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Transforming Growth Factor-β, Interleukin-10, and Serological Markers in EBV-associated Gastric Carcinoma

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Abstract. *Background/Aim: The association between Epstein-Barr virus (EBV) and gastric cancer (GC) has been reported by many researchers. Immunosuppressive cytokines, such as interleukin-10 (IL-10) and transforming growth factor β (TGFβ), play an important role in the tumor process. The aim of the present study was to detect antibodies against EBV and explore the levels of TGFβ and IL-10 in Polish GC patients. Patients and Methods: Fifty patients with GC and 50 hospitalized individuals without GC (control group) were enrolled in the study. Frozen tumor tissue fragments were tested using nested PCR assay for EBV DNA detection. ELISA test was used to detect the presence of anti-VCA IgG, anti-EBNA IgG, anti-EA IgG, TGFβ and Il-10 in sera from all individuals. Results: EBVCA was detected in 88.0%, EBNA in 90.0%, and EA in 72.0% of patients. Levels of TGFβ and IL-10 were significantly higher in patients with high levels of anti-EA antibodies (25.4 ng/ml; 7.8 pg/ml) compared to patients with low levels of anti-EA antibodies (12.61 ng/ml; 4.29 pg/ml). Conclusion: The significantly higher level of EA in patients' sera indicates EBV reactivation. TGFβ level was significantly higher in GC than in the control group, especially in EA-positive patients, indicating its possible role in gastric carcinogenesis.*

Gastric cancer (GC) is the fifth most commonly diagnosed cancer and the third leading cause of cancer-related mortality worldwide (1). According to Cancer Atlas Research Network, GC is divided into 4 molecular types: GC positive for Epstein-Barr virus (EBV), GC with microsatelite instability, genomically stable GC and GC with chromosomal instability (2).

Every year, more than 950,000 cases of gastric adenocarcinoma are diagnosed in the world and it is estimated that about 10% of these cases are associated with EBV (3, 4). Each year worldwide it is estimated that there are about 84,000 cases of EBV-associated gastric carcinoma (5). EBV-positive gastric cancer cases constitute the largest group of EBV-associated cancers (4). In Poland, EBV-positive GC was 12.5%, in 2012 (2). Though, there are very few publications on the serological status of GC patients, in the available world literature. In a meta-analysis by Chenan *et al.* (6), the authors found only 4 publications comparing the presence of anti-EBV antibodies between patients with GC and healthy individuals. There are specific serological markers of EBV infection that are useful indicators of exposure to infection and reactivation. For example, antibodies against Epstein-Barr virus capsid antigen (EBVCA), that is expressed in latent infection, anti-Epstein-Barr virus nuclear antigen (EBNA) that is primarily epidemiological, and anti-early antigen (EA), which might indicate the reactivation of latent infection (6, 7). In addition, many studies indicate that transforming growth factor-β (TGFβ) induces EBV reactivation in certain types of cancer (8, 9).

Therefore, the aim of the present study was to evaluate the levels of antibodies against EBV antigens: anti-EBVCA IgG, anti-EBNA IgG and anti-EA IgG, in order to determine the possible reactivation of the virus. Furthermore, levels of IL-10 and TGF-β in sera of GC patients compared to the control group were estimated. Finally, we tested the incidence of latent membrane protein (LMP-1), a well-known oncoprotein that takes part in neoplastic transformation of EBV-infected B lymphocytes, and the 30 bp deletion variant del-LMP-1 (10).

Materials and Methods

Patients. The present study consisted of a group of 50 patients with diagnosed GC that were hospitalized at the Surgery Ward of Private Hospital in Nałęczów and AMG Hospital Center in Ryki, Poland. All patients were *Helicobacter pylori*-negative (based on the medical

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history of the patients). The results were compared to the control group, consisted of 50 individuals hospitalized at the same Ward due to diseases other than GC. There were no statistically significant differences between the patients and the control group (age, sex, tobacco and alcohol consumption). The socio-demographic and clinicopathological characteristics of the study group are shown in Table I. The research material consisted of sera and fresh frozen tumor tissue fragments. This research was approved by the Ethics Committee and is in accordance with the GCP regulations (no. KE-0254/133/2013/Medical University of Lublin, Poland).

