered in

Hodgkin's disease and identical to those observed in nasopharyngeal carcinoma. Blood 1993;82:2937-42.

11. Brinkmann MM, Schulz TF. Regulation of intracellular signalling by the terminal membrane proteins of members of the Gammaherpesvirinae. J Gen Virol 2006;87:1047-74.

12. Neves M, Marinho-Dias J, Ribeiro J, Sousa H. Epstein–Barr virus strains and variations: Geographic or disease-specific variants? J Med Virol 2017;89:373-87.

13. Mainou BA, Raab-Traub N. LMP1 strain variants: Biological and molecular properties. J Virol 2006;80:6458-68.

14. Li S-N, Chang Y-S, Liu S-T. Effect of a 10-amino acid deletion on the oncogenic activity of latent membrane protein 1 of Epstein-Barr virus. Oncogene 1996;12:2129-35.

15. BenAyed-Guerfali D, Ayadi W, Miladi-Abdennadher I, Khabir A, Sellami-Boudawara T, Gargouri A, et al. Characteristics of epstein barr virus variants associated with gastric carcinoma in Southern Tunisia. Virol J 2011;8:1-9.

16. Chen JN, Ding YG, Feng ZY, Li HG, He D, Du H, et al. Association of distinctive Epstein–Barr virus variants with gastric carcinoma in Guangzhou, Southern China. J Med Virol 2010;82:658-67.

17. Chen J-n, Jiang Y, Li H-g, Ding Y-g, Fan X-j, Xiao L, et al. Epstein-Barr virus genome polymorphisms of Epstein-Barr virus-associated gastric carcinoma in gastric remnant carcinoma in Guangzhou, Southern China, An endemic area of nasopharyngeal carcinoma. Virus Res 2011;160:191-9.

18. Cheng N, Hui D-y, Liu Y, Zhang N-n, Jiang Y, Han J, et al. Is gastric lymphoepithelioma-like carcinoma a special subtype of EBV-associated gastric carcinoma? New insight based on clinicopathological features and EBV genome polymorphisms. Gastric Cancer 2015;18:246-55.

19. Ai J, Xie Z, Liu C, Huang Z, Xu J. Analysis of EBNA-1 and LMP-1 variants in diseases associated with EBV infection in Chinese children. Virol J 2012;9:1-8.

20. Fukayama M, Ushiku T. Epstein-Barr virus-associated gastric carcinoma. Pathol Res Pract 2011;207:529-37.

21. See HS, Yap YY, Yip WK, Seow HF. Epstein-Barr virus latent membrane protein-1 (LMP-1) 30-bp deletion and Xho I-loss is associated with type III nasopharyngeal carcinoma in Malaysia. World J Surg Oncol 2008;6:1-10.

22. Huen D, Henderson S, Croom-Carter D, Rowe M. The Epstein-Barr virus latent membrane protein-1 (LMP1) mediates activation of NF-kappa B and cell surface phenotype via two effector regions in its carboxy-terminal cytoplasmic domain. Oncogene 1995;10:549-60.

23. Moorthy RK, Thorley-Lawson DA. Biochemical, genetic, and functional analyses of the phosphorylation sites on the Epstein-Barr virus-encoded oncogenic latent membrane protein LMP-1. J Virol 1993;67:2637-45.

24. Kanai K, Satoh Y, Saiki Y, Ohtani H, Sairenji T. Difference of Epstein–Barr virus isolates from Japanese patients and African Burkitt's lymphoma cell lines based on the sequence of latent membrane protein 1. Virus Genes 2007;34:55-61.

25. Jovanovic T. Analysis of the variability of epstein-barr virus genes in infectious mononucleosis: Investigation of the potential correlation with biochemical parameters of hepatic involvement. J Med Biochem 2016;35:337-46.

26. Miller WE, Edwards RH, Walling DM, Raab-Traub N. Sequence variation in the Epstein–Barr virus latent membrane protein 1. J Gen Virol 1994;75:2729-40.