Molecular methods. DNA extraction from fresh frozen tumour tissue, detection of EBV DNA, amplification of EBNA-2 gene (the nested PCR) and serological tests for antibody level detection were performed as previously described (11). PCR screening for the EBV LMP-1 subtype based on exon 3, defined as wild-type (wt-LMP-1) or del-LMP-1, was analyzed as previously described (12).

Statistical analysis. Descriptive statistics were used to characterize patient baseline characteristics. The Mann-Whitney *U*-test and Kruskal-Wallis test were used to compare the antibody and cytokine levels. Pearson's Chi-square test was used to investigate the relationship of LMP-1, clinical and demographical parameters between patients and control group. Statistical significance was defined as $p<0.05$.

Results

Men aged over 60 years and rural residents were predominant. GC was most frequently localized to the pylorus region (58.0%), then to the body region (24.0%), and the cardiac region of the stomach (18.0%). Anti-EBVCA IgG were detected in 88.0% of GC patients and in 86.0% of control individuals, while anti-EBNA IgG respectively in 90.0% and 84.0%. Antibodies against early antigen (EA) were more frequent in patients (72.0%) than in the control group (8.0%) ($p<0.001$). Levels of the studied antibodies and cytokines TGFβ and IL-10, were higher (24.4 ng/ml; 7.2 pg/ml, respectively) in patients with EBV-associated GC (EBVaGC) than in control patients (10.1 ng/ml; 3.5 pg/ml, respectively) ($p<0.001$) (Table II). An association was detected between serum levels of TGFβ, Il-10 and the level of the studied antibodies ($p<0.001$). The patients infected with wild type LMP-1 had a higher concentration of TGFβ (24.8 ng/ml *vs*. 18.1 ng/ml in del-LMP-1; $p<0.01$) (Table III).

Discussion

The number of publications on various aspects of GC is steadily increasing (13, 14). EBVaGC is more common in younger patients, between 50 and 68 years of age, while EBV-negative GC is more frequent among patients aged 56-72. Several risk factors for developing EBVaGC have been identified, *i.e*. tobacco smoking (15, 16, 17). In our own study 64.0% were men over the age of 60, most of them tobacco smokers (74%).

Epidemiological analyses of EBV infection in GC (also in other cancers) are based on the results of laboratory tests detecting the presence of the virus in tumor tissue, *i.e*. principally *in situ* hybridization (ISH) or PCR and serological tests to assess the presence and/or titer of specific antibodies in patients' sera (18). In the present study, PCR was used to detect the presence of EBV DNA.

Table I. *Socio-demographic and clinicopathological characteristics of patients and healthy individuals (control group).*

Parameters	Patients N=50		Control group N=50		*p*-Value
	N	%	N	%	
Gender					
M	31	64.0	33	66.0	>0.05
F	18	36.0	17	34.0	
Age					
40-59	15	30.0	16	32.0	>0.05
>60	35	70.0	34	68.0	
Place of residence					
Urban	10	20.0	10	20.0	>0.05
Rural	40	80.0	40	80.0	
Tobacco smoking					
Yes	37	74.0	35	70.0	>0.05
No	13	26.0	15	30.0	
Alcohol abuse					
Yes	35	70.0	33	66.0	>0.05
No	15	30.0	17	34.0	
G					
G1	8	16.0	-		
G2	32	64.0	-		
G3	10	20.0	-		
T					
T1	6	12.0	-		
T2	38	76.0	-		
T3	6	12,0	-		
T4	0	0	-		
N					
N0	20	40.0	-		
N1	13	26.0	-		
N2	7	14.0	-		
N3	10	20.0	-		
M					
M0	50	100.0	-		
M1	-	-			
Location					
Pylorus	29	58.0	-		
Body	12	24.0	-		
Cardia	9	18.0	-		
EBV type					
Wild	39	78.0	-		
del-LMP-1	11	22.0	-		
Antibodies positive					
EBVCAIgG	44	88.0	43	86.0	>0.05
EBNAIgG	45	90.0	42	84.0	>0.05
EA IgG	36	72.0	4	8.0	0.001*

*Statistically significant. M, Male; F, female; EBV, Epstein-Barr virus; del-LMP-1, LMP1 30bp-deletion variants; EBVCA, Epstein-Barr virus capsid antigen; EBNA, Epstein-Barr virus nuclear antigen; EA, early antigen.

Table II. *Serum level of anti-EBVCA, anti-EBNA, TGFβ and IL-10 in patients and healthy individuals (control group).*

anti-EBV	Means±SD	*p*-Value	Cytokine	Means±SD	*p*-Value
EBVCA			TGFβ		
Control	33,04±2.8		Controls	10.1±3.1	0.001*
GC Patients	66,7±6.3	0.000045*	Patients	24.4±6.8	
EBNA			IL-10		
Control	45,5±5.7		Controls	3.5±1.4	0.001*
GC Patients	69,4±2.1	0,00001*	Patients	7.2±3.1	

*S tatistically significant. anti-EBVCA, Anti-Epstein-Barr virus capsid antigen; anti-EBNA, anti-Epstein-Barr virus nuclear antigen; TGFβ, transforming growth factor β; IL-10, interleukin-10.

EBV is very widespread in the world, so approximately 95% of the population is detected with EBV-related antibodies. In a meta-analysis by Chenan *et al*. (6), EBNA IgG and EBVCA IgG were more frequently detected than the other antibodies, in both patients and control group. In our study, the prevalence of anti-EVBCA and anti-EBNA was similar in the GC and the control group, though titers of these antibodies differed between the groups, as both were significantly higher in EBVaGC patients. Anti-EA antibodies were found in 72% of patients with GC, of which 50% in high titer, while they were detected only in 17% of the control group, at very low titers. High anti-EA titers at the same time with high anti-EBVCA and anti-EBNA levels might indicate the reactivation of EBV infection in patients with GC.

A scientific research in Japan has shown statistically higher levels of EBVCA among GC than in control (19). On the other hand, Shinkura *et al*. (7) and Kayamba *et al*. (20) showed higher anti-EA antibody titers in patients with EBVaGC. This fact would suggest a re-activation of infection in the early stages of neoplastic transformation (21, 22).

An important feature of EBV, as well as of the entire Herpesviridae family, is the ability to enter into latency (23, 24), in which only some genes are expressed (25, 26).

EBVaGC is considered as a latency type I or II EBV infection, (18), in which *i.e*. EBNA-1, EBNA-2, LMP-1 and LMP-2 are expressed. EBNA-1 is present in all types of latency, and as the only EBNA type protein is also produced during activation into the lytic cycle. EBNA-2 is the major transcription factor regulating the expression of viral genes. It activates, among others, the LMP-1 and LMP-2 promoters and also transactivates many cellular genes that play a key role in cell proliferation and immortalization after infection (18).

Wang *et al*. (27) state that EBVaGC exhibits type I latency and, therefore, LMP-1 is not expressed. The authors show that the expression of LMP-1 occurs much more frequently in nasopharyngeal cancer (NPC). Some studies indicate low,

Table III. *Correlation between serum levels of TGFβ, IL-10, EBNA, EBVCA, anti-EA and LMP-1 type.*

Parameters	TGFβ	IL-10	EBNA	EBVCA
EA +	25.45±6.79	7.81±3.7	78.4	67.7
EA –	12.61±6.23	4.29±1.7	47.9	38.0
p-Value	0.0003*	0.016*	0.0001*	0.0001*
wt-LMP-1	24.8	7.8	64.8	68.5
del-LMP-1	18.1	7.4	69.4	76.6
p-Value	0.01*	>0.05	>0.05	>0.05

*Statistically significant. Del-LMP-1, LMP1 30bp-deletion variants; TGFβ, transforming growth factor β; IL-10, interleukin-10; EBNA, Epstein-Barr virus nuclear antigen; EBVCA, Epstein-Barr virus capsid antigen; anti-EA, anti-early antigen; LMP-1 type, latent membrane protein 1; wt-LMP-1, wild-type latent membrane protein 1.

while others show high LMP-1 expression in the EBVaGC (18). The type of virus with a deletion is more common in NPC (28). However, in a study in Japanese GC patients, the incidence of del-LMP-1 was similar in both the study and control group (29).

The results obtained from this study indicate that the LMP-1 protein was detected in all cases, which would indicate the presence of type II latency. The wild-type LMP-1 was detected in a predominant proportion (78%), while a del-LMP-1 was found in 22%. Tsao *et al*. (30) suggest that LMP-1 might play a different role in early and late stages of carcinogenesis. High expression of LMP-1 can lead to deregulation of the cytotoxic effect of tumor cells and increased rates of mutation and methylation EBVaGC, though authors state the need for further research on this field.

We were further demonstrated that all patients with EBV del-LMP-1 had a high serum anti-EA antibody titer, while 30% of GC patients with the wild type had high anti-EA titer and 60% low. In addition, there were observed high TGFβ

levels (24.8 ng/ml) in patients with wild-type LMP-1, which stimulated the reactivation of latent infection. It can, therefore, be assumed that the wild type plays a greater role in the pathogenesis of GC. However, the limitation of our study was that the sample size was quite small to make inferences about the Polish population means.

Reactivation of the virus and entry into the lytic cycle might be induced by various causes, including decrease in host resistance, various chemicals (31), and TGFβ (32).

Immunosuppressive cytokines, such as IL-10 and TGFβ, play an important role in the tumor process (33-39). In the present study, IL-10 was significantly higher in patients with GC (7.2 pg/ml), and in patients with high titers of anti-EA (7.8 pg/ml). In patients with high levels of IL-10 a high titer of anti-EBNA was antibody also observed. Viral IL-10 (vil-10), which exhibits high homology with human IL-10, is secreted late in the lytic phase and also at the onset of B lymphocytes infection. (40). IL-10 levels, as shown by other studies, are statistically higher in the serum of GC patients than in the control group and therefore might predict GC and, particularly, could be used for the assessment of disease progression (41, 42). Our results showed higher serum levels of TGFβ in patients with EBVaGC (24.4 ng/ml) compared to the control group (10 ng/ml). In studies by Shukla *et al.* (32) carried out on a group of 95 patients with EBVaGC, TGFβ mRNA expression was detected in 89.5% of cases, indicating the role of TGFβ in the development of GC. In addition, they found that TGFβ expression was closely related to the lytic phase of infection in patients without *H. pylori*. These researchers suggest that TGFβ plays a role in the reactivation of EBV. In this study, TGFβ concentration correlated with the high anti-EA antibody titer (25.44 ng/ml *vs.* 7.8 ng/ml, respectively). On the other hand, there was no such relationship between EBVCA and EBNA.

Genetic and epigenetic changes also take part in the process of tumor transformation of gastric epithelial cells. Up to now, 9 high-expression viral genes in EBVaGC have been described (43). It has also been found that in EBVaGC cases, mutations can occur in approximately 205 cell genes, including TGFβ receptor TGFBR1, which is a serine-threonine kinase protein. Induced EBV infection with TGFBR1 mutation may be of importance for EBVaGC pathogenesis (43). *In vitro* studies of EBVaGC have shown that epigenetic changes also affect the expression of many genes. A correlation between EBVaGC and CpG methylation was detected suggesting that hypermethylation of entire groups of genes promotes gastric epithelial cell transformation (43).

Conclusion

Our results show that antibodies against EBV are frequent (EBVCA - 88.0%, EBNA - 90.0%, EA - 72.0%) and the wild-type LMP-1 is predominant (78.0%) in Polish patients with GC. The significantly higher level of EA in the patients points to EBV reactivation. TGFβ level was significantly higher in GC than in control group, especially in EA-positive patients, suggesting its role in gastric carcinogenesis. However, further studies are necessary to understand the complex mechanisms of tumor transformation that gastric epithelial cells undergo.

Ethics Approval and Consent to Participate

This research was approved by the Ethics Committee and is in accordance with the GCP regulations (no. KE-0254/133/2013/ Medical University of Lublin, Poland).

All participants provided written informed consent to participate in this study according to forms required by the Local Ethics Committee.

Conflicts of Interest

The Authors declare that they have no competing interests.

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References

1 Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. CA Cancer J Clin *65(2)*: 87-108, 2015.

2 Cancer Genome Atlas Research Network: Comprehensive molecular characterization of gastric adenocarcinoma. Nature *513(7517)*: 202-209, 2014.

3 Liu S, Zhao Z, Han L, Liu S and Luo B: Epstein-barr virus infection in gastric remnant carcinoma and recurrent gastric carcinoma in qingdao of northern China. PloS One *11(2)*: e0148342, 2016.

4 Jacome AA, de Lima EM, Kazzi AI, Chaves GF, de Mendonca DC, Maciel MM and Dos Santos JS: Epstein-Barr virus-positive gastric cancer: a distinct molecular subtype of the disease? Rev Soc Bras Med Trop *49(2)*: 150-157, 2016.

5 Cohen JI, Mocarski ES, Raab-Traub N, Corey L and Nabel GJ: The need and challenges for development of an Epstein-Barr virus vaccine. Vaccine *31(02)*: B194-B196, 2013.

6 Chen XZ, Chen H, Castro FA, Hu JK and Brenner H: Epstein-Barr virus infection and gastric cancer: a systematic review. Medicine *94(20)*: e792, 2015.

7 Shinkura R, Yamamoto N, Koriyama C, Shinmura Y, Eizuru Y and Tokunaga M: Epstein-Barr virus specific antibodies in Epstein-Barr virus -positive and -negative gastric carcinoma cases in Japan. J Med Virol *60(4)*: 411-416, 2000.

8 Diaz Orea MA, Muñoz Perez V, Gómez Conde E, Castellanos Sánchez VO, Gonzalez Lopez R, Flores Alonso JC, Cárdenas ME, Galicia AL and Mendoza A: Expression of cytokines interleukin-2, interleukin-4, interleukin-10 and transforming growth factor β in gastric adenocarcinoma biopsies obtained from Mexican patients. Asian Pac J Cancer Prev *18(2)*: 577-582, 2017.

9 Ishimoto T, Miyake K, Nandi T, Yashiro M, Onishi N, Huang KK, Lin SJ, Kalpana R, Tay ST, Suzuki Y, Cho BC, Kuroda D, Arima K, Izumi D, Iwatsuki M, Baba Y, Oki E, Watanabe M, Saya H, Hirakawa K, Baba H and Tan P: Activation of transforming growth factor beta 1 signaling in gastric cancer-associated fibroblasts increases their motility, *via* expression of rhomboid 5 homolog 2, and ability to induce invasiveness of gastric cancer cells. Gastroenterol *153(1)*: 11-204, 2017.

10 Matsuda G, Nakajima K, Kawaguchi Y, Yamanashi Y and Hirai K: Epstein-Barr virus (EBV) nuclear antigen protein (EBNA-LP) forms complexes with a cellular anti-apoptosis protein Bcl-2 or its EBV counterpart BHRF1 through HS1-associated protein X-1. Microbiol Immunol *47(1)*: 91-99, 2003.

11 Polz-Dacewicz M, Strycharz-Dudziak M, Dworzański J, Stec A and Kocot J: Salivary and serum IL-10, TNF-α, TGF-β, VEGF levels in oropharyngeal squamous cell carcinoma and correlation with HPV and EBV infection. Infect Agent Cancer *11*: 45-53, 2016.

12 Foltyn S, Strycharz-Dudziak M, Drop B, Boguszewska A and Polz-Dacewicz M: Serum EBV antibodies and LMP-1 in Polish patients with oropharyngeal and laryngeal cancer. Infect Agent Cancer *12*: 31, 2017.

13 Bae JM and Kim EH: Epstein-Barr virus and gastric cancer risk: A meta-analysis with meta-regression of case-control studies. J Prev Med Public Health *49(2)*: 97-107, 2016.

14 Chen CJ, Hsu WL, Yang HI, Lee MH, Chen HC, Chien YC and You SL: Epidemiology of virus infection and human cancer. Recent Results Cancer Res *193*: 11-32, 2014.

15 Cho J, Kang MS and Kim KM: Epstein- Barr virus associated gastric carcinoma and specific features of the accompanying immune response. J Gastr Cancer *16(1)*: 1-7, 2016.

16 Kang MS and Kieff E: Epstein–Barr virus latent genes. Exp Mol Med *47*: e131, 2015.

17 Murphy G, Pfeiffer R, Camargo MC and Rabkin CS: Meta-analysis show that prevalence of Epstein-Barr virus-positive gastric cancer differs based on sex and anatomic location. Gastroenterol *137(3)*: 824-833, 2009.

18 Shinozaki-Ushiku A, Kunita A and Fukayama M: Update on Epstein-Barr virus and gastric cancer. Int J Oncol *46(4)*: 1421-1434, 2015.

19 Tajima M, Takeda F, Takeshima T, Yokohata T and Okinaga K: Human herpesviruses infections (I) – EBV infection and immunity in cancer patients. Kansenshogaken Zasshi *65(7)*: 799-807, 1991.

20 Kayamba V, Monze M, Asombang AW, Zyambo K and Kelly P: Serological response to Epstein-Barr virus early antigen is associated with gastric cancer and human immunodeficiency virus infection in Zambian adults: a case-control study. Pan Afr Med J *23*: 45-52, 2016.

21 Schetter AJ, You WC, Lennette ET, Gail M and Rabkin CS: Association of Epstein-Barr virus antibody levels with precancerous gastric lesions in high-risk cohort. Cancer Sci *99(2)*: 350-354, 2008.

22 Sairenji T, Tajima M, Kanamori M, Takasaka N, Gao X, Murakami M, Okinaga K, Satoh Y, Hoshikawa Y, Ito H, Miyazawa Y and Kurata T: Characterization of EBV-infected epithelial cells lines from gastric cancer-bearing tissues. Curr Top Microbiol Immunol *258*: 185-198, 2001.

23 Imai S, Koizumi S, Sugiura M, Tokunaga M, Uemura Y, Yamamoto N, Tanaka S, Sato E and Osato T: Gastric carcinoma: monoclonal epithelial malignant cells expressing Epstein-Barr virus latent infection protein. Proc Natl Acad Sci USA *91(19)*: 9131-9135, 1994.

24 Niedobitek G and Yung LS: Epstein-Barr virus persistence and virus-associated tumours. Lancet *343(8893)*: 333-335, 1994.

25 Reza MA, Reza MH, Mehdieyeh L, Mehdi F and Hamid ZN: Evolution frequency of Merkel cell polyoma, Epstein-Barr and mouse mammary tumor viruses in patients with Breast cancer in Kerman, southeast of Iran. Asian Pac J Cancer Prev *16(16)*: 7351-7357, 2015.

26 Ryan JL, James RJ, Kenney SC, Rivenbark AG, Tang W, Knight ER, Coleman WB and Gulley ML: Epstein-Barr virus – specific methylation of human genes in gastric cancer cells. Infect Agent Cancer *5*: 27, 2010.

27 Wang A, Zhang W, Jin M, Zhang J, Li S, Tong F and Zhou Y: Differential expression of EBV proteins LMP-1 and BHFR1 in EBV-associated gastric and nasopharyngeal cancer tissues. Molecular Medicine Reports *13(5)*: 4151-4158, 2016.

28 Da Costa VG, Ariany C, Marques-Silva AC and Moreli ML: The Epstein-Barr virus latent membrane protein-1 (LMP-1) 30-bp deletion and XhoI-polymorphism in nasopharyngeal carcinoma: a meta-analysis of observational studies. Syst Rev *4*: 46, 2015.

29 Mori S, Itoh T, Tokunaga M and Eizuru Y: Deletions and single-base mutations within the carboxy-terminal region of the latent membrane protein 1 oncogene in Epstein-Barr virus-related gastric cancers of Southern Japan. J Med Virol *57(2)*: 152-158, 1999.

30 Tsao SW, Tsang CM, To KF and Lo KW: The role of Epstein-Barr virus in epithelial malignancies. J Pathol *235(2)*: 323-333, 2015.

31 Choi CK, Ho DN, Hui KF, Kao RY and Chiang AKS: Identification of novel small organic compounds with diverse structures for the induction of Epstein-Barr Virus (EBV) lytic cycle in EBV-positive epithelial malignancies. PloS One *10(12)*: e0145994, 2015.

32 Shukla SK, Khatoon J, Prasad KN, Rai RP, Singh AK, Kumar S, Ghoshal U and Krishnani N: Transforming growth factor beta 1 (TGFβ-1) modulates Epstein-Barr virus reactivation in absence of *Helicobacter pylori* infection in patients with gastric cancer. Cytokine *77*: 176-179, 2016.

33 Ngan RK, Lan WH and Yip TT: Remarkable application of serum EBV EBER-1 in monitoring response of nasopharyngeal cancer patients to salvage chemotherapy. Ann NY Acad Sci *995*: 73-79, 2001.

34 Rosa MD, Gottlieb E, Lerner MR and Steitz JA: Striking similarities are exhibited by two small Epstein-Barr virus-encoded ribonucleic acids and the adenovirus associated ribonucleic acids VAI and VAII. Mol Cell Biol *1(9)*: 785-796, 1981.

35 Ruf IK, Rhyne PW, Yang C, Cleveland JL and Sample JT: Epstein-Barr virus small RNAs potentiate tumorigenicity of Burkitt Lymphoma cells independently of an effect on apoptosis. J Virol *74(21)*: 10223-10228, 2000.

36 Căinap SS, Fetică B, Buiga R, Căinap C, Constantin AM and Şovrea AS: The prognostic role of EBER in pediatric cancer. Rom J Morphol Embroyol *56(4)*: 1269-1277, 2015.

37 Lamberdt SL and Martinez OL: Latent membrane protein 1 of EBV activates phosphatidylinositol 3-kinase to induce production of IL-10. J Immunol *179(12)*: 8225-8234, 2007.

38 Mosser DM and Zhang X: Interleukin-10: new perspectives on an old cytokine. Immunol Rev *226*: 205-218, 2008.

39 Ng TH, Britton GJ, Hill EV, Verhagen J, Burton BR and Wraith DC: Regulation of adaptive immunity; the role of interleukin-10. Front Immunol *4*: 129, 2013.

40 Chao Y, Jing Y, Jia Y, Wang, Y, Zhao C and Luo B: Conservation and mutation of viral interleukin-10 gene in gastric carcinomas and nasopharyngeal carcinomas. J Med Virol *83(4)*: 644-650, 2011.

41 McKenzie J, Lopez-Giraldez F, Delecluse HJ, Walsh A and El-Guindy A: The Epstein-Barr virus immunoevasins BCRF1 and BPLF1 are expressed by a mechanism independent of the canonical late pre-initiation complex. PLOS Pathogens *12(11)*: e1006008, 2016.

42 De Vita F, Orditura M, Galizia G, Romano C, Infusino S, Auriemma A, Lieto E and Catalano G: Serum interleukin-10 levels in patients with advanced gastrointestinal malignancies. Cancer *86(10)*: 1936-1943, 1999.

43 Zhao J, Liang Q, Cheung KF, Kang W, Lung RW, Tong JH, To KF, Sung JJ and Yu J: Genome-wide identification of Epstein-Barr virus-driven promoter methylation profiles of human genes in gastric cancer cells. Cancer *119(2)*: 304-309, 2013.

